HYPOTENSIVE ACTIVITY AND TOXICOLOGICAL PROFILE OF STEM BARK EXTRACT OF *SYZYGIUM GUINEENSE* (Willd, D.C. *Myrtaceae*) IN LABORATORY ANIMALS

BY

Hafeez MUHAMMAD, M.Sc. PHARM (ABU) 2006
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DECLARATION

I declare that the work in this Dissertation titled "Hypotensive Activity and Toxicological Profile of stem bark extract of *Syzygium guineense* (*Myrtaceaea*) in laboratory animals*"* has been carried out by me in the Department of Pharmacology and Therapeutics. 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.

_____ _____

Muhammad Hafeez Date

CERTIFICATION

This dissertation entitled HYPOTENSIVE ACTIVITY AND TOXICOLOGICAL PROFILE OF STEM BARK EXTRACT OF *SYZYGIUM GUINEENSE* (*Myrtaceaea*) IN LABORATORY ANIMALS by Hafeez Muhammad meets the regulations governing the award of the degree of Doctor of Philosophy Pharmacology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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Prof. O.A. Salawu Chairman Supervisory Committee	Date
_____	_____
Prof. I. M. Hussain Member, Supervisory Committee	Date
_____	_____
Dr. N.M. Danjuma Member Supervisory Committee	Date
_____	_____
Dr. A.U. Zezi Head of Department	Date
_____	_____
Prof. A.A. Joshua Dean, School of Postgraduate Studies	Date

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DEDICATION

To my parents

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ABBREVIATIONS

ACE	Angiotensin converting enzyme
ACEI	Angiotensin converting enzyme inhibitor
ADH	Antidiuretic hormone
ADR	adrenaline
ALDN	Aldosterone
ALLHAT	Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
Ang II	Angiotensin II
ANP	Atrial natriuretic peptide
ARB	Angiotensin receptor blocker
AST	Aspartate Aminotransferase
ASI	African Scientific Institute
AV	Atrio-ventricular
BHR	Borderline hypertensive rat
BMI	Body mass index
BRFSS	Behavioral Risk Factor Surveillance System
BUN	Blood urea nitrogen
BP	Blood pressure
Ca^{2+}	Calcium ion
CCB	Calcium channel blocker
CO	Cardiac output
CMF	Chloroform methanol fraction

CNS	Central nervous system
CVS	Cardiovascular System
DAG	Diacylglycerol
DBP	Diastolic blood pressure
DOCA	11-desoxycorticosterone acetate
DP	Diastolic pressure
DR	Dahl salt-resistant
DS	Dahl salt-sensitive
EEG	Electroencephalograph
ENaCs	Epithelial sodium channels
ESC	European Society of Cardiology
ESH	European Society of Hypertension
FDA	Federal Drug Administration
GGT	Gamma Glutamyl Transferase
Hb	Heamoglobin
HCT	Heamatocrit
HDL	High Density Lipoproteins
HOT	Hypertension Optimal Treatment
HR	Heart rate
HTN	Hypertension
ie	id est (that is)
IHME	Institute for Health Metrics and Evaluation
IMT	Intima-media thickness
IP_3	Inositol triphosphate
ISH	International Society of Hypertension

JNC7	Seventh report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure.
K^+	Potassium ion
Kg	Kilogram
L-NAME	Nitro-L- argentine methyl ester
LA	Left atrium
Lb.	Pound
LDL	Low Density Lipoproteins
LV	Left ventricle
LYM	Lymphocytes
mV	Millivolt
MAP	mean arterial pressure
MTD	Maximum Tolerated Dose
MNC	Monocytes
Na^+	Sodium ion
NEUT	Neutrophils
NHANES	National Health and Nutrition Examination Survey
NIPRD	National Institute for Pharmaceutical Research and Development
N.M.J.	Neuromuscular Junction
NO	Nitric oxide
NOEL	No Observable Effect Level
NPRs	Natriuretic peptide receptors
OECD	Organization for Economic Co-operation and Development
PCV	Packed Cell Volume
PLT	Platelets

PNS	Parasympathetic nervous system
PR	Peripheral resistance
R.A	Renin-angiotensin
R.A	Right atrium
R.A.A	Renin-angiotensin-aldosterone
ROS	Reactive oxygen species
RPM	Rounds Per Minute
RV	Right ventricle
SA	Sino-atrial
SAD	Denervation of sinoaortic baroreceptors
SBP	Systolic blood pressure
SEM	Standard Error of the Mean
SG	Syzygium guineense
SGF	Syzygium guineense fraction
SHR	Spontaneously hypertensive rat
SNS	Sympathetic nervous system
SP	Systolic pressure
SV	Stroke volume
T/Bil	Total Bilirubin
T/Chol	Total Cholesterol
TCA	Tricyclic antidepressant
TGR	Transgenic hypertension rat
Trig.	Triglycerides
VEDV	Ventricular end diastolic volume
VIP	Vasoactive intestinal peptide

VSMC	Vascular smooth muscle cell
WBC	White Blood Corpuscles
WHO	World Health Organization
WKY	Wister-Kyoto

ABSTRACT

Hypertension of unknown cause is called essential or primary hypertension while hypertension of a known cause is called secondary hypertension. Hypertension is called a silent killer because it is usually asymptomatic. It is a global problem affecting 1 in 3 adults in the United States. It is preventable and treatable disease. Orthodox drugs for its management are expensive and have associated side effects. Alternative source with good efficacy and minimum toxicity from natural products are explored. Hypotensive activity and toxicological profile of methanolic stem bark extract of *Syzygium guineense* (Willd, D.C. *Myrtaceae*) were evaluated. It is a medicinal plant used in folk medicine to treat hypertension, diarrhoea and infection. Phytochemical analysis of the stem bark extract was carried out using standard procedures. Bioassy-guided fractionation and finger printing of the crude extract were carried out. Five fractions(SGF_1-SGF_5) were isolated. The oral and intraperitoneal acute toxicity studies were carried out in mice using Lorke's method with modification. Subchronic oral toxicity studies of the methanolic stem bark extract in male and female Wistar rats for 90 days. The assessment of the toxic effect was evaluated at 42 and 90 days respectively, on clinical observations, feed and water intake, body weight, heamatology, blood chemistry, urinalysis, gross necropsy, organ weight and histopathology. The *in vivo* assessment of hypotensive activity of extract and fractions were carried in anaesthesized normotensive cat. The *in vitro* investigations to assess the possible mechanisms of hypotensive action of the extract were carried out on isolated tissue experiments. These included: guinea pig atria, rabbit perfused heart, rabbit ileum and rat uterus. The oral median lethal dose of the methanolic stem bark extract of *Syzygium guineense* was greater than 5000mg/Kg body weight while the intraperitoneal median lethal dose was 2154.1mg/Kg. There was significant ($p<0.05$) increase in feed intake in extract- treated male and female rats when compared to the corresponding control. There was also significant increase in water intake in male and female rats treated with *Syzygium guineense* in a dose-dependent manner in 90 days. The extract produced significant ($p<0.05$) increase in body weight of male and female rats. The relative organ body weight ratio values of extract-treated rats were not significantly different from those in the control groups. The haematological parameters and the biomarkers of liver function test and lipid profile were all normal in male and female rats when compared to the control. There

was also, no significant change in the serum levels of electrolytes, urea and creatinine in male and female rats. All the values were within normal range. The histopathology results revealed no significant changes in all the organs. Phytochemical tests revealed the presence of carbohydrates, alkaloids, saponins, tannins, phytosterols, anthranol glycosides, cardiac glycosides, phenols, terpenoids, and flavonoids. The extract and fraction F_3 significantly ($p<0.0001$) reduced both systolic and diastolic blood pressure of anaesthetized normotensive cat. The extract significantly ($P<0.05$) reduced adrenaline induced rise in systolic and diastolic pressure in anaesthetized cat. Atropine competitively reversed the hypotensive effect of the extract towards basal rhythm obtained with normal saline. The extract produced significant ($p<0.05$) concentration-dependent decrease in height of contraction of guinea pig atria. The extract also produced a concentration-dependent decrease in contraction of perfused rabbit heart. Adrenaline alone increased the height of contraction of the heart muscles. In the presence of the methanolic stem bark extract of *Syzygium guineense* the height of the amplitude produced was markedly lower than that due to adrenaline alone. The extract abolished all the spontaneous contractions of the rat uterus. These results showed that methanolic extract *Syzigium guineense* is safe and possesses significant blood pressure lowering effect in anaesthetized normotensive cat thus justifying its folkloric use in the treatment of hypertension.

CHAPTER 1.0 INTRODUCTION

1.1 Preamble

Hypertension (HTN) or high blood pressure is a chronic medical condition in which the blood pressure (BP) in the arteries is elevated and sustained. It is the second leading cause of premature death; kills 1 in 6 adults in the United States; and 1 in 5 Americans is completely unaware of the risk (Olives *et al*., 2013). Significant differences across the United States were revealed in the first ever analysis of awareness, treatment and control of hypertension conducted by the Institute for Health Metrics and Evaluation (IHME) at the University of Washington. Also, in the united states, estimate trends in prevalence, awareness, treatment and control were derived from data on National Health and Nutrition Examination Survey (NHANES) and Behavioral Risk Factor Surveillance System (BRFSS). The estimate of median prevalence of total hypertension in 2009 was; 37.6% (range: 26.5 to 54.4%) in men and 40.1% (range 28.5 to 57.9%) in women. The median country-level control was 57.7% (range 43.4 to 65.9%) in men and 57.1% (range 43.0 to 65.46%) in women.Although control of hypertension is on the rise, prevalence of total hypertension continues to increase in the US (Olives *et al*., 2013).

Hypertension is a multifactorial disease involving a complex interplay of genetic and environmental factors (Lerman *et al*., 2005); it is one of the most common global Health ailments affecting more than 10% of the world's population (Chen *et al*., 2007). It is the leading preventable cause of cardiovascular diseases such as stroke, myocardial infarction, left ventricular hypertrophy, nephropathy, retinopathy and dementia. Every 20mmHg of systolic or 10mmHg diastolic increase in blood pressure

above 115mmHg/75mmHg in middle and old age groups, results in doubling of mortality from ischemic heart disease and stroke (Lewington *et al*., 2002).

Over 50 million Americans have, as indicated by the data from the National Health and Nutrition Examination survey (NHANES), hypertension that warrant treatment (Burt *et al.,* 1995; Hajjar and Kotchen, 2003). The global prevalence estimates for hypertension is about 1 billon individuals; and approximately 7.1 million deaths per year may be attributable to it (W H O, 2002). Prevalence increases with advancing age; more than half of people 60-69 years of age and approximately three quarter of those 70 years and older are affected (Burt *et al.,* 1995). The life time risk of hypertension is approximately 90 percent for men and women who were non-hypertensive at 55 or 65 years and survived to the age of 80-85 years (Vasan *et al*., 2002).

The reduction in cardiovascular, and renal morbidity and mortality is the ultimate goal of antihypertensive therapy. A number of drugs are available for reducing blood pressure. Two or more antihypertensive agents from different classes of drugs are required in most hypertensive individuals before a control can be attained (Cushman *et al*., 2002; Hansson *et al.,* 1998; Black *et al*., 2003; Dahlof *et al*., 2002; Materson *et al.,* 1993).

Advances have been made in the efficacy and tolerability of orthodox antihypertensive drugs. Nevertheless, target blood pressure (i.e. <140/90mmHg) is not achieved in 25% of individuals treated with them. (Evans *et al*., 2005); and apart from being expensive, each agent is associated with relative drawbacks, often within the context of various patient characteristics such as age, co-morbidities and risks status (Germino, 2009).

Plants have been used for prevention and treatment of various diseases since ancient times. The use of different plants in the form of botanical drugs, dietary suppliments, and medicinal foods is increasing (Raskin *et al*., 2002). Human use of plants as medicines is dated from fossil records at least to the middle Paleolithic age some 60,000 years ago (Solecki and Shenidar, 1975).

Almost 65% of the world's population have, according to the World Health Organisation, incorporated medicinal plants into their primary modality of health care (Farnsworth *et al*., 1985). The World Health Organisation (WHO) also estimates that 80% of the population living in developing countries depend on traditional medicine for their primary health care needs (WHO, 2001).

Natural products can be considered as one of the potential sources for more effective and better tolerated antihypertensive agents. The present study is attempts to evaluate the antihypertensive effect of methanolic stem bark extract of *Syzygium guineense* to support its folkloric use in the treatment of hypertension.

1.2 Statement of Research Problem

Hypertension is a global cardiovascular disease. It affects as many as 73 million Americans including children (Rosamond *et al.,* 2008). It increases the risks of serious health conditions, including strokes, heart attacks,heart and kidney failure. Symptoms of hypertension are often undetectable, many people live with untreated high blood pressure and hence the disease is called silent killer disease.

In 2005, a report by the National Health Examination Surveys (NHANES), in the United State indicated that the population aged 20 years or older, an estimated 41.9 million men and 27.5 million women have prehypertension, 12.8 million men and 12.2 million women have stage I hypertension and 4.1 million men and 6.9 million women have stage II hypertension (Qureshi *et al*., 2005). Approximately 20% of the world adults are estimated to have hypertension when hypertension is defined as blood pressure [BP] in excess of 140/90 mmHg. The prevalence increases in patients over 60 years. It is also estimated to cause approximately 7.1 million death annually (WHO, 2002). Prescription (synthetic) drugs normalize the blood pressure but do not treat it. They are expensive and have associated side effects which could be life ridden (Barton, 2008).

Medicinal plants have, since time immemorial, been used in virtually all cultures as a source of medicine. In most developing countries, the use of traditional medicine and medicinal plants as normative basis for the maintenance of good health has been observed (UNESCO, 1996). Dietary change is important in lowering blood pressure. The Dietary Approaches to Stop Hypertension (DASH) includes fruits and vegetables, low fat dairy food, beans and nuts as well as sodium restriction (Wong, 2008).

Natural remedies have been used in traditional medicine for thousands of years to support the health of the heart and circulatory system. Herbal medicines that contribute to the commercial drug preparations manufactured today include, ephedrine from *Ephedra sunica*, Salicin (source of aspirin) from *Salix alba,* digitoxin from *Digitalis purpurea* and reserpine from *Rauwolfia serpentina* (Nick *et al*., 1998). A number of published clinical studies demonstrated the ability of range of herbs to support cardiovascular system and to maintain blood pressure within normal range

(Leuchtgens, 1993). Further studies demonstrated the benefits of certain herbs in maintaining cardiac health and blood flow (Hempel *et al*., 2005).

Different parts of *Syzygium guineense* have various folkloric uses. Decoction of the bark is used as antidiarrhoeal and antihypertensive (Orwa *et al*., 2009). The seed of the plant is used to treat Diabetes Mellitus (Adebayo, 2009). It is used in combination with other medicinal plants to treat Herpes zoster (Kisangau *et al*, 2007; Kazlila and Hedmbi, 2010). It is used to treat malaria, has antibacterial effect (Tsalaka, 1997) and molluscicidal activity (Oketch-Rabah and Dossaji 1998).

1.3 Justification

Hypertension is gradually becoming a global killer disease. The incidence is increasing in the developed countries, in spite of the availability of effective antihypertensive drugs. Nearly one in three Americans has, according to federal health officials; high blood pressure and more than half of those cases are "out of control" (Rosamond *et al*., 2008; Olives *et al*., 2013). In developing countries especially Africa, access to effective, inexpensive and good quality medicines is vital especially at primary care level. In Nigeria prevalence of hypertension ranges from 8% - 46.6% depending on the study target population, type of measurement and cut-off value used for defining hypertension. Prevalence is similar in men and women (7.9% - 50.2%) vs 3.5% - 68.8% respectively) and in the urban (8.1% - 42.0%) and rural setting (13.5% - 46.4%). Awareness, treatment and control are generally low with attendant high burden of hypertension related complications (Ogah, *et al*., 2012).

In developing countries, conventional treatment of hypertension is expensive, inaccessible to most rural populace and is associated with severe adverse or toxic effects. Poorly treated or untreated cases of hypertension especially primary (essential) type predisposes to serious end organ damage such as heart failure, kidney damage, hypertensive encephalopathy and hypertensive retinopathy. In Sub-Saharan Africa, more than 80% of the population relies on traditional medicines and healers as the primary source of health care (WHO, 2002). This is mainly due to easy accessibility and affordability of consulting with traditional healers. The use of plant remedies has steadily increased worldwide in recent years, as well as the search for new phytochemicals that potentially could be developed as useful drugs for the treatment of hypertension and other chronic diseases. Problems of access to and high cost of conventional antihypertensive therapy, particularly by the rural dwellers in low income settings like Nigeria necessitated this research into the hypotensive activity and toxicological evaluation of methanolic extract of *Syzygium guineense* stem bark in experimental animals.

1.4 Theoretical Frame Work

Phytotherapy is a modern botanical medicine that applies scientific research and professional standards to the practice of herbal medicine. The methods used to evaluate herbal medicines are similar to those used by orthodox medicines. A herb may however, contain many active ingredients and may have several actions to support the body's health, unlike the single chemical in a drug. The initial evaluation of phytotherapeutic agents is acute toxicity studies which may reveal clinical manifestations elicited by the drug on investigation and provide viable information for

determining median lethal dose (LD_{50}) based on method described by Lorke (1983) with slight modifications. The results could also be used to identify the different dose levels for chronic or subchronic toxicity studies.

1.4.1 *In-Vitro* Studies

These are experiments that are conducted using component of an organism (tissue) that have been isolated from their usual biological surroundings in order to permit a more detailed or more convenient analysis than can be done with whole organisms. Tissue organ bath are used to maintain the integrity of the tissue for several hours, in a temperature controlled environment while physiological measurements are performed. Experiments involve the addition of drugs to the organ-bath or direct/field stimulation of the tissue. Reaction of the tissue is by contraction or relaxation. Isolated tissue preparations are used to study the effects of drugs on specific type of receptors. They are also used for bioassay of drugs, characterisation of specific receptors or its subtypes to determine concentration response curve of an agonist, to study antagonism of drug in a new drug discovery. Other advantages of isolated tissue over intact animals are that several preparations can be tested on tissue obtained from single animal; relatively small amount of test materials is required and the drug effect is tested directly and its action as the drug is free from the factors of absorption, metabolism and excretion (Narkhede *et al.*, 2012). Tissues can be sourced from various species (e.g. rabbit, guinea pig, rat, chick or toad). Some of the common tissues that are studied with organ bath system include: heart tissues, aortic rings, ileum, phrenic diaphragm and gastrenemius muscles (www.adinstruments.com).

1.4.2 *In-Vivo* Research

In vivo research is experimentation using a whole living organism. It is better suited for observing the overall effect of drugs and chemical agents on living organisms. Experiments are conducted in animal models to support clinical drug candidate selection. Clinical trial is also a form of *in vivo* research. Pharmacodynamic and pharmacokinetic properties of drugs in normal pathophysiological animal models are studied. Toxicology *in vivo* research includes acute, sub-chronic and chronic studies which are usually designed to meet international guidelines (OECD, FDA etc.).

1.4.3 Sub-chronic toxicity Study

In drug discovery research, the primary objective of toxicology studies is to evaluate the safety of potential drug candidates. The ultimate goal is to translate the animal responses into the under of the risk for human subject.

Subchronic toxicity studies are 12-26 weeks duration. The animals are dosed daily by the same route (oral) that the chemical substance would normally be administered to humans. They are then observed for any physical changes as well as changes in body weight or food consumption. At the end of the dosing regimen, the animals are euthanisized and their tissues evaluated for evidence of toxicity.

1.4.4 High-Performance liquid Chromatography

This is a chromatographic technique used to separate mixture of compounds with the purpose of identifying, quantifying and purifying the individual components of the mixture. Chemical separation can be accomplished by utilising the fact that certain compounds have different migration rates given a particular column and mobile phase. Compounds can be separated; the extent or degree of separation is determined

by the choice of stationary phase and mobile phase. One method of separation would involve a column packing that is polar so that a solution of solute of differing polarity would have the solutes held on the column packing for differing periods of time, depending on the polarity of the solute. Thus less polar solutes would be eluted first and very polar solutes would be eluted last.

1.5 Objective of the study

The main objective is to determine the hypotensive activity and safety profile of methanolic stem bark extract of *Syzygium guineense.* Specific objectives include:

i. To evaluate the acute toxicity of the extract to detect behavioural signs of toxicity and determine the median lethal dose ($LD_{50)}$ following oral and intraperitoneal administration in rats.
ii. To carry out fractionation of the crude extract using Column Chromatography.
iii. To carry out phytochemical screening of the crude extract.
iv. To determine the effect of the extract and fractions on anaesthetized normotensive cats' blood pressure.
v. To determine the effect of the extract and fractions on the adrenergic and cholinergic receptors on isolated guinea pig atria.
vi. To determine the effect of the extract and fractions on isolated pefussed rabbit heart.
vii. To determine the effect of the extract and fractions on the adrenergic and cholinergic receptors on isolated rabbit ileum.

viii. To determine the effect of the extract and fractions on isolated non-pregnant rat uterus.

ix. To carry out sub-chronic (90 days) toxicity studies of the crude extract to determine its long term effects in rats.

1.6 Research Hypothesis

Syzygium guineense stem bark is safe and contains pharmacologically active chemical constituent(s) with hypotensive activity.

CHAPTER 2.0 LITERATURE REVIEW

2.1 Overview of the Design of the Circulatory System

2.1.1 Physiology of the circulatory system

The circulatory system, the chief transport system of the body is made up of three principal components, the heart, blood vessels and blood.

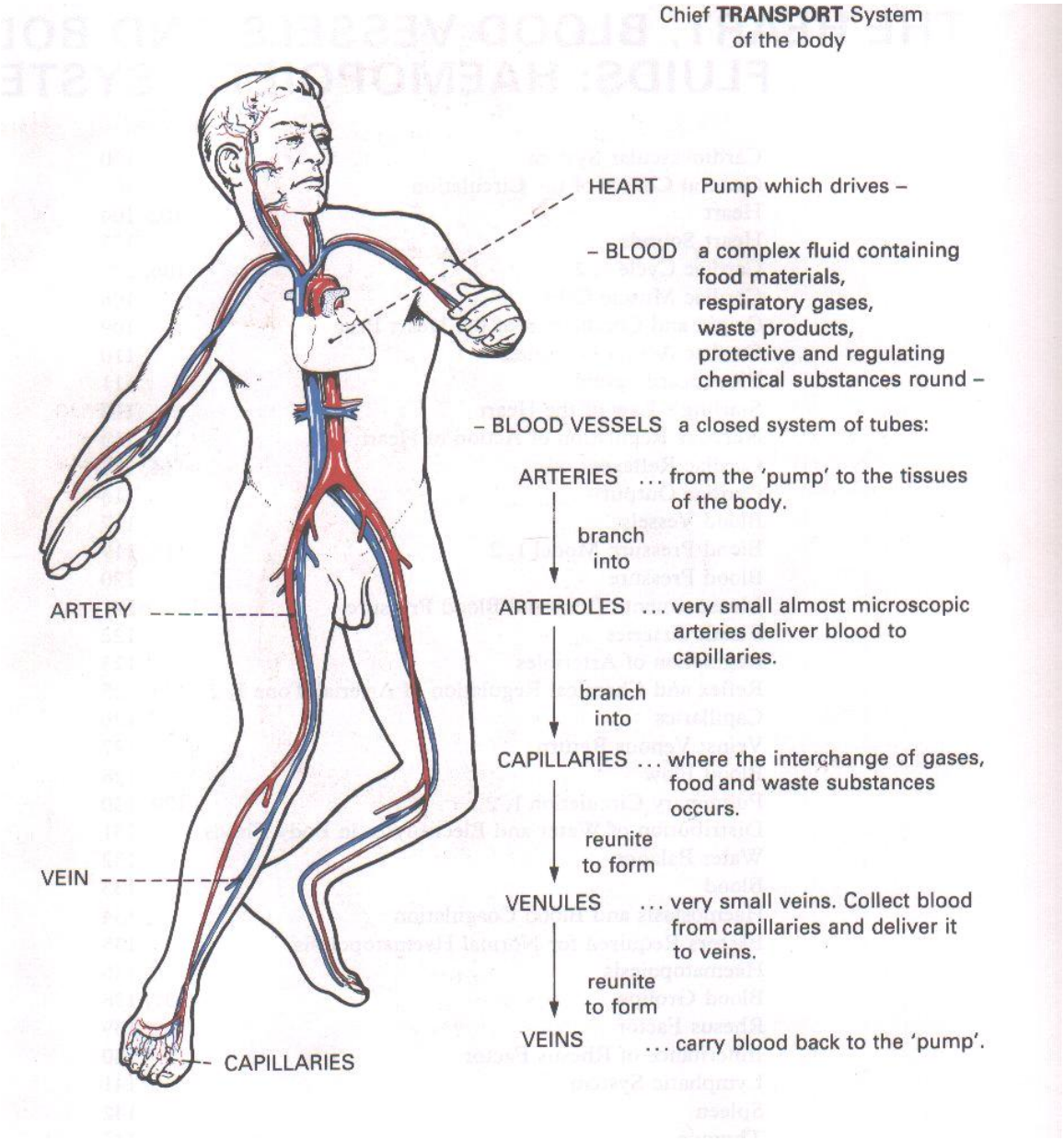


Fig. 2.1: Component of Circulatory System (Mackenna and Callender, 2003)

The cardiovascular system forms a closed loop. Blood ejected out of the heart through one set of vessels returns to the heart by different set.

The heart is cone shaped, approximately the size of a closed fist and is located in the thorax. It is divided into left and right halves by a septum. Each half contains two chambers: an upper chamber – the atrium- and a lower chamber the ventricle. The atrium on each side empties into the ventricle on that side. The heart receives blood through the veins and propels it out through the heart in only one direction because of the four valves that guard the openings of the ventricles to prevent back flow, (Fig 2.2).

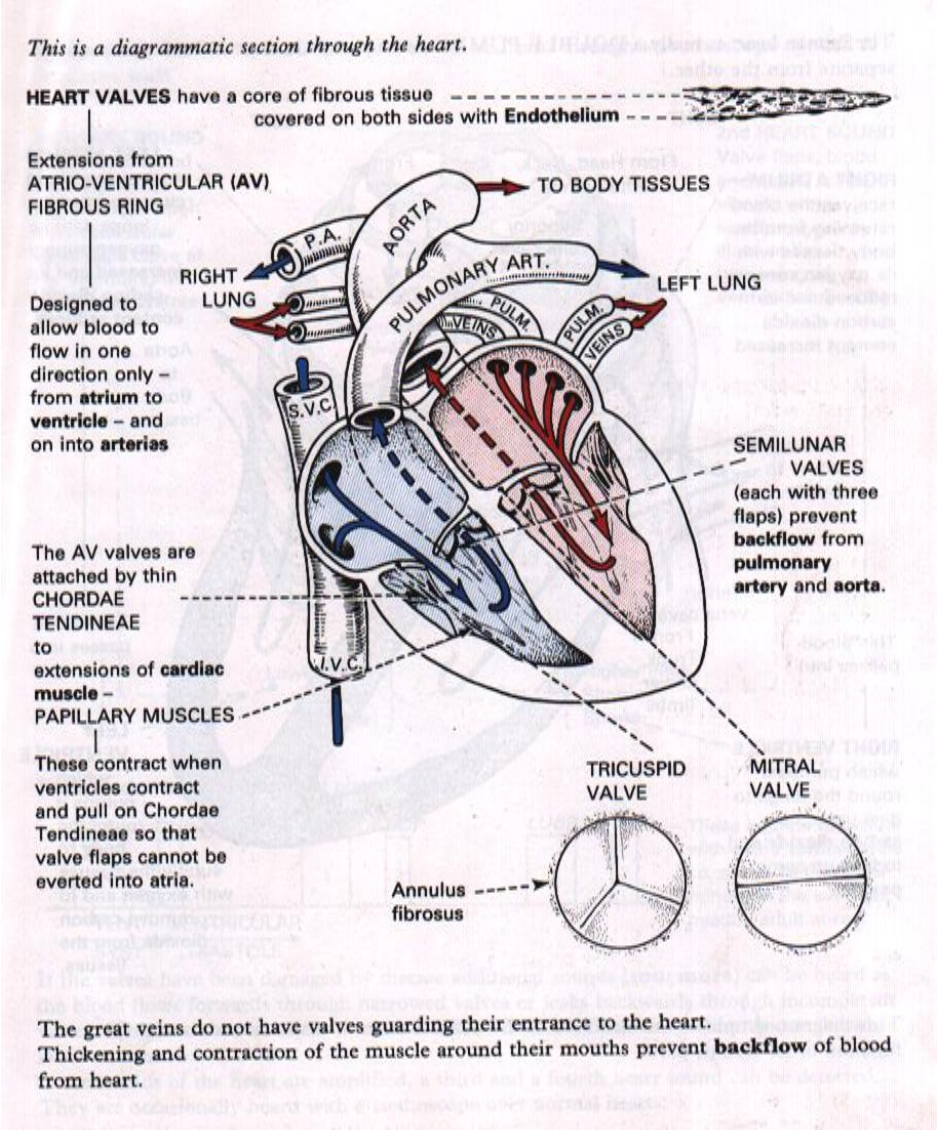


Fig. 2.2:Diagrammatic Section through the Heart (Mackenna and Callender, 2003)

The path of blood from the heart (right ventricle), through the lungs and back to the heart (left atrium) constitutes the Pulmonary Circulation. Oxygen-rich blood in the left atrium enters the left ventricle and is pumped out into a very large, elastic artery-the aorta. Arterial branches from the aorta supply oxygen-rich blood to all organ systems. These arteries divide into progressively smaller vessels. The smallest arteries branch into arterioles, which also branch into a large number of small vessels, the capillaries, which unite to form large diameter venules. The venules unite to form

larger vessels veins. The veins from various organs unite to produce two large veins, the inferior *vena cava* from below the heart and the superior *vena cava* from above the heart. These two veins return blood to the right atrium; constituting systemic circulation (Widmaier *et al*., 2004).

In the circulatory system, the heart functions as the primary pump, large arteries as conduits, the arterioles as resistance vessels, the capillaries as exchange mechanisms and the veins as capacitance vessels.The functional and structural characteristics of blood vessels change with successive branching. The entire cardiovascular system from the heart to the smallest capillary has one structural component in common, a smooth, single-celled layer of endothelial cells which lines the inner surface of the vessels. Capillaries consist of only endothelium; other vessels have additional layers of connective tissue and smooth muscle. The aorta and other systemic arteries have thick walls containing large quantities of elastic tissue. They are viewed as elastic and because of large radii they serve as low-resistance tubes. They also act as pressure reservoir for maintaining blood flow through tissues during diastole.

Arterioles in individual organs are responsible for determining the relative blood flow to those organs at any given mean arterial blood pressure. They are also, as a whole a major factor in determining mean arterial blood pressure itself. Capillaries contain at any given moment, approximately five percent of the total circulating blood; it is this 5 percent that is performing the ultimate function of the entire cardiovascular system i.e the exchange of nutrients and metabolic end products (Widmaier *et al*., 2004). The volume of blood in an averaged sized person (70kg; 1541b) is approximately 5.5L. The rapid flow of blood throughout the body is produced by pressures created by the pumping action of the heart. This flow is known as bulk flow because all constituents of blood move in one direction together. All the blood pumped by the right side of the

heart is received by the lungs, whereas each of the peripheral organs and tissues receives only a fraction of the blood pumped by the left ventricle in an adult at rest (Table 1.1).

Table 2.1: Distribution of systemic blood flow to the various organs and tissues of the body at rest.

Organ	Flow at rest	Ml/min
Brain	650	(13%)
Heart	215	(4%)
Skeletal muscle	1030	(20%)
Skin	430	(9%)
Kidney	950	(20%)
Abdominal organs	1200	(24%)
Other	525	(10%)
Total	5000	(100%)

2.1.2 Electrical activity of the heart and electrocardiogram

The rhythmic contraction of the heart is called the heartbeat. Special cells, called 'pacemaker' cells in the sino-atrial (SA) node which lies in the wall of the right atrium originate the electrical activity which produces contraction of the cardiac muscles. This pacemaker exhibits a spontaneous depolarisation that causes action potentials which results in the automatic beating of the heart. Electrical impulses are conducted by myocardial cells in the atria and are transmitted to the ventricles by specialized conducting tissues (Stuart, 2004).

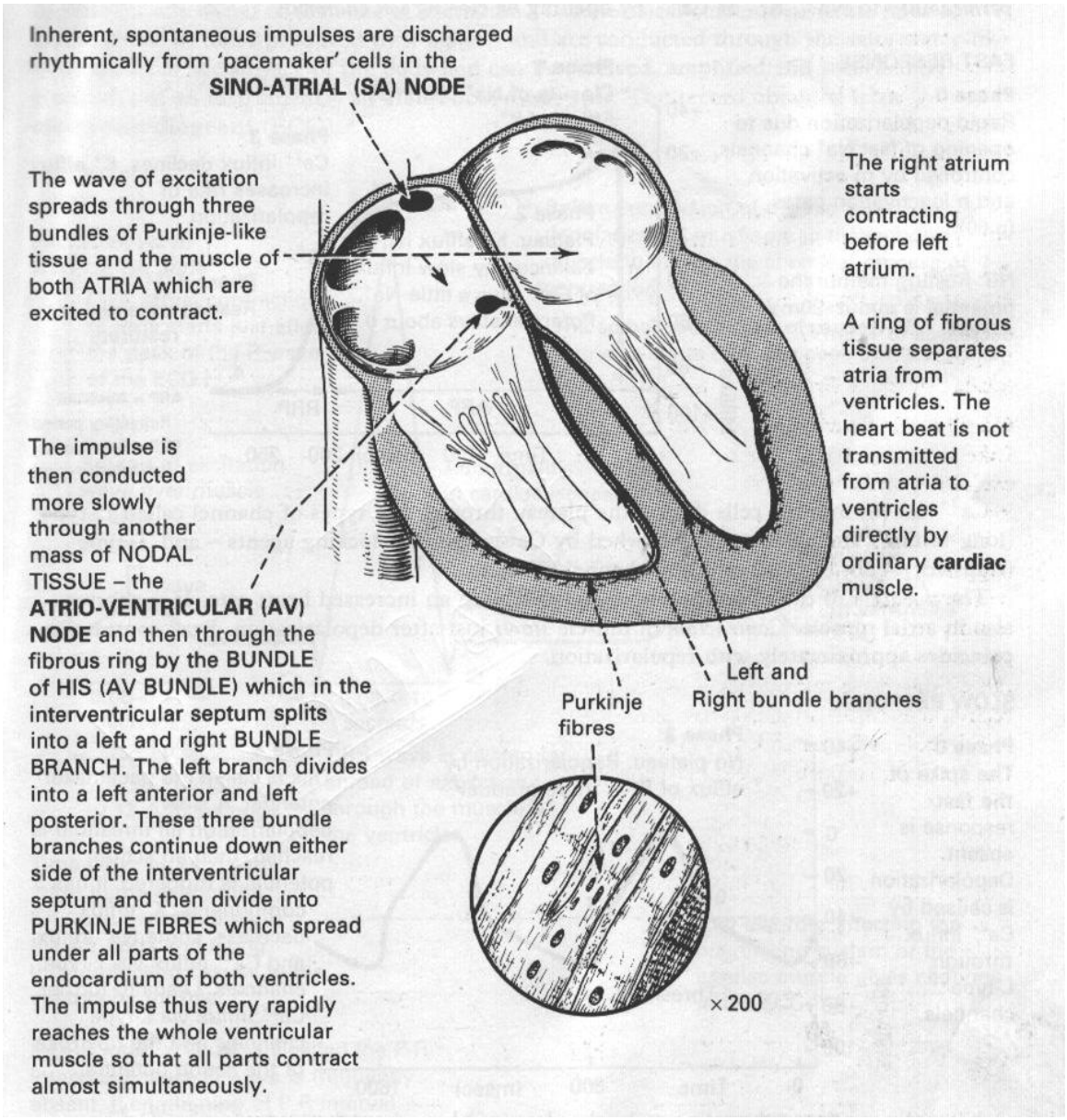


Fig. 2.3: Conduction System of the Heart (Mackenna and Callender, 2003)

Two types of action potential occur in the heart. These slow response, pacemaker potential and fast response, and myocardial action potential [Fig. 2.4 and Fig. 2.5].

In the pacemaker potential, the SA node exhibits a slow spontaneous depolarisation. The membrane potential begins at about -60mv and gradually depolarise to -40mv which is the threshold for producing an action potential in these cells. Diffusion of Ca^{2+} through openings in the membrane called *slow calcium channels* produce the

spontaneous depolarisation. At the threshold level of depolarisation, other channels, called fast calcium channels, open, and Ca^{2+} rapidly diffuses into the cells. The opening of voltage-regulated Na^{+} gates and the inward diffusion of Na^{+} contribute to the upshot phase of the action potential in the opening of K^{+} gates and outward diffusion of K^{+}.

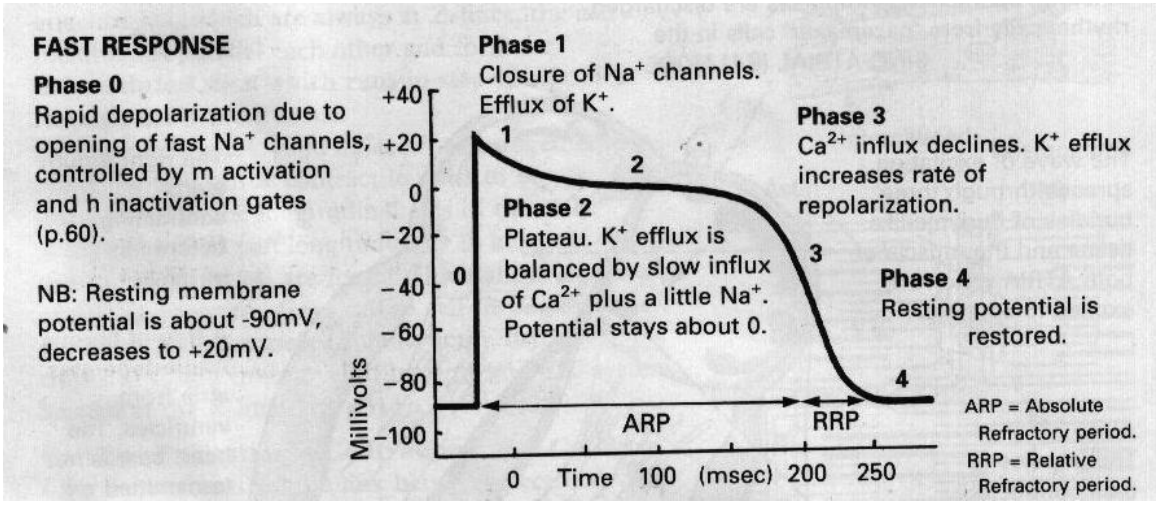


Fig. 2.4: Cardiac Action Potential (Mackenna and Callender, 2003)

The myocardial action potential is stimulated by action potential from pacemaker region. The myocardial cells have resting potential of about -90mv. When stimulated by action potential from pacemaker region, the cells become depolarized to threshold at which point their voltage-regulated Na^{+} gates open. The upshoot phase of the action potential of non-pacemaker cells is due to the inward diffusion of Na^{+}. The membrane potential quickly declines to about – 15mv following the rapid reversal of membrane polarity; the depolarisation is maintained for 200 to 300msec before repolarisation. The plateau phase results from a slow inward diffusion of Ca^{2+} which balances a slow outward diffusion of cations. At the end of Plateau phase rapid repolarisation is achieved; and is due to the opening of K^{+} channels and the rapid out ward diffusion of K^{+}. The refractory periods absolute and relative, last almost as long as the contraction;

so that the myocardial cells cannot be stimulated a second time until they have completed their contraction from the first stimulus (Fox, 2004).

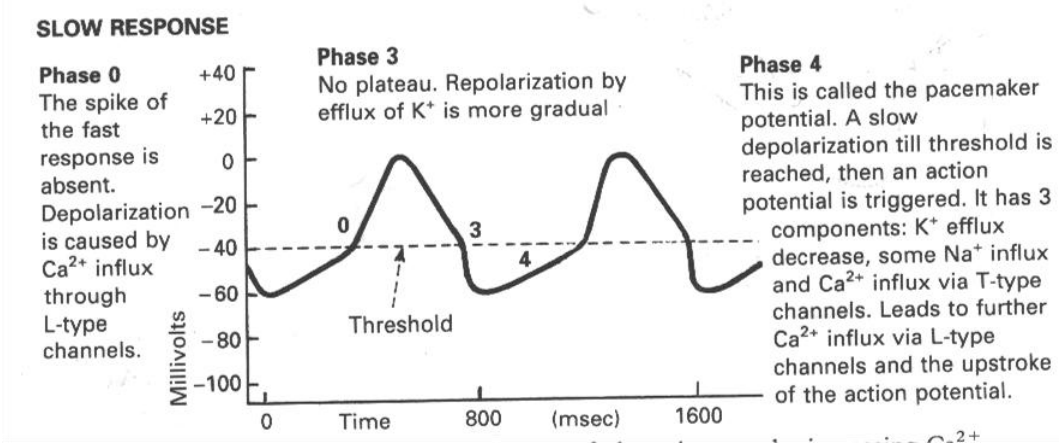


Fig. 2.5: Cardiac Action Potential (Mackenna and Callender, 2003)

The wave of excitation which spreads through the heart wall consists of changes in electrical activity of the membrane of cardiac muscle cells. The electrical currents generate lines of force similar to those produced by a magnet and are conducted through the salty water-like body fluids to the surface of the body and are received, amplified and recorded by an electrocardiograph. This produces a record called electrocardiogram (ECG), (Fig. 2.6). Each cardiac cycle produces three distinct ECG waves designated P, QRS and T. These waves represent changes in potential between two regions on the surface of the heart that are produced by composite effects of action potentials in numerous myocardial cells. Any disorder affecting the conducting system or the cardiac muscle gives changes in the ECG. (Mackenna and Callender, 2003; Fox, 2004).

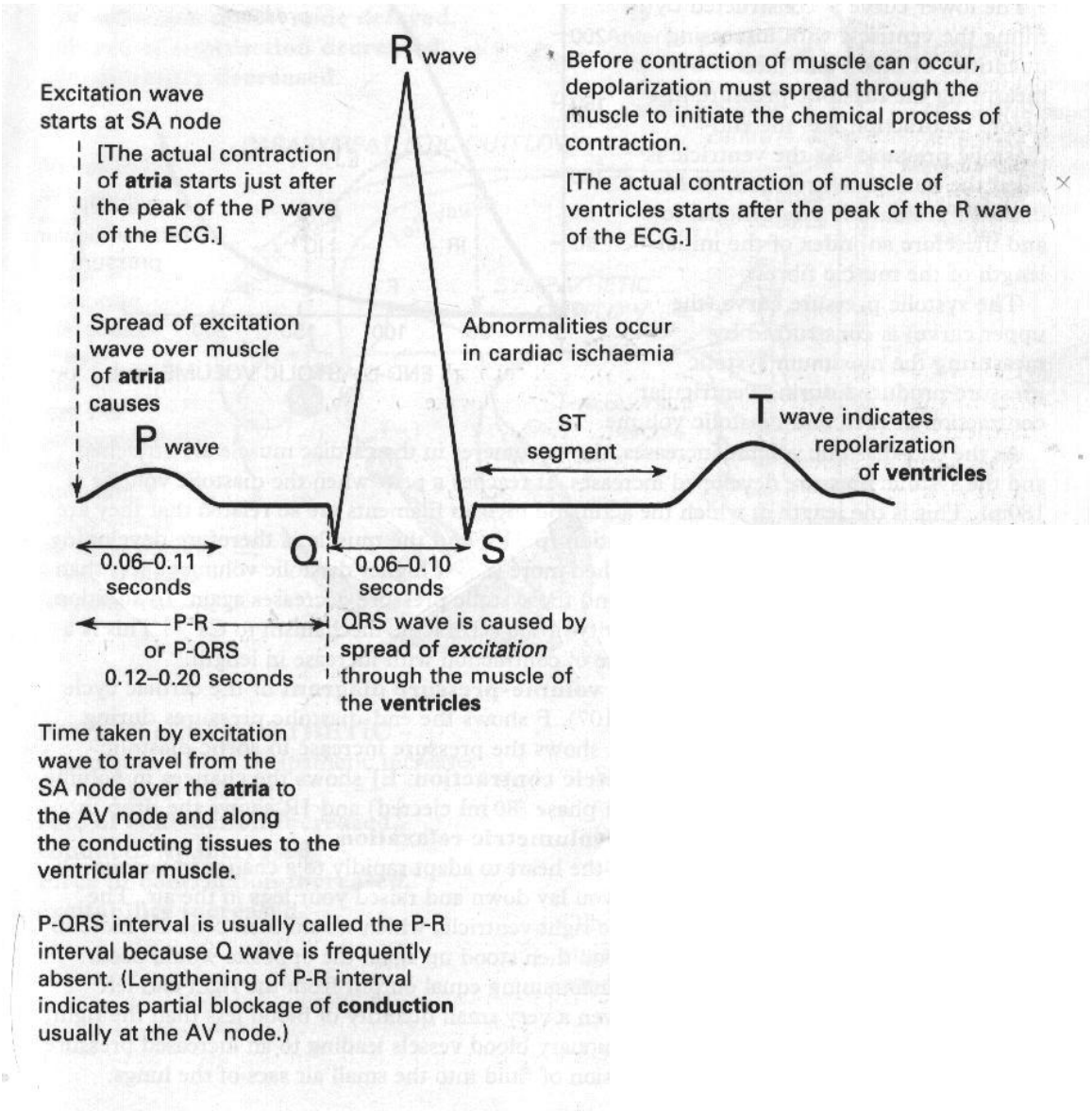


Fig. 2.6: Electrocardiogram (Mackenna and Callender, 2003)

2.1.3 Mechanical events of the cardiac cycle

The pacemaker potentials which stimulates myocardial action potentials produce an orderly process of depolarisation which triggers a recurring Cardiac cycle of atrial and ventricular contractions and relaxations.

The cycle is divided into two major phases, both named for events in the ventricles. The period of ventricular contraction and blood ejection is called systole, and the period of ventricular relaxation and blood filling is called diastole. At an average heart rate of 72 beats/min, each cardiac cycle lasts 0.8s with 0.3s in systole and 0.5s in diastole (Widmaier, 2004). Pressure changes during one cardiac cycle which occur in the aorta, the left ventricle and left atrium, along with changes in the left ventricular volume are indicated in Fig 2.7. The figure also indicates correlating ECG and heart sounds (Mackenna and Callender, 2003).

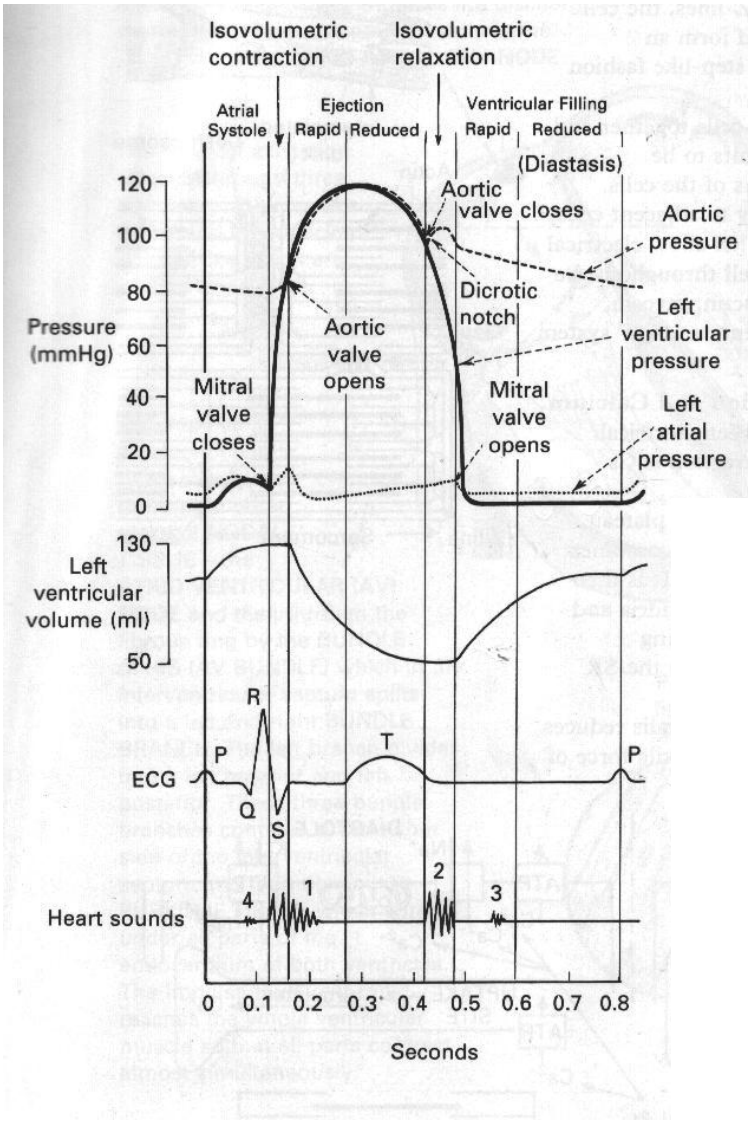


Fig. 2.7: Cardiac Cycle (Mackenna and Callender, 2003)

During the first part of the systole, the ventricles are contracting but all valves in the heart are closed and so no blood can be ejected. This period is termed isovolumetric ventricular contraction because the ventricular volume is constant. The ventricular walls are developing tension and squeezing on the blood they enclose, raising the ventricular blood pressure. Once the pressure in the aorta and pulmonary trunk is exceeded by the rising pressure in the ventricles, the aortic and pulmonary valves open and ventricular ejection period of systole occurs. Blood is forced into the aorta and pulmonary trunk as the contracting ventricular muscle fibres shorten. The volume of blood ejected from each ventricle during systole is termed stroke volume (Mackenna and Callender, 2003)

During the first part of diastole, ventricles begin to relax, and the aortic and pulmonary valves close. The atrioventicular valves are also closed; no blood is entering or leaving the ventricles. This is termed isovolumetric relaxation. The atrioventricular valves then open and Ventricular filling occurs as blood flows in from the atria. Atrial contraction occurs at the end of diastole, period of diastasis, after most of ventricular filling has taken place. The ventricle receives blood throughout most of diastole, not just when the atrium contracts (Widmaier *et al*., 2004).

With ejection of each stroke volume about 50ml of blood remains in the ventricle as residual volume which becomes less with increased contractility during exercise, and increases in severe heart failure. The time of diastasis is reduced more than the other phase of the cardiac cycle with increase in heart rate in exercise(Mackenna and Callender, 2003).

2.1.4 Cardiac output

The cardiac output [CO] is the volume of blood ejected by one ventricle in one minute. It is usually expressed in litres per minute. It is also the volume of blood flowing through either the systemic or the pulmonary circuit per minute. The cardiac output is determined by multiplying the heart rate [HR] i.e the number of beats per minute and the stroke volume (SV) i.e the blood volume ejected by each ventricle with each beat. Mathematically it is expressed as:

CO = HR x SV

2.1.4.1 Control of heart rate

Autonomic innervation of the SA node represents the major means by which cardiac rate is regulated. Activity in the vagal nerve causes the heart rate to decrease, whereas activity in the sympathetic nerves increases the heart rate. In the resting state, there is considerably more vagal activity on the heart than sympathetic; this maintains the resting heart rate at about 70 beats per minute. Adrenaline from the adrenal medulla, speeds the heart rate by acting on beta-adrenergic receptors in the sino-atrial node. The heart rate is also sensitive to changes in the body temperature, plasma electrolyte concentration and a metabolite adenosine, produced by myocardial cells (Widmaier *et al.*, 2004). This is elaborated as follows:

Fever in infectious illness is due to resetting of the anterior hypothalamic thermostat so that the temperature is regulated with the same exquisite sensitivity as in the normal person, but the regulation is at a higher level. The resetting of the thermostat is due to synthesis of prostaglandin(s)- PGs, probably PGE_2 in the brain. The stimuli for prostaglandin synthesis are the three monokines, interleukin I (IL-1), interleukin 6

(IL-6) and tumour necrosis factor (TNF- ∝). All of them are proteins and each one is pyrogenic and can stimulate prostaglandin synthesis in the hypothalamus. Fever, like sepsis, is another manifestation of the leakage of inflammatory mediators, IL-1, IL-6 and TNF-∝, from a local site of infection into the bloodstream. This also induces acute phase serum protein changes and neutrophil leucocytosis. The clinical hallmark is peripheral circulatory failure, with raised cardiac output, low systemic vascular resistance and generalized capillary leakage (Murphy, 2000). Fever occurs in heat stroke caused by inability to cool the body fast enough to compensate for the existing heat load. Progressive rise in body temperature above $38^{o}C$ leads to irritability and confussion, and ultimately to heat stroke with cardiovascular collapse and heat denaturation of proteins (Keatinge, 2000). Drug induced fever include neuroleptic malignant syndrome an idiosyncratic reaction to therapeutic doses of phenothiazines, thioxanthines and butyrophenones and is characterized by hyperthermia, muscle rigidity, impaired consciousness and tachycardia. Malignant hyperpyrexia syndrome (MHS), an autosomal dominant condition affecting the sarcoplasmic reticulum of skeletal muscles in which there is intracellular accumulation of Ca^{2+} in response to inhaled halothane or nitrous oxide is another drug induced fever. Muscle contraction develops and the skin becomes extremely hot after injection of anaesthesia; central temperature can rise by $1^{o}C$ in a few minutes, and thence to total levels (Craston, 2000).

Acidosis has adverse effects on cardiac contractility the negative inotropic effects are particularly related to changes in cardiac intracellular pH and are greater in acute respiratory than in acute metabolic acidosis. The effects are due to a combination of mechanisms connected with excitation contraction coupling and energy supply. Among other mechanisms acidosis inhibits the inward flow calcium current during

action potential and the release of calcium from saroplasmic reticulum, thereby depressing glycolysis. The consequence is that circulatory collapse is often seen after some hours of metabolic acidosis not primarily due to shock (Cohan and Woods, 2000).

Adenosine produces transient slowing of the SAN and atrioventricular (AV) nodal block, and is thus effective for the termination of arrhythmias involving the AV node (Cobbe and Rankin, 2000).

2.1.4.2 Control of stroke volume

The stroke volume is regulated by three variables:-

i. Changes in Ventricular end diastolic volume [VEDV] which is the volume of blood in the ventricles at the end of diastole prior to contraction (preload). The stroke volume is directly proportional to preload. An increase in preload thus results in an increase in stroke volume. The relationship between preload, contraction strength, and stroke volume is an intrinsic property of heart muscles and is known as the Frank-starling law of the heart. It is a length-tension relationship. It explains how the heart can adjust to a rise in total peripheral resistance which causes a decrease in stroke volume of the ventricle such that more blood remains in the ventricle and the preload is greater in the next cycle; this results in the ventricle being stretched to a greater degree in the next cycle. The heart then contracts more strongly to eject more blood.

ii. Total peripheral resistance. This is the frictional resistance or impedance to blood flow in the arteries (after load). The stroke volume is inversely proportional to the total peripheral resistance so that the greater the peripheral

resistance the lower the stroke volume. The return of blood to the heart via veins, venous return, controls VEDV and thus the stroke volume and cardiac output. The rate at which atria and ventricles are filled with venous blood depends on the total blood volume and the venous pressure which is the driving force for the return of blood to the heart. Veins have higher compliance and can expand to hold more blood than arteries and are therefore called capacitance vessels.

iii. The strength of ventricular contraction. The effect of the sympathetic mediator, nor-adrenaline acting on beta adrenergic receptors is to increase ventricular contractility; this is defined as the strength of contraction at any given end-diastolic volume. Plasma adrenaline acting on these receptors also increases myocardial contractility. The increased force of contraction and stroke volume resulting from sympathetic nerve stimulation or adrenaline is independent of a change in ventricular end diastolic volume. A change in contraction force due to increased ventricular end diastolic volume [Frank-starling mechanism] doesn't reflect increased contractility. Increased contractility is specifically defined as an increased contraction force at any given end diastolic volume. The increased contractility leads to a more complete ejection of the end diastolic ventricular volume (Widmaier *et al*., 2004).

The major factors that determine cardiac output can be summarized as follows:-

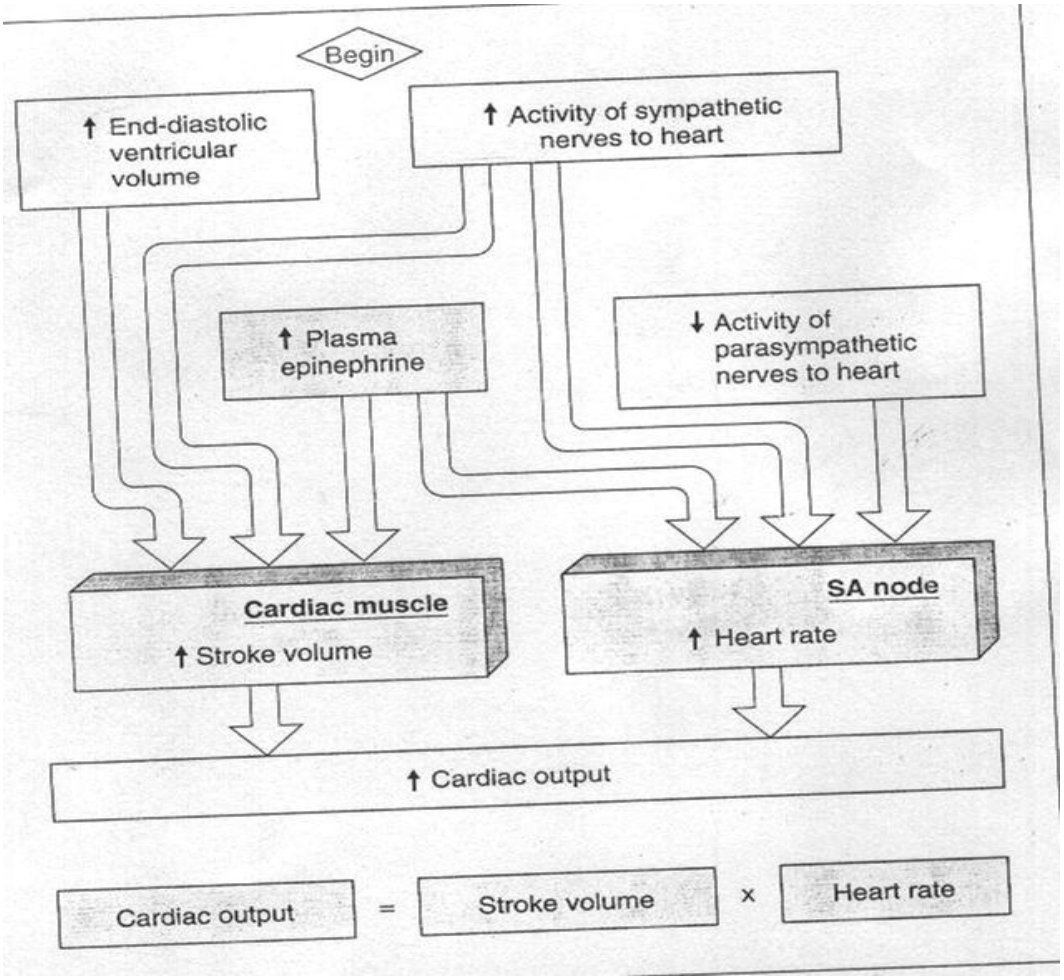


Fig. 2.8: Major factors determining cardiac out put (Widmaier *et al*., 2004)

2.2 Blood Pressure

2.2.1 Physical relationship of blood circulation

The circulatory system is made up of arteries, veins and capillaries which vary in diameter, length, capacity and composition of their walls. Some vessels are in series and others in parallel and there is a continuous flow of blood from the heart into the arterial system and from the venous system back to the heart. Major differences in pressures, rates of flow and volume of blood exist in the components of the system.

The circulation of blood involves the inter-relationship between the following:

i. Cardiac output

ii. Calibre of the blood vessel (cross sectional area)

iii. Velocity of the blood flow

iv. Resistance to flow

v. Dispensability and elasticity of the vessels

vi. Blood viscosity and

vii. Blood volume

At any point within a tube filled with a circulating fluid, force is excerted at right angle to the direction of flow. This force is termed Lateral pressure. This is the pressure measured and referred to as Blood pressure. Blood pressure is therefore the force applied to the walls of arteries by the blood (Langley et al., 1969). The role of the circulation is to deliver blood to the tissues and flow occurs because of the

pressure difference established by the pumping action of the heart. The driving pressure is the pressure difference between the lateral pressures at any two points.

Blood flow is directly proportional to the pressure difference between two ends and is inversely proportional to the frictional resistance to blood flow through the vessels.

$$\textbf{Blood flow} \propto \frac{\Delta P}{\textbf{Resistance}}$$

where ΔP is pressure difference in mmHg

The resistance to flow through a vessel is directly proportional to the length of the vessel and to the viscosity of the blood and is inversely proportional to the fourth power of the radius of the vessel.

$$\textbf{Resistance} \propto \frac{L\eta}{r^4} \quad \textbf{- - - - - - (1)}$$

The rate of blood flow can be calculated according to Poiseuille's law when physical constants are added to the above relationship.

$$\textbf{Blood flow} = \frac{\Delta P r^4(\pi)}{\eta L(8)} \quad \textbf{- - - - (2)}$$

by rearranging equation 2 we get the following expression for pressure

$$\textbf{Pressure} = \frac{\textbf{flow} \times \eta L(8)}{r^4(\pi)} \quad \textbf{- - - - (3)}$$

Where L = length of vessel

η = viscosity of blood (eta)

r = radius of vessel

$\frac{8}{\pi}$ = a mathematical constant.

(Fox, 2004)

The radius of blood vessel is important in regulating blood pressure. The smaller the cross-sectional area of the vessel the greater will be the drag or resistance; also a small change in radius is multiplied to the fourth power in its effect on blood pressure.

Vessel length and blood viscosity do not vary significantly in normalcy although viscosity is increased in severe dehydration and polycythemia. The longer the vessel and the greater the viscosity of the fluid the greater will be the resistance (Langley et al., 1969).

2.2.2 Arterial blood pressure

The pressure within a chamber represents the ratio between the size of the chamber and the volume of fluid forced into it. If the fluid doesn't fill the available space there is no pressure. In order to exert a positive pressure the chamber must be over filled. The greater the volume of fluid in ratio to the capacity of the chamber the greater will be the pressure. If the wall of the chamber is inelastic, a small increase in volume will cause a sharp rise in internal pressure. If however, the wall of the chamber is stretchable, large quantities of fluid can be added with only a small rise in pressure. The ability for a structure to be stretched is called compliance.

$$\textbf{Compliance} = \frac{\Delta \text{ volume}}{\Delta \text{ pressure}}$$

The higher the compliance of a structure the more easily it can be stretched.

These principles are applied to the analysis of arterial blood pressure. The contraction of the ventricles ejects blood into pulmonary and systemic arteries during systole. When the ventricular contraction ends the stretched arterial walls recoil passively and blood continuous to be delivered into arterioles during diastole.

As the blood leaves the arteries, the arterial volume and therefore the arterial pressure slowly falls. The next ventricular contraction occurs while there is still adequate blood in the arteries to stretch them partially. The arterial pressure therefore doesn't fall to zero.

The maximum arterial pressure reached during peak ventricular ejection is called systolic pressure (SP); and the minimum arterial pressure occurs just before ventricular ejection begins and is called diastolic pressure (DP). Arterial pressure is generally recorded as systolic / diastolic mmHg.

The difference between systolic pressure and diastolic pressure is called pulse pressure. It can be felt as pulsation or throb in the arteries of the wrist or neck with each heart beat. Pulse pressure is a reflection of stroke volume.

The arterial pressure is continuously changing throughout the cardiac cycle. The average pressure (Mean arterial pressure, MAP) in the cycle is approximately equal to the diastolic pressure plus one-third of the pulse pressure.

MAP = DP + 1/3 (SP – DP)

The mean arterial pressure is the major cardiovascular variable regulated. It is the driving force for blood flow through all the organs except the lungs. Its maintenance is a prerequisite for ensuring adequate blood flow to these organs.

The mean arterial blood pressure is also considered as the product of two factors:

i. Cardiac output (CO) and

ii. Total peripheral resistance (TPR).

MAP = CO x TPR

These two factors, cardiac output and total peripheral resistance, set the mean systemic arterial pressure because they determine the average volume of blood in the systemic arteries over time, and it is this blood volume that causes the pressure. All changes in mean arterial pressure must be the result of changes in cardiac output and / or total peripheral resistance.

The scheme of factors that determine the mean systemic arterial pressure are depicted in Fig. 2.9 below; a change in only one single variable will produce a change in mean systemic arterial pressure by altering cardiac output or total peripheral resistance (Widmaier *et a*l., 2004).

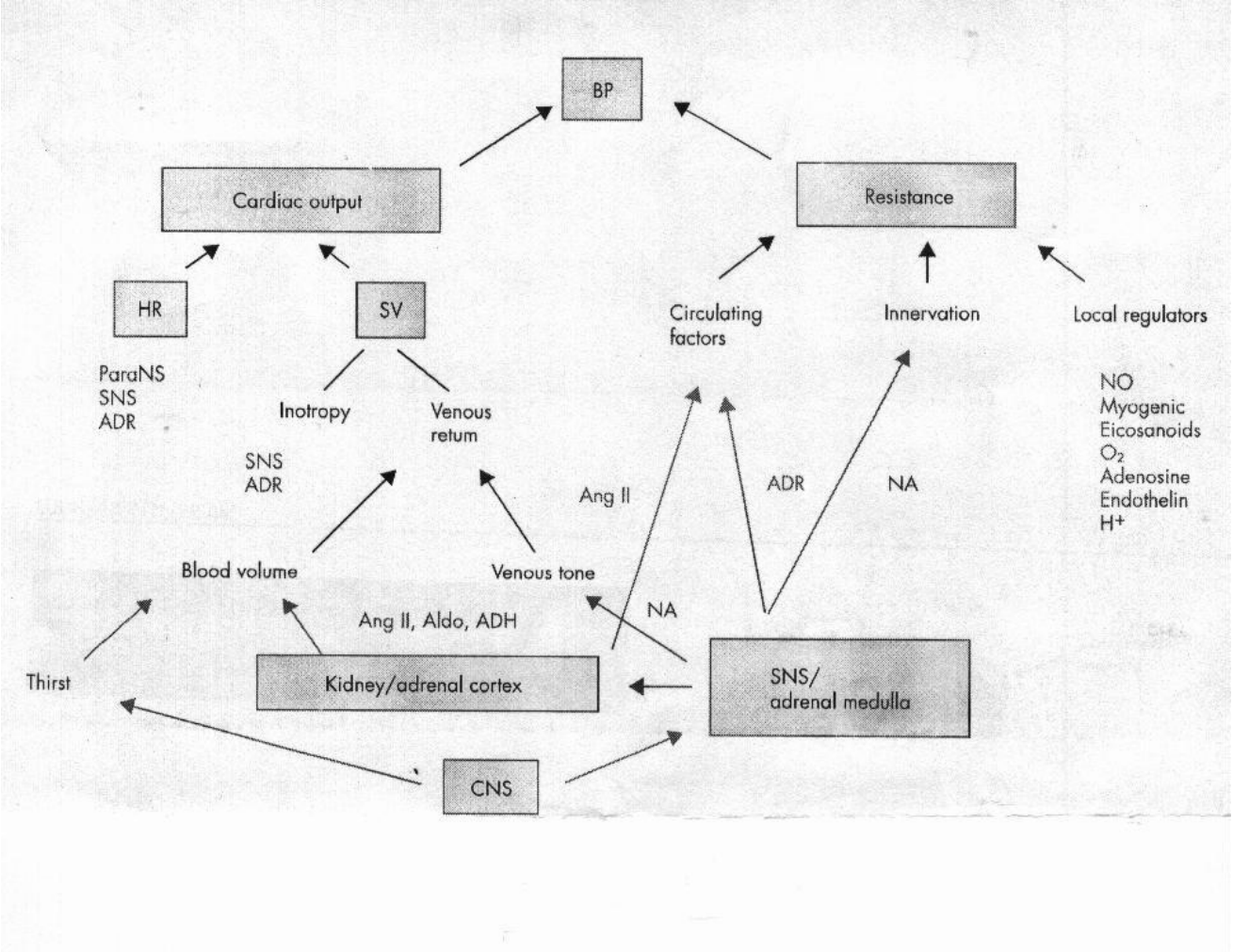


Fig. 2.9: Factors regulating mean arterial blood pressure (Mayet and Huges 2003).

2.2.3 Physiological regulation of blood pressure

Blood pressure is maintained by moment to moment regulation of cardiac output and peripheral vascular resistance. This regulation is offered at the level of arterioles, post capillary venules (capacitance vessels) and the heart (Fig. 2.10). The kidney offers another anatomic control site to the maintenance of blood pressure by regulating the volume of intravascular fluid.

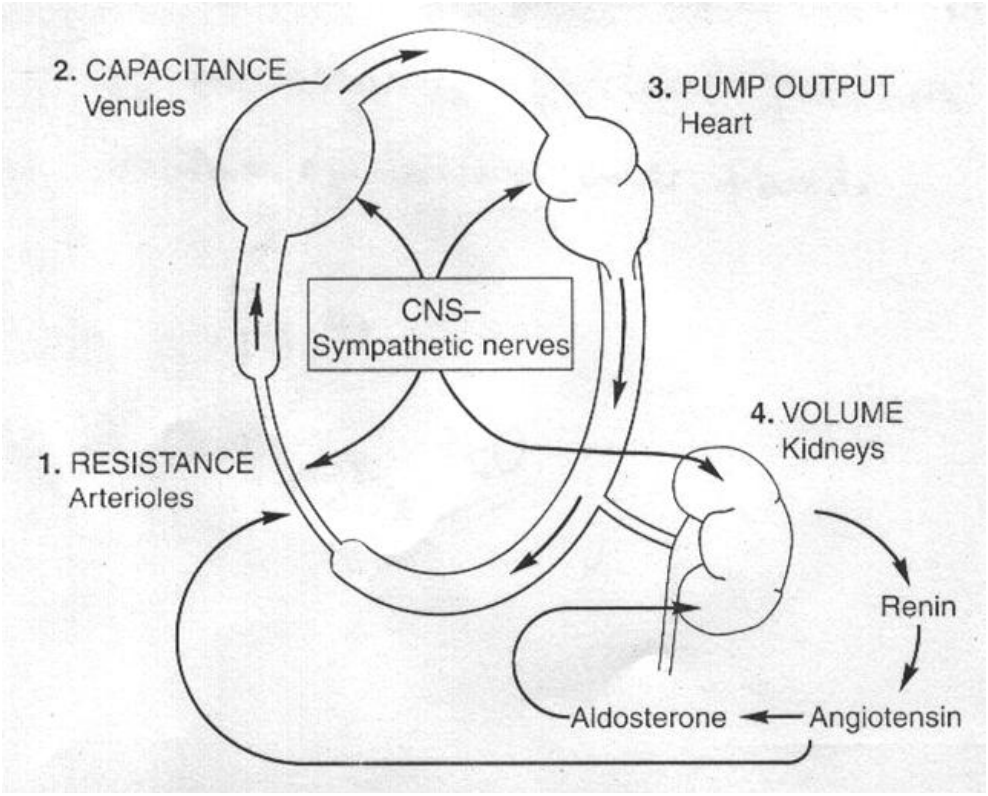


Fig. 2.10: Anatomic sites of blood pressure control

The release of vasoactive substances example, endothelin-l which constricts and nitric oxide which dilates blood vessels, from vascular endothelium may be involved in the regulation of vascular resistance (Benowitz, 2007).

Cardiac output is the product of heart rate and stroke volume. The factors that determine the stroke volume and heart rate have been mentioned above. The peripheral resistance fluctuates in response to nervous and chemical stimuli (Langley *et al*., 1969).

2.2.3.1 Vasomotor nerves

The efferent nerves supplying the cardiovascular system belong to the autonomic nervous system. The reflex mechanisms that affect the activity of the heart also affect the calibre of blood vessels. The heart beat is normally under the continuous influence of both parasympathetic and sympathetic tones, so that the resting heart is the resultant of their opposing influences.

The nerves to the blood vessels, *the vasomotor nerves*, are principally from the sympathetic division.

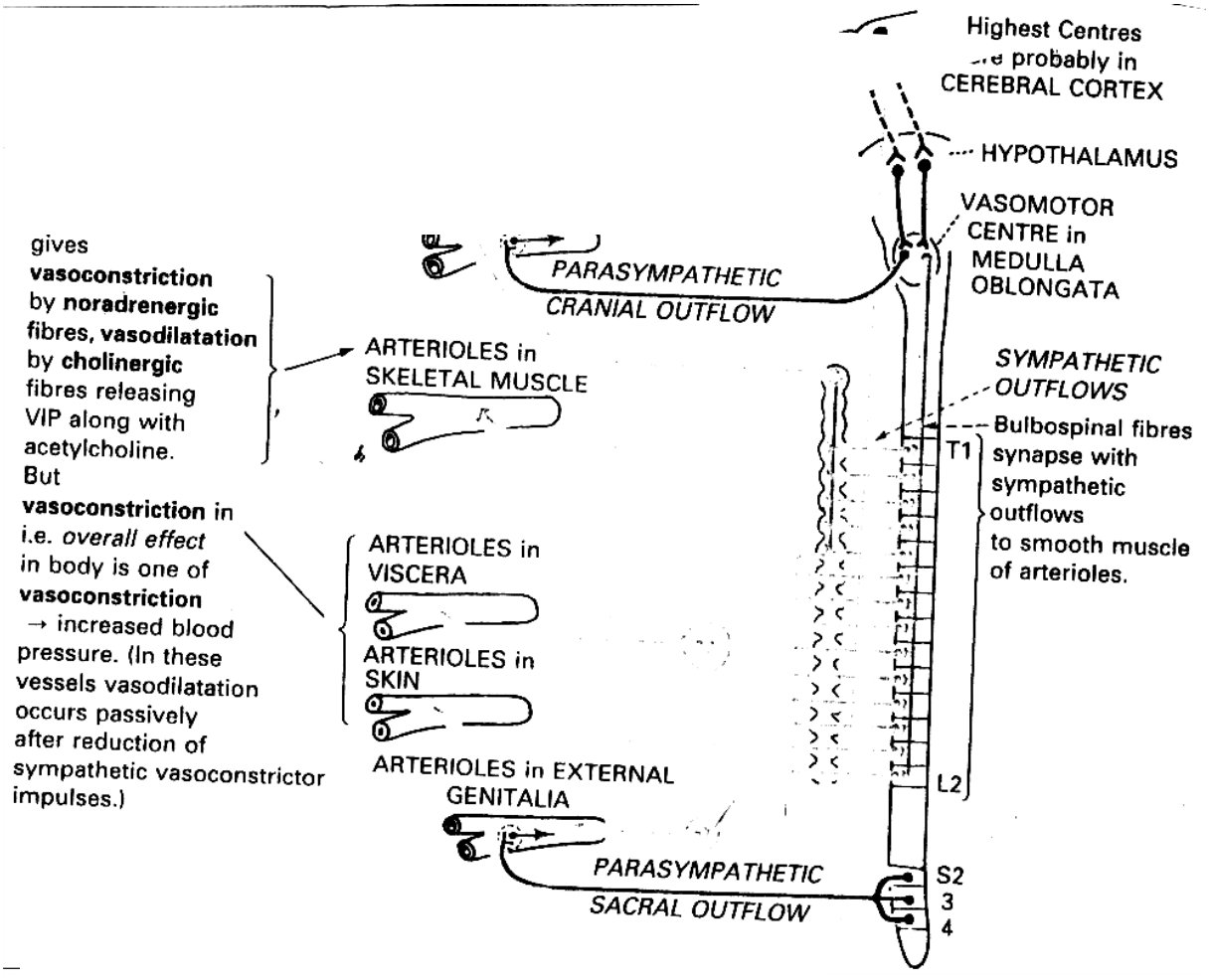


Fig. 2.11: Vasomotor nerves (Mackenna and Callender, 2003)

In general, blood vessels are not innervated by the parasympathetic nervous system, except the nerve supply to the erectile tissue in which stimulation of the sacral parasympathetic nervi eregentes produces dilatation of the arterioles and constriction of the veins so that the tissue becomes engorged with blood. Stimulation of the nerves to the salivary and sweat glands in conjunction with released bradykinin and vasoactive intestinal peptide also produce vasodilatation.

Almost all vasomotor nerves are adrenergic, the transmitter nor-adrenaline producing vasoconstriction by acting on the α-adrenoceptor of the vascular smooth muscles. Many vessels particularly in the skeletal muscles are innervated with β_2 type adrenoceptors subserving vasodilatation. In most blood vessels, the adrenergic nerves form a plexus at the adventitial medial border. The density of the adrenergic plexus depends on the vascular region and on the size of the blood vessels. The densest innervation occurs at the junction of small muscular arteries (Bowman and Rand, 1980).

2.2.3.2 Baroreceptor reflexes

The level of systemic arterial blood pressure is detected by pressure sensitive receptors or baroreceptors located at strategic points in the arterial side of the circulation. Baroreceptors are stretch receptors comprising complex nerve endings. They are present in the adventitia of the walls of carotid sinus, the arch of the aorta and the wall of the left ventricle. The nerves from these baroreceptors are termed buffer nerves because they serve to restore the blood pressure to normal by reflex inducing alterations in the cardiac output and in the calibre of the blood vessels. The level towards which the baroreceptor reflexes tend to return the blood pressure is

termed the *set-point*. The cardiovascular changes elicited by baroreceptor reflexes are mostly mediated through cardiac sympathetic (accelerens) and parasympathetic (vagus), the splanchnic nerve to the adrenal medulla and sympathetic vasomotor nerves to the splanchnic region (Fig 2. 12). Afferent axons from carotid sinus and aortic arch form synapse with the dorsal vagal nuclei in the medulla oblongata, cardioinhibitory centre and with the cell bodies of the sympathetic vasoconstrictor nerves. At normal blood pressure levels these afferent axons discharge at a low rate (Bowman and Rand, 1980).

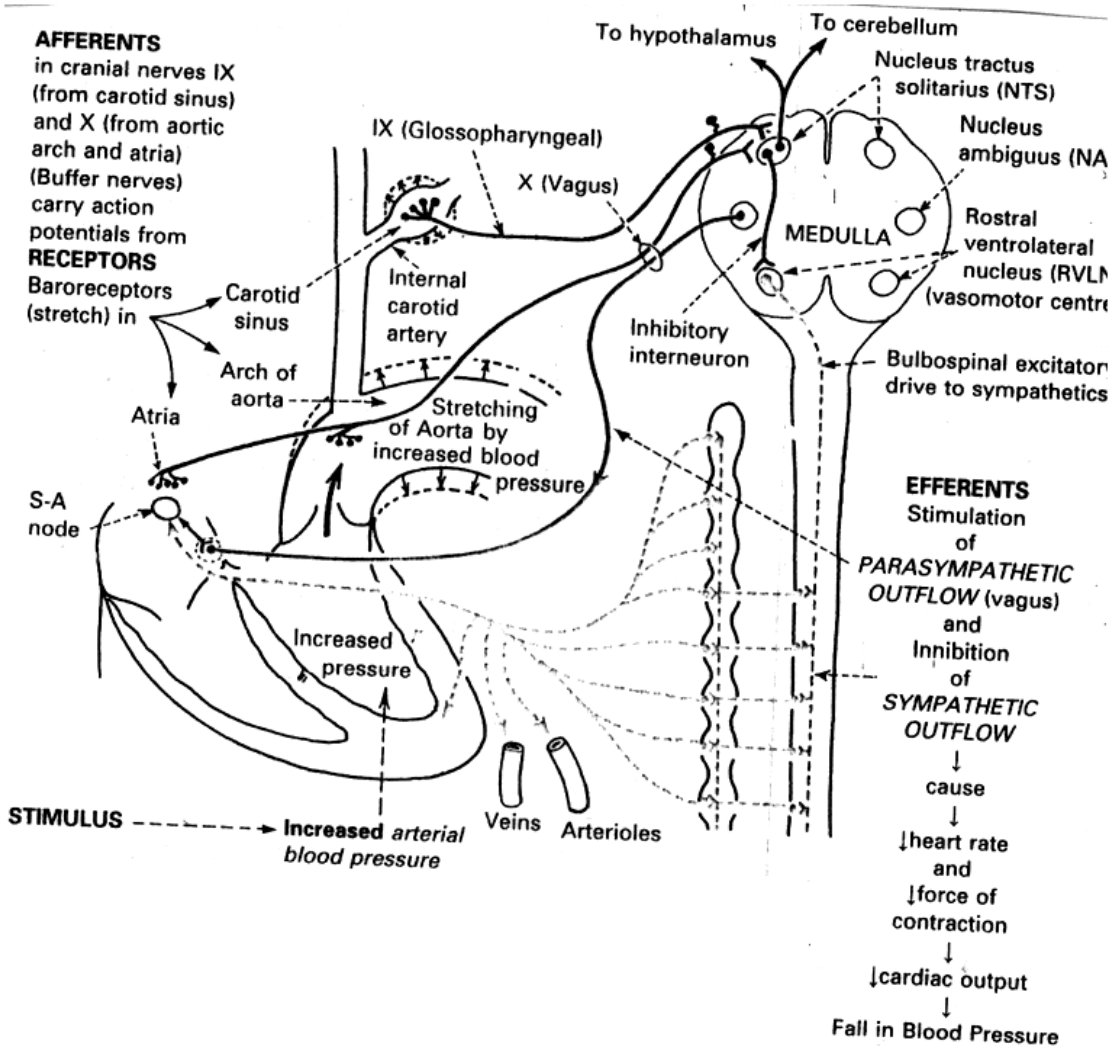


Fig. 2.12: Cardiac Reflexes (Mackenna and Callender, 2003)

The vasomotor control centres in the medulla control vasoconstriction/ vasodilatation; it helps to regulate total peripheral resistance. The cardiac control centres in the medulla regulates the cardiac rate. The baroreceptor reflex is activated whenever blood pressure increases or decrease. The reflex is more sensitive to decrease in pressure than increases and is more sensitive to sudden changes in pressure than to more gradual changes (Fox, 2004).

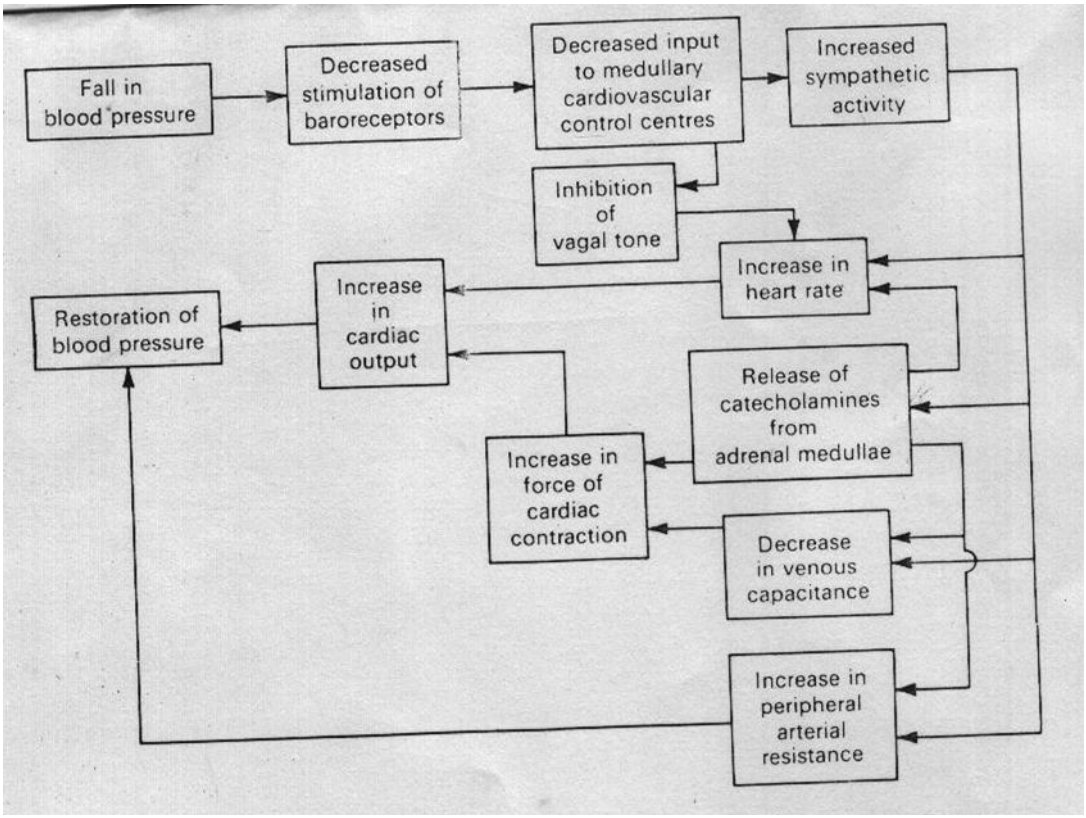


Fig. 2.13: The baroreceptor reflex loops for homeostatic control of blood pressure, showing the peripheral receptors responding to a fall in blood pressure. Opposite effects are elicited by a rise in blood pressure (Bowman and Rand, 1980).

2.2.3.3 Medulla oblongata and cardiovascular control

The primary centre for the baroreceptor reflexes is a diffuse network of highly interconnected neurones called the medullary cardiovascular centre. The neurones in this centre receive input from the various baroreceptors. Afferent nerves from the carotid sinus and the aortic arch baroreceptors enter the medulla oblongata and form a descending tract on each side termed tractus solitarius. Synaptic connections with the cell bodies in the nucleus of tractus solitarius are formed by primary afferent axons. The axons of the cell bodies of the tractus solitarius project into the medullary reticular formation (Bowman & Rand 1980).

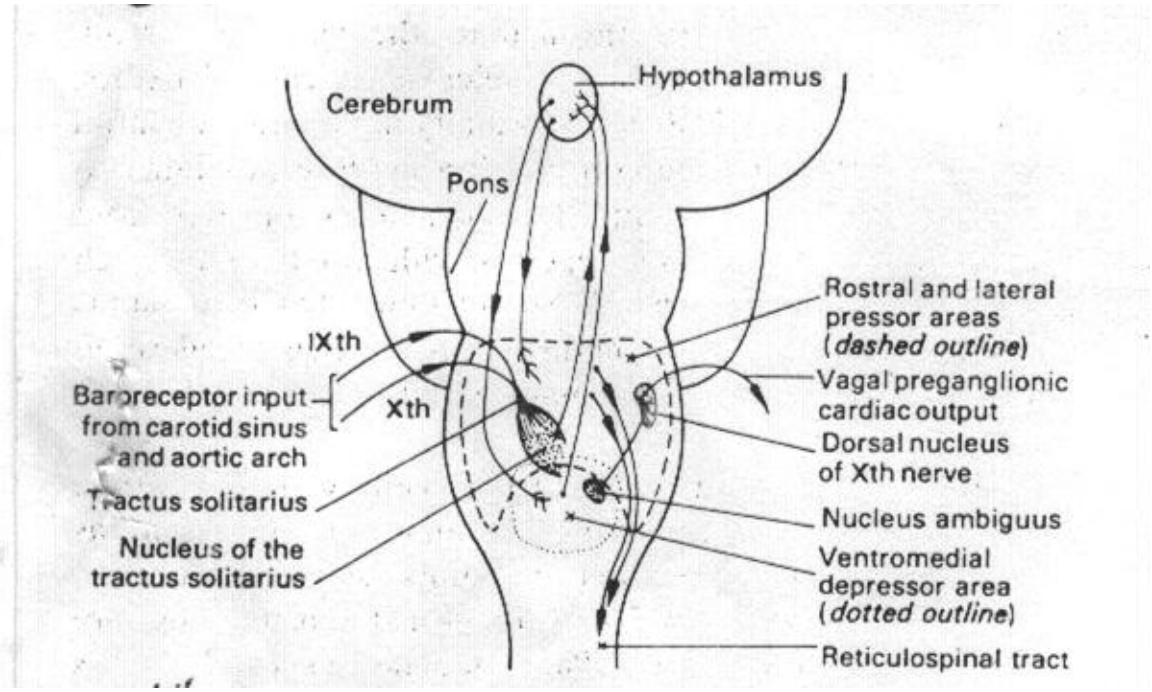


Fig. 2.14: Diagrammatic representation of cardiovascular control centres in the medulla oblongata, showing inputs on the left and outputs on the right. The areas enclosed by the dashed and dotted outlines represent the pressor and depressor areas, respectively, of the reticular system of the medulla oblongata. There are many other interconnections in addition to those shown between the medulla and hypothalamus

Stimulation of the ventromedial reticular area produces depressor responses which are due to increase in cardiac vagal activity and inhibition of activity in cardiac, vasomotor and adrenal sympathetic nerves. Stimulation of lateral and rostral reticular areas produces pressor response which are due to increased activity in cardiac, vasomotor and adrenal sympathetic nerves and inhibition of cardiac vagal activity (Bowman and Rand, 1980).

2.2.3.4 Central connection with cardioregulatory centres of medulla oblongata

The nucleus of tractus solitatarius receives a descending pathway from the neocotex which modulates the input from baroreceptors before the connections with the reticular cardiovascular centres. The medullary centres give rise to an ascending pathway to the cerebellum and receive an input from it. These may be concerned with the integration of cardiovascular control with muscle activity. Two main pathways for the ascending projections of baroreceptor input exist. One originates from the nucleus of the tractus solitarius and ventromedial reticular depressor area and runs to the hypothalamus; the other passes through the centromedian nucleus to the thalamus. Stimulation of the hypothalamus at discrete point can elicit activity in specific pathways concerned with localized components of cardiovascular control (Bowman and Rand 1980).

2.2.3.5 Renin-angiotensin-aldosterone system, blood pressure and electrolyte homeostasis

The renin-angiotensin-aldosterone system regulates sodium balance, fluid volume and blood pressure. Renin is a proteolytic enzyme that is secreted by a group of cells in the kidneys called Juxtaglomerular apparatus. Renin is cleared rapidly from the

plasma. The rate at which renin is secreted by the kidneys is the primary determinant of activity of the renin-angiotensin-aldosterone (RAA) system. It acts on angiotensinogen, a plasma globulin made in the liver, splitting off a decapeptide, angiotensin I from the N-terminal end of the protein. A membrane-bound enzyme on the surface of endothelial cells in the lungs, Angiotensin-converting enzyme converts angiotensin I to a potent vasoconstrictor octapeptide, anagiotensin II. Aminopeptidase A and N act on angiotensin II to produce Angiotensin III and angiotensin IV (Rang, *et al*., 2007), (Fig 2.15).

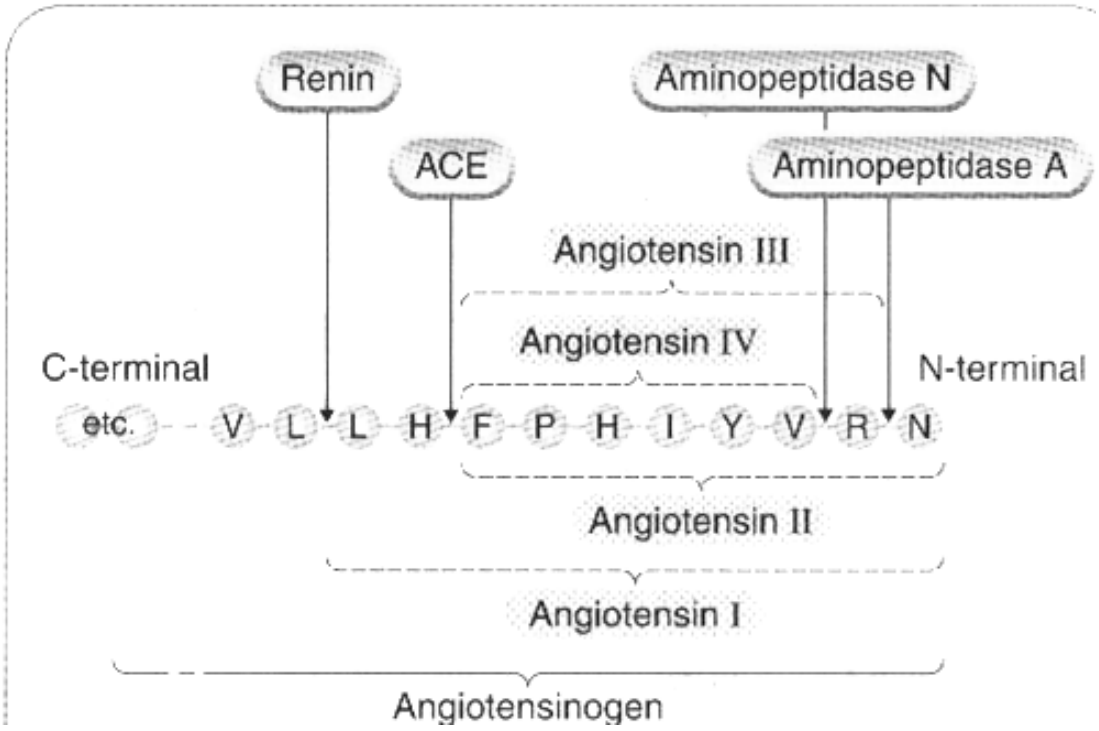


Fig. 2.15: Formation of angiotensins I-IV from the N-terminal of the precursor protein angiotensinogen (Rang *et al.*, 2007).

Active renin is released immediately upon stimulation of the juxtaglomerular apparatus. The control of its secretion involves responses to various physiological stimuli which include:-

i. Renal vascular receptor located in the apparent arterioles in the juxtaglomerular cells which function as stretch receptor. Decrease stretch leads to increase in renin release and *vice versa*.

ii. *Macula Densa*, a specialized part of the distal tubule apposed to the juxtaglomerular apparatus. It contains a different type of receptor which is sensitive to changes in the rate of delivery of sodium or chloride to the distal tubule. Decreases in distal delivery result in stimulation of renin secretion and *vice versa*.

iii. Renal sympathetic nerve activity, β-adrenoceptor agonists and prostaglandinI_2 (PG I_2) stimulate renin secretion. Noradrenaline stimulates renin secretion by a direct action on the juxtaglomerular cells.

iv. Angiotensin II the primary product of (RA) system inhibits renin release, a direct action on the juxtaglomerular cells and forms the basis of a short-loop negative feedback mechanism.

v. Atrial natriuretic peptide (ANP), a naturally occurring substance synthesized by cardiac myocytes in predominantly the atria oppose the activity of R-A system. Two related natriuretic peptides, brain natriuretic peptide ((BNP) and C-type natriuretic peptide, (CNP), are found, respectively in the ventricular muscle and vascular endothelium. Release of atrial natriuretic peptide occurs during volume overload in response to stretching of atria. Their main effects

are increase in Na^+ and water excretion by the kidney; relaxation of vasucular smooth muscles; increase in vascular permeability and inhibition in the release or action of many hormones and mediators such as aldosterone, angiotensin II, endothelin and autidiuretic hormone. The effects are exerted by combining with membrane receptors, natriuretic peptide receptors (NPRs) which exist in at least two subtypes, NPR-A and NPR-B; they incorporate a catalytic guanylate cyclase moiety and leads to intracellular generation of cyclic guanosine monophosphate (cGMp).(Rang *et al*., 2007; Reid, 2007).

The control of renin release, formation and actions of angiotensin II, and sites of action of drugs that inhibit the cascade are depicted in Fig. 2.16 below.

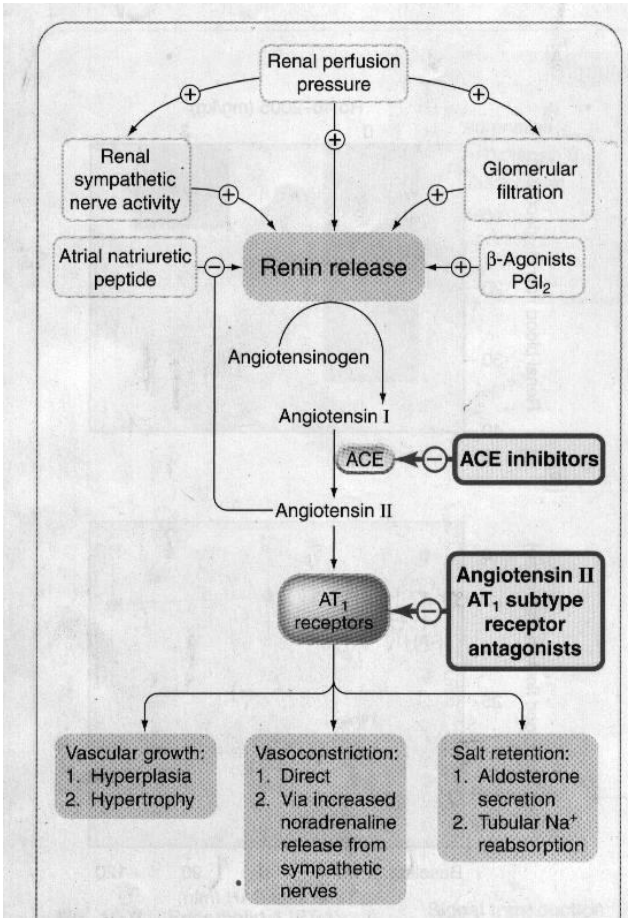


Fig. 2.16: Control of renin release and formation, and action of angiotensin II. (Rang *et al*., 2007)

The main pharmacological actions of angiotensin II are mediated via angiotensin II receptor subtypes AT_1 and AT_2 which belong to the family of G-protein coupled receptors. Effects mediated by AT_1 receptors include:-

- Generalized vasoconstriction, especially in efferent arterioles of the kidney.
- Increased release of noradrenaline from sympathetic nerve terminals, reinforcing vasoconstriction and increasing the rate and force of contraction of the heart.
- Stimulation of re-absorption of Na^+ at proximal tubule.
- Adrenocortical synthesis and secretion of aldosterone.
- Growth of cell in the heart and arteries. This is initiated by the G-protein-coupled AT_1 receptor acting via the same intracellular tyrosine phosphorylation pathways as are used by cytokines-Jak/Stat pathway.

The AT_2 receptors are involved in growth, development, exploratory behaviour and cardiovascular effect. The cardiovascular effects involve cell growth and lowering of blood pressure (Rang *et al*., 2007).

Important actions are exerted by angiotensin II at vascular smooth muscle, adrenal cortex, kidney, heart and brain; through these actions, a key role is played by the rennin-angiotensin system in the regulation of fluid and electrolyte balance and arterial blood pressure. Hypertension and disorders of fluid and electrolyte homeostatsis can result from excessive activity of the renin-angiotensin system. (Katzung, 2007).

Aldosterone, a steroid hormone secreted by zona glomerulosa of the adrenal cortex, stimulates the reabsoption of salt by the kidneys. Retension of salt indirectly promotes retention of water. The action of aldosterone produces an increase in blood volume (Fox, 2004).

Low plasma Na^+ or high plasma K^+ concentrations also affect the zona glomerulosa cells of the adrenal gland directly, stimulating aldosterone release. Aldosterone enhances Na^+ reabsorption and K^+ excretion. The promotion of Na^+ re-absorption is by:-

- a rapid effect, stimulation of Na^+/H^+ exchange, an action on membrane aldosterone receptors.
- a delayed effect, via nuclear receptors. The interactions of aldosterone with its specific receptor in the nucleus initiates transcription and translation of specific proteins, resulting in an increased number of epithelial sodium channels (ENaCs) in the apical membrane of the cell.
- long-term effects, by increasing Na^+/K^+ ATPase molecule in basolateral membrane (Rang *et a*l., 2007).

Angiotensinogen and angiotensin-converting enzyme (ACE) are normally present in excess; the rate limiting factor in angiotensin II formation is the plasma renin concentration. The chain of events in salt depletion is thus, increased renin secretion increase plasma renin concentration increased plasma angiotensin concentration increased aldosterone release increased plasma aldosterone concentration (Widmaier *et al,* 2004). By changing the plasma concentrations of effector components, angiotensin II and aldosterone, this hormonal interaction simultaneously regulates:

i. Body sodium and water content and thus hydrostatic pressure

ii. Arterial blood pressure

iii. Potassium balances (John, 1974).

2.2.3.6 Arginine vasopressin and blood pressure control

Argenine vasopressin also called antidiuretic hormone (ADH) is a peptide hormone secreted by supra optic nuclei in the hypothalamus; it migrates (attached to carrier peptide, neurophysin) along the fibres in the supraopticohypophyseal tract to the posterior pituitary for storage (in bulbous nerves endings containing accumulations of secretory granules) and release into capillaries (Mackenna and Callender, 2003)).

ADH is released by the posterior pituitary in response to rising plasma tonicity (an increase in plasma osmolality), which produces a sensation of thirst, or falling blood pressure. It plays an important role in the long term control of blood pressure through increasing water reabsoption in the kidney. Final urine concentration is determined at the collecting tubule. ADH controls the permeability of this segment to water by regulating the insertion of preformed water channels (aquoporin-2) into the apical membrane via G-protein coupled cAMP-mediated process. ADH markedly increases water permeability and this leads to the formation of a more concentrated final urine. It activates urea transporters; urea concentration in the medulla contributes in maintaining the high osmolarity of the medulla and in the concentration of urine. ADH also plays an important role in the short term regulation of arterial pressure by its constrictor action (Reid, 2007). ADH has three classes of receptors: V_1, V_2 and V_3. V_2 receptors coupled to adenylate cyclase mediate its main physiological actions in the kidney, while V_1 and V_3 receptors are coupled to phospholipase C/ inositol triphosphate system (Rang *et al*., 2007).

2.2.3.7 Endothelins

Endothelins are the most potent vasoactive peptides known. They were first isolated from aortic endothelial cells. Endothelins and their receptors are widely distributed in the body. Three isoforms of endothelin ET-1, ET-2 and ET-3 have been identified and are differently expressed in organs such as brain, adrenal glands, cardiovascular system, kidney and intestine. ET-1 is the predominant endothelin secreted by the vascular endothelium, ET-2 by the kidneys and intestine, while ET-3 is found in highest concentration in the brain. Endothelin receptors are of two subtypes ET_A and ET_B. ET_A receptors have high affinity for ET-1 and a low affinity for ET-3 and are located on smooth muscle cells where they mediate vasoconstrion. ET_B receptors, located on vascular endothelial cells have approximately equal affinities for ET-1 and ET-3; they mediate release of PG I_2 and nitric oxide. Both receptor sub-types belong to the G protein-coupled seven-transmembrane domain family of receptors. The binding of ET-1 to its receptors triggers signal transduction mechanisms which include stimulation of phospholipase C, formation of inositol triphosphate, and release of calcium from the endoplasmic reticulum These result in vasoconstriction, stimulation of PGI_2 and nitric oxide synthesis which results in decreased intracellular calcium concentration and vasodilation, the predominant overall response is vasoconstriction (Reid, 2007). Several other possible functions associated with endothelins include:

- release of various hormones, including atrial natriuretic peptide, aldosterone, adrenaline and hypothalamic pituitary hormones.
- thyroglobulin synthesis (the concentration of ET-1 in thyroid follicle is extremely high).

- control of uteroplacental blood flow (ET-1 is present in very high concentrations in amniotic fluid).
- renal and cerebral vasospasm.
- development of the cardiorespiratory system (Rang *et al*., 2007).

2.2.3.8 Neuropeptide Y

This is one of the most abundant neuropeptides both in central and peripheral nervous system. Neuropeptide Y is frequently localized in noradrenergic neurones and apparently functions both as a vasoconstrictor and a co-transmitter with nor adrenaline.

Neuropeptide Y produces central nervous system effects which include increase feeding, hypotension, hypothermia and respiratory depression. It also produces vasoconstriction of cerebral blood vessels, positive chronotropic and inotropic actions on the heart and hypertension. The peptide is a potent renal vasoconstrictor and suppresses renin secretion but can cause diuresis and natriuresis. Vascular actions include direct vasoconstriction, potentiation of the action of vasoconstrictors and inhibition of the action of vasodilators (Reid, 2007).

2.2.3.9 Urotensin

Human urotensin II is an 11-amino acid peptide expressed mainly in the brain, spinal cord and kidneys. Its activity depends on the type of blood vessels. Vasoconstriction occurs primarily in arterial vessels where it can be more potent than endothelin-1. The actions of urotensin II are mediated by a G protein-coupled receptor referred to as UT

receptor which is widely distributed in the brain, spinal cord, heart, vascular smooth muscles, skeletal muscle, and pancrease. Some effects of the peptide including vasoconstriction are mediated by the phospholipase C, IP_3-DAG signal transduction pathway (Reid, 2007).

2.2.3.10 Kinins

These are potent vasodilator peptides formed by the action of enzymes known as kallikreins or kininogenases on protein substrates called kininogens. Three kinins have been identified in mammals: Bradykinin, Lysylbradikinin (kallidin), and methionyllysylbradykinin. Each contains bradykinin in its structure. Bradykinin is the predominant kinin in the plasma.

Kinins produce marked vasodilation in several vascular beds; these include the heart, kidney, intestine, skeletal muscle and liver. The vasodilation may result from a direct inhibitory effect of kinins on arteriolar smooth muscles or may be mediated by the release of nitric oxide or vasodilator prostaglandins such as PGE_2 and PGI_2 (Reid, 2007).

Other actions of kinins particularly bradykinin include:-

- increased vascular permiability
- stimulation of pain nerve endings.
- stimulation of epithelial ion transport and fluid secretion in airways and gastrointestinal tract.
- contraction of intestinal and uterine smooth muscles (Rang *et al*., 2007).

2.2.3.11 Vasoactive intestinal peptide

Vasoactive Intestinal Peptide (VIP) is widely distributed in central and peripheral nervous systems where it functions as neurotransmitter or neuromodulator. It is also found in the gastrointestinal tract, heart, lungs, and thyroid gland. VIP exerts significant effect on the cardiovascular system; it produces marked vasodilation in most vascular beds. In the heart it causes coronary vasodilation and also exerts positive inotropic and chronotropic effects. It is thought to participate in the regulation of coronary blood flow, cardiac contraction and heart rate (Reid, 2007).

2.2.3.12 Neurotensin

Neurotensin, a tridecapeptide first isolated from the central nervous system and subsequently the gastrointestinal tract and circulation, is synthesized as part of a larger precursor that also contains neuromedin N. Processing of the precursor in the brain leads to the formation of neurotensin and neuromedin N which are released together from nerve endings. Neurotensin serves a dual function as a neurotransmitter or neuromodulator in the central nervous system and as a local hormone in the periphery. When centrally administered, neurotensin exerts potent effects including hypothermia, antinociception and modulation of dopamine neurotransmission. When administered into the peripheral circulation, it causes vasodilation, hypotension, increased vascular permeability, increased secretion of several anterior pituitary hormones, hyperglycaemia, inhibition of gastric acid pepsin secretion, and inhibition of gastric motility (Reid, 2007).

2.2.3.13 Substance P

Substance P belongs to the tachykinin family of peptides. It is present in the central nervous system where it is a neurotransmitter, and in the gastrointestinal tract where it plays a role as a transmitter in the enteric nervous system. It is a potent arteriolar vasodilator producing marked hypotension in humans and several animal species. The vasodilation is mediated by release of nitric oxide from the endothelium (Reid, 2007).

2.2.3.14 Adrenomedullin

Adrenomedullin is a member of the calcitonin family of peptides. It is widely distributed in the body. The highest concentrations are found in the adrenal glands, hypothalamus, anterior pituitary, kidney, lungs, cardiovascular system and gastrointestinal tract.

Adrenomedullin dilates resistance vessels in the kidney, brain, lungs, hind limbs and mesentery, resulting in a marked, long-lasting hypotension. The hypotension causes reflex increases in heart rate and cardiac output (Reid, 2007).

2.3 Hypertension

2.3.1 Definition

Blood pressure, like height, weight, heart rate, respiratory rate and body temperature, is a biological characteristic of the individual depending on genetic and environmental factors. It is a physiological measurement. It normally falls with repeated measurements, so that definition using a single reading will yield a much higher prevalence than with an average value taken over several occasions. Blood pressure shows great variability. Exposure to pain, mental stress, exercise or sexual intercourse give rise to rapid elevation of pressure. It also changes over the 24-h period, reaching its lowest point during the early hours of the morning and maximum on rising (Swales, 2000).

Blood pressure is a 'mathematical representation' of two forces. The top number (systolic) is the force applied against the walls of the arteries when the heart beats and the bottom number (diastolic) is the force applied against the walls between the two beats of the heart (i.e. when heart is in a relaxed state). A blood pressure range of 110/70 to 120/80 is considered normal

High blood pressure or hypertension (HTN) is a condition in which the pressure of the blood increases beyond the normal range. It is defined conventionally as a sustained rise in blood pressure $\geq$ 140/90mmHg, a criterion that characterizes a group of patients whose risk of hypertension related cardiovascular diseases are high enough to merit medical attention. Hypertension is the most common cardiovascular disease whose prevalence increases with advancing age as people above sixty years have hypertension. HTN or untreated/uncontrolled/poorly controlled HTN causes pathological changes in the vasculature and left ventricular hypertrophy. The risk of both fatal and non-fatal cardiovascular disease in adult is lowest with systolic pressure

of less than 120mmHg and diastolic blood pressure less than 80mmHg. Progressively, these risks increase with higher systolic and diastolic pressures. The recognition of this continuously increasing risk provides a simple classification of hypertension (Table 2.2) (Hoffman, 2006).

Table 2.2: Classification of hypertension

	JNC 7		WHO/ISH		ESH/ESC	
CLASSIFICATION	SBP*	DBP*	SBP*	DBP*	SBP*	DBP*
OPTIMAL	–	–	–	–	<120	<80
NORMAL	<120	<80	–	–	120–129	80–84
PREHYPERTENSION/ HIGH NORMAL	120–139	80–89	–	–	130–139	85–89
STAGE 1/GRADE 1	140–159	90–99	140–159	90–99	140–159	90–99
STAGE 2/GRADE 2	≥160	≥100	160–179	100–109	160–179	100–109
GRADE 3	–	–	≥180	≥110	≥180	≥110
ISOLATED SYSTOLIC HYPERTENSION	–	–	–	–	≥140	<90

Key

- = Pressure Measured in mmHg

JNC 7 = Seventh report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (2003).

WHO/ISH = World Health Organisation/International Society of Hypertension

ESH/ESC = European Society of Hypertension/European Society of Cardiology.

DBP = Diastolic blood pressure

SBP = Systolic blood pressure

Consequent on the announcement of the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) results at the end of 2002, the US hypertension guidelines were completely revised and reissued as the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and the Treatment of High Blood Pressure (JNC 7) in the Spring of 2003. This was soon

followed by guideline updates jointly issued by the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC). At the 2004 meeting of International Society of Hypertension (ISH), in Sao Paulo, Brazil, these updates and the updated World Health Organization (WHO)/ISH guidelines for the management of hypertension were discussed.

Unlike the current US hypertension guidelines (JNC 7) and European guidelines, the WHO/ISH guidelines are aimed at a global audience and are intended to serve as a template for the development of national, regional, and local guidelines. However, they resemble the European guidelines more closely than they do JNC 7.

The WHO/ISH blood pressure classification includes 3 grades of hypertension (Table 2.2).

Unlike JNC 7, in the WHO/ISH statement, there is no "prehypertension" classification and the WHO/ISH guideline authors were concerned about the treatment implications of such a label.

The severity of hypertension can be classified by diastolic pressure. Progressive elevation of the systolic pressure is also predictive of adverse cardiovascular events. Systolic blood pressure tends to rise disproportionately greater in elderly due to decrease compliance in blood vessels associated with aging and atherosclerosis.

Fulminant arteriopathy characterized by endothelial injury and a marked proliferation of cells in the intima which lead to intimal thickening and ultimate arteriolar occlusion is developed by some patients with very high blood pressures (Systolic $\geq$210 and/or diastolic $\geq$ 120mmHg). This is the pathological basis of the syndrome of immediately life-threatening hypertension, which is associated with rapidly

progressive microvascular occlusive disease, the kidney (with renal failure), brain (hypertensive encephalopathy), congestive heart failure, and pulmonary edema. Pathological changes in certain target organs herald worse prognosis. Retinal heamorrhages, exudates, and papilledema indicate a far worse short-term prognosis. Left ventricular hypertrophy is associated with worse long-term outcome that include a higher risk of sudden cardiac death. Hypertension is the principal cause of stroke, a risk factor for coronary artery disease and its attendant complications, myocardial infarction and sudden cardiac death. It also causes dissecting aneurysm of the aorta (Hoffman, 2006).

2.3.2 Pathogenesis of hypertension

The blood pressure, under normal circumstances is maintained by the interplay of various mechanisms. It is a function of cardiac output and peripheral vascular resistance.



Blood Pressure = Cardiac output (CO) x Peripheral vascular resistance

Cardiac output is the major determinant of systolic blood pressure. Diastolic blood pressure is determined by the resistance in the arterioles. Elevation of blood pressure comes as a result of an increase in cardiac output, and increase in total peripheral vascular resistance or a combination of both (Mayet and Huges, 2003).

For the blood pressure to remain high more than one mechanism involved in the control of blood pressure must be affected. Considering the heamodynamic components of blood pressure, the significant factors involved in its control, the

possible mechanisms of hypertension can be depicted as follows (Fig. 2.17) (Kassavan, 1998).

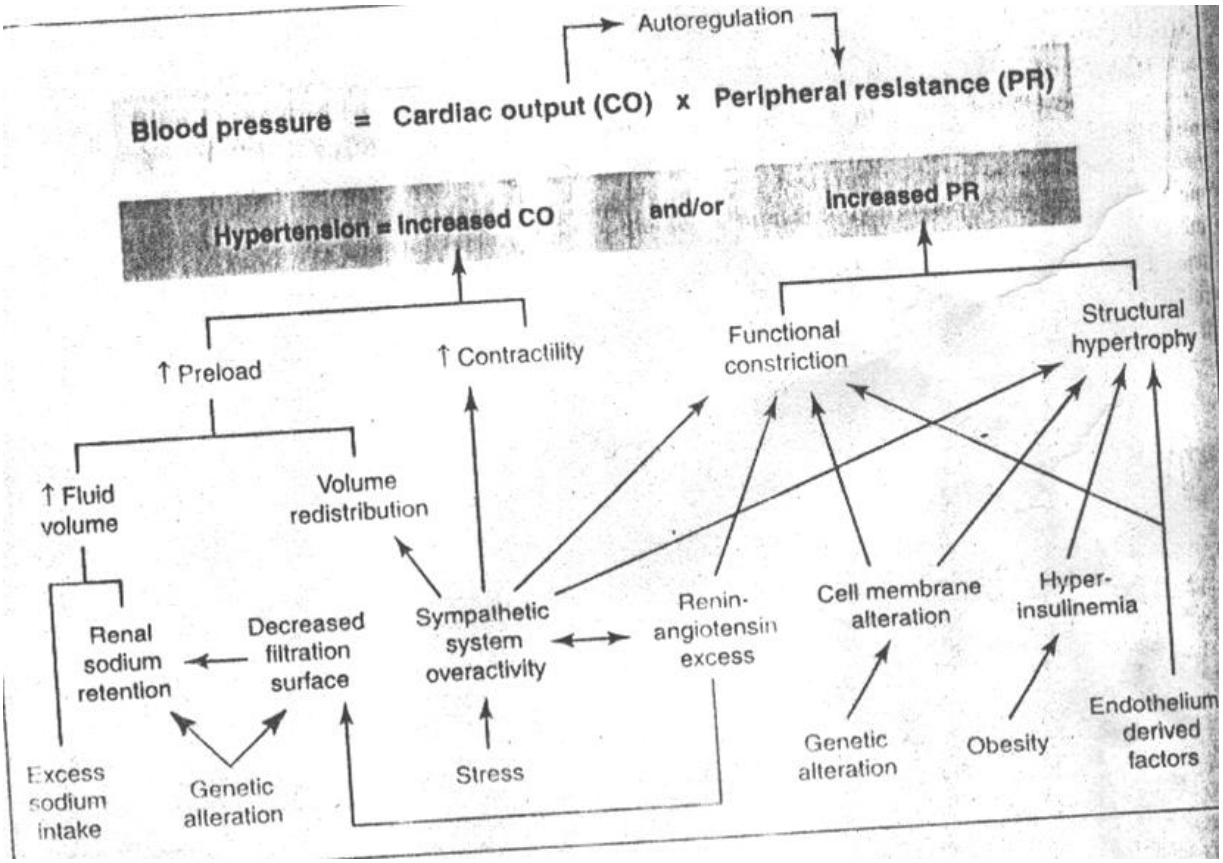


Fig. 2.17: Pathogenesis of Hypertension (Kassavan, 1998)

The final common pathway in the genesis of hypertension appears to be in the vessels that maintain peripheral resistance as cardiac output may be normal in chronic hypertension. There is a reduction in luminal diameter associated with an increase in wall thickness in patients with hypertension.

Activation of the sympathetic nervous system (SNS) is among the triggers of essential hypertension; it is also a potential early step in its development (Beevers *et al*, 2001).

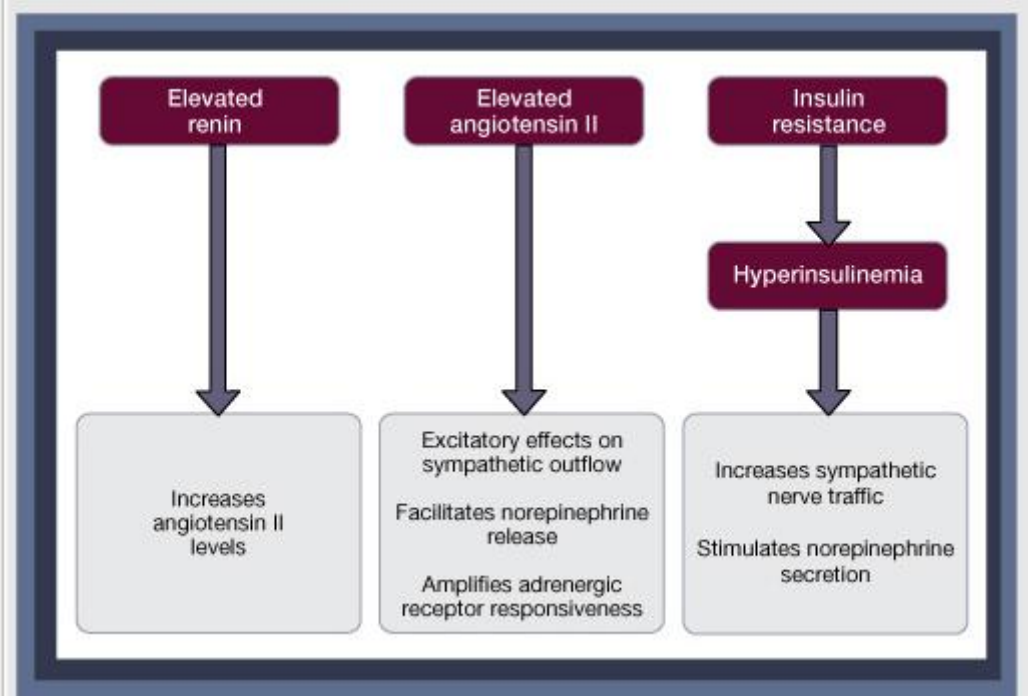


Fig. 2.18: Potential Mechanisms for Activation of the Sympathetic Nervous System in Essential Hypertension (Mancia *et al* 1999)

The activation of the sympathetic nervous system has, apart from increasing blood pressure, many downstream cardiovascular consequences, such as structural alteration of the heart and/or vasculature (Fig 2.19) (Mancia *et al*., 1999).

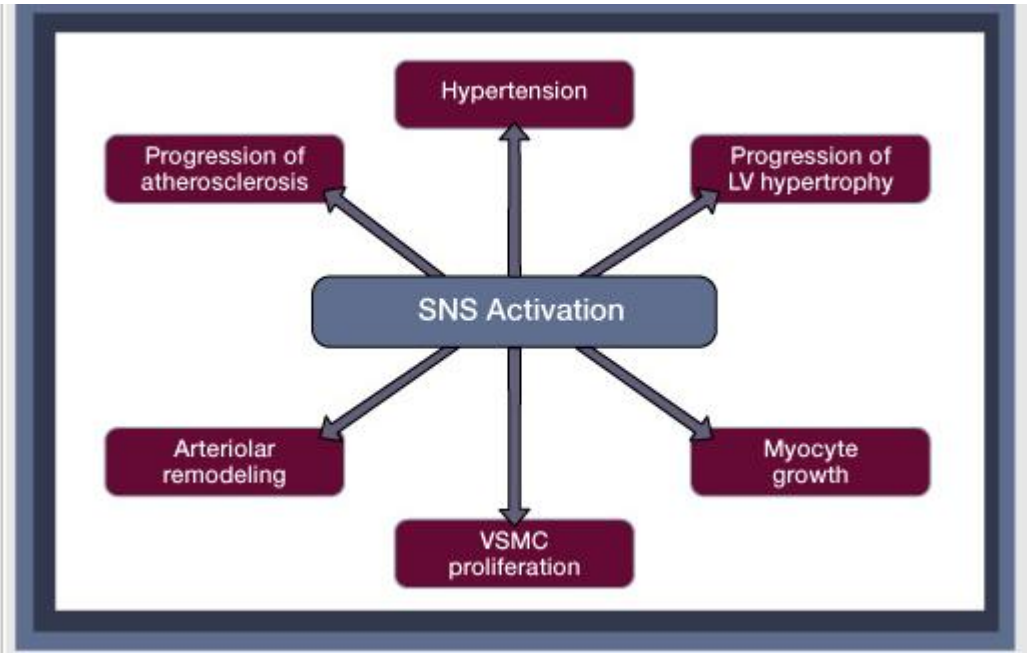


Fig. 2.19 Potential Cardiovascular Effects of Sympathetic Nervous System Activation

The baroreceptor sensitivity is reduced in hypertensive patients, probably as a result of structural changes in the walls of the arteries in which the baroreceptors are situated causing them to become more inelastic (Swales, 2000).

Theories of hypertension implicate kidney. In non-malignant hypertension renal blood flow is reduced but glomerular filtration rate is preserved with an increase in filtration fraction. Intracapillary glomerular pressure is increased. Hyaline degeneration is observed in the afferent arterioles. Progressive hyalinisation of nephrons account for the slow decline in glomerular filtration rate with age. The rate of this loss is increased in essential hypertension.

Severe renal impairment causes hypertension; the raised arterial pressure is an early feature of all glomerular diseases and of adult polycystic disease. Salt and water retention in advanced renal failure is an important mechanism.

Atheromatous disease and fibromuscular hyperplasia are two most common causes of renovascular hypertension. Other rare causes include dissecting aneurysm of the aorta, renal arterial thrombosis or embolism, abdominal trauma, neurofibromatosis, post irradiation fibrosis, and Tokayasus arteritis. Hypertension arises in part because of under perfusion of the kidney which leads to activation of the renin-angiotensin system which may cause the initial rise in blood pressure.

Hypertension appears to lead to progressive renal impairment. It is a major risk factor for both large –vessel and microvascular atheroselerotic disease. In the elderly, essential hypertension sustained over many years results in significant atherosclerotic narrowing of intrarenal small vessels, and an ischaemic nephropathy with an increased rate of glomerulosclerosis. In malignant hypertension endothelial integrity of renal vasculature is lost in afferent arterioles and glomerular tuft with leakage of plasma constituents and fibrin deposition in vessel walls, forming characteristic fibrinoid necrosis. Another characteristic pathological change observed is proliferative endarteritis of interlobular arteries. The resulting narrowing or obliteration of the arterial lumen causes both glomerular and tubulointerstitial ischemia and fibrosis, with resulting loss of renal function (Raine, 2000). Renal damage in patients with hypertension could be either a cause or a consequence (Green *et al*., 1993).

Blood pressure is remarkably sodium dependent in most patients with chronic renal failure. Essential hypertensives as a group show no increase sodium intake, no impairment of sodium excretion and no increased body sodium content. Dietary sodium may amplify, at most, blood pressure raising mechanisms and contribute to secondary rise in blood pressure with age (Swales, 2000).

The renin – angiotension – aldosterone – kallikrein – kinin, atrial nitriuretic peptide, endothelin, prostaglandin, and renomedullary lipid systems have all been implicated at one time or another in the development of essential hypertension. Although they may respond to the rise in blood pressure, there is no evidence to implicate any of them as a primary cause of essential hypertension (Swales, 2000). The major theories in the pathogenesis of hypertension are summerized in table 2.3(Green and Harris,1993).

Table 2.3: Pathogenetic Theories of Essential Hypertension

Site	Lesion	Possible consequences
CVS centre	Activity ↑	↑ SNS outflow → ↑ vasoconstriction and/or contractility
Kidney	Na/water clearance ↓	Fluid retention → ↑ CO Higher pressure set by CVS centre to maintain normal fluid/electrolyte clearance (the 'pressure natriuresis' theory) (Fig. 3.16)
	Renin ↑	Vasoconstriction and fluid retention
Circulating Na pump inhibitor	[Na] ↑	Vascular wall oedema → lumen ↓ ↑ Intracellular Ca availability and/or ↓ resting membrane potential → increased constriction
Endocrine		Renin/ angiotensin/ aldosterone ↑ Bradykinin/ prostaglandin (vasodilator) ↓ Atrial natriuretic peptide ↓
Arterial structure	Hypertrophy (growth factors)	↑ Vascular lumen → ↑ PR → ↑ BP → further vascular damage

2.3.3 Hemodynamics of hypertension

The heamodynamic features of hypertension can be correlated with current physiological concepts. They are considered in relation to the various functional subdivisions of the cardiovascular system.

Hypertension of several years standing without target organ damage is associated with increase peripheral resistance with a normal cardiac output. The cause of the elevation may not be the result of increased sympathetic tone, but rather a narrowing of the resistance vessels brought about by progressive arteriosclerosis and, occasionally degenerative changes in elastic tissue (Bender, 1966). The increase in peripheral vascular resistance is present in all vascular beds, such as renal, splanchnic, skeletal muscles, coronary and pulmonary. The vasculature of skeletal muscles does not share in the increase resistance.

In established hypertension, abnormal cardiac performance contributes significantly to elevated blood pressure particularly in subjects with concentric hypertrophy of the heart. There is also a reduction in arterial compliance and a central shift in blood volume, which may be secondary to reduced venous compliance. Blood volume and extra cellular fluid volume are generally found to be normal (Mayet and Huges,2003).

Young individuals less than 19 years with highest blood pressure have increased peripheral vascular resistance and sometimes increase in cardiac index. The peripheral vascular resistance in adults, 18 – 40yrs, with boarder line hypertension is often in the normal range at rest; the raised blood pressure is often attributable to increased cardiac index and heart rate.

2.3.4 Structural changes in hypertension

The heart and arteries adapt their structure in response to altered load; this occurs physiologically in somatic growth and pathologically in hypertension. Increased pressure exerts an increased load on a thin walled chamber or tube by increasing wall tension according to Laplace's law (fig 20).

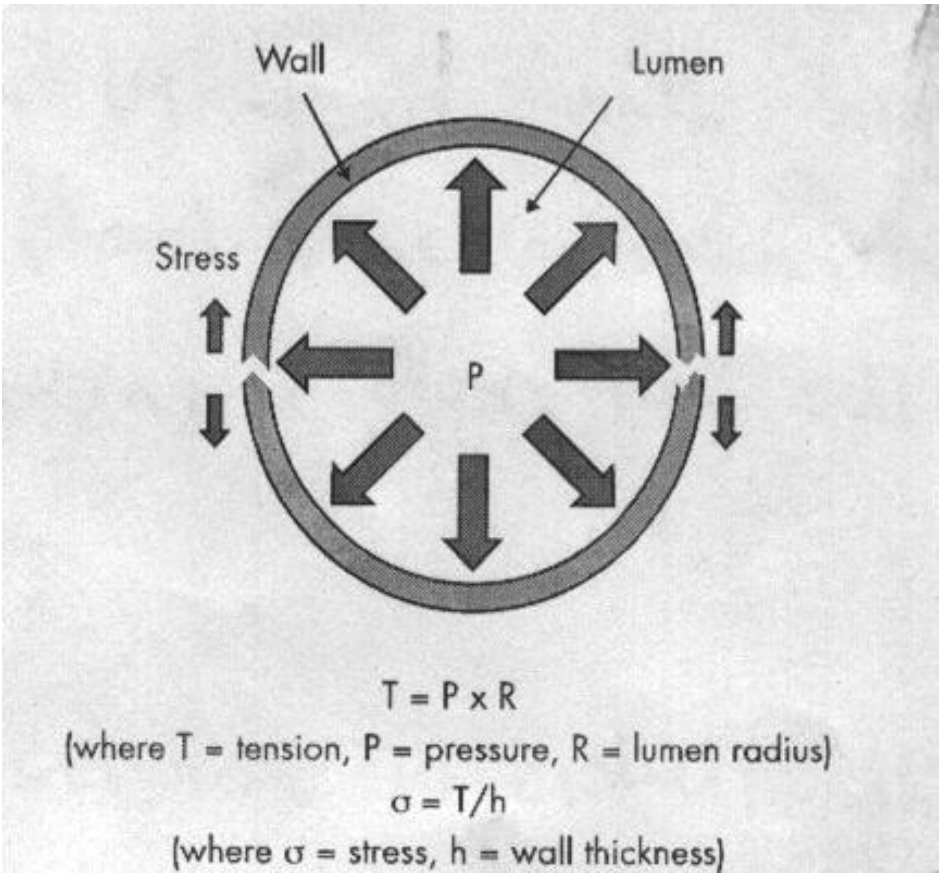


Fig 2.20: Wall stress and tension in a thin walled tube or chamber (Laplace's law) (Mayet and Huges, 2003).

A rise in tension results in increased wall tensile stress. Normalisation of wall tensile stress can be achieved by an increase in wall thickness or by reduction in chamber/lumen diameter, or both. Cardiac structure is influenced by pressure and volume loads. The increase pressure load in hypertension is primarily caused by the increased resistance although reduced compliance and altered magnitude and timing of reflected pressure waves make a contribution. Spectrum of structural change is seen in hypertension. The pattern of change may reflect differences between individuals in terms of age, heamodynamics, 24 hrs blood pressure profile, arterial stiffness, plasma volume, myocardial performance, neurohormonal status or genetic influences (Mayet and Huges, 2003).

The left ventricle might adjust to an elevated peripheral resistance by three possible mechanisms: -

i. an increase in residual volume, and, hence, diastolic fibre length

ii. augmentation of intrinsic contractility of the myocardial fibres independent of fibre length, and

iii. hypertrophy (FreRis, 1960).

Hypertrophy involves myocytes and interstitial network. Left ventricular hypertrophy predicts a poor prognosis.

The increase in ventricular arrythmias and increased QT duration and QT dispersion seen in left ventricular hypertrophy may account for increased risk of sudden death. Other mechanisms such as impaired coronary perfusion could also be important. Hypertrophy and remodelling of vascular smooth muscles and cardiac impaired active relaxation. This may implicate changes in intracellular calcium handling, ion exchange and ion channels.

Cardiac hypertrophy is associated with impaired coronary reserve which may be caused by mechanisms like endothelial dysfunction, narrowing of small arteries, microvascular rarefaction, perivascular fibrosis, altered wall mechanisms, and relative myocyte hypertrophy. A diminished coronary reserve will lead to myocardial ischeamia and may further impair relaxation. Impaired relaxation results in prolongation of isovolumic relaxation time because it takes longer for the left ventricular pressure to decrease below arterial pressure.

Structural changes in hypertensive vasculature show similarities to those of the heart. The elevated pressure causes an increase in wall tension that is largely experienced by

vascular myocytes and the extracellular matrix of the blood vessels. Remodelling of the vasculature depends on the size and function of the artery. Flow affects arteries via frictional force experienced by the wall shear stress. The wall shear stress is sensed by endothelial cells which respond by releasing a number of vasoactive factors that affect arterial tone and growth.

Large elastic arteries do not contribute to peripheral vascular resistance, but influence total arterial compliance and wave reflection. There is also increase in wall thickness (intima – media thickness – IMT). Arterial stiffness is increased in hypertension, with the exception of carotid artery of young hypertensive. The stiffness is a result of the increased distending pressure. Blood vessels do not obey Hooke's law and are non-linearly elastic (they become stiffer when distended).

The lumen diameter as a ratio of wall thickness is reduced in small arteries in all forms of hypertension. Arteriolar tone may be increased but there is little structural changes except in accelerated or malignant hypertension. The density of microvasculature is reduced. This can be visualised in sites like sclera, retina or skin. The reduction in the microvascular density could either be functional (non-perfused vessels) or structural (obliterated vessels) (Mayet and Huges, 2003).

2.4 Types of Hypertension

Hypertension, the most common cardiovascular disease, is a leading cause of morbidity and mortality worldwide. The prevalence of hypertension increases with age. There are two types of high blood pressure or hypertension:

i. Primary (essential) hypertension

ii. Secondary hypertension

2.4.1 Primary or essential hypertension

Hypertension is referred to as a "essential" or "idiopathic" when the cause is unknown. No specific cause can be located. It affects 95% of high blood pressure patients. While there is no underlying cause to it, some risk factors have been identified which include:

i. **Genetics:** primary hypertension can be genetic or hereditary. Family history of high blood pressure gets manifested in the next generation. The genes that contribute to human essential hypertension have not been identified. There is a family linkage of angiotensinogen gene. Some rare forms of secondary hypertension are due to mutation in a single gene. These include Liddle's syndrome (epithelial sodium channel gene) and glucocorticoid suppressible hyperaldosteronism (Swales, 2000).

ii. **Age:** with advancement in age, the arteries in the bodies become ateriosclerotic, resulting in increased blood pressure.

iii. **Insulin resistance:** individuals suffering from metabolic "syndrome X" have increased risk of having hypertension and other health problems such as diabetes and stroke. Insulin insensitivity is the key abnormality in metabolic "syndrome X" in which it is associated with hypertension, high low-density lipoprotein cholesterol, low high-density lipoprotein cholesterol, and an increased risk of atheroma formation (Garrow, 2000).

iv. **Obesity:** obesity can lead to structural changes in the kidney and abnormal control of sodium. Those with Body Mass Index (BMI) greater than 25 are

considered to be obese. Obese people face at least 5 times the risk of high blood pressure than those with normal BMI.

v. **Innate aberration in sympathetic nervous system:** the sympathetic nervous system controls the heart rate, blood pressure and the diameter of blood vessels. Abnormality in this system may account for this type of hypertension.

vi. **Renin homeostasis:** high activity of the enzyme renin can cause an increase of blood pressure.

vii. **Vitamin D:** based on clinical observations, an inverse relationship has been described among Vitamin D3, blood pressure and plasma renin activity (Burgess *et al*., 1990). Vitamin D_3 supplement was also observed to reduce blood pressure in hypertensive patients. Other studies showed that the effects of Vitamin D_3 and vitamin D receptor on renin expression were direct ones, mediated by calcium metabolism or parathyroid hormones, both of which are known to independently influence renin expression (Smith *et al*., 1983).

viii. **Salt Sensitivity:** Genes play a role in sensitivity to salt intake. 10% increase blood pressure after a high salt meal indicates salt sensitivity (blood-pressure-update.com/bp, 2009).

ix. **Low level of Nitric Oxide:** The release of Nitric oxide from endothelium mediates vasodilation and subsequent hypotension; its low level as seen in hypercholesterolaemia, a risk factor for atherosclerosis which contributes to atherogenesis predispose to hypertension. Failure to increase endogenous nitric oxide biosynthesis during pregnancy contribute to eclampsia (Rang *et al*., 2009).

Some of the characteristics of severe essential hypertension are as follows:

- The mean arterial pressure is increased by 40-60 percent.
- In more severe stages of essential hypertension, the renal blood flow is decreased to about one half normal.
- The resistance to blood flow through the kidneys is increased two fold to four fold.
- Despite the great decrease in renal blood flow, the glomerular filtration rate is often near normal as the higher arterial pressure in hypertension still causes adequate filtration of fluid through glomeruli into the renal tubules.
- The cardiac output is about normal.
- The total peripheral resistance is increased by about 40-60 percent, the same amount the arterial pressure is increased.

Finally, the most important finding in people with essential hypertension is the following:

The kidneys will not excrete adequate amount of salt and water unless the arterial pressure is high. In other words if the mean arterial pressure in essential hypertensive person is 150 mmHg, reduction of the arterial pressure artificially to 100 mmHg (but without otherwise altering the renal function except for the decreased pressure) will cause almost total anuria and the person will retain salt and water until the pressure rises back to elevated value of 105 mmHg. The reason is unknown (Guyton and Hall, 2004).

2.4.2 Secondary hypertension

It is the result of certain identifiable underlyimg causes which include kidney diseases, endocrine diseases and tumors. Hypertension secondary to Renal and Renovascular disorders include: renal artery stenosis, Polycystic kidney, Chronic reflux nephropathy, Chronic glomerulonephritis, Arterio nephrosclerosis, Diabetic nephropathy, Polyarteritis nodosa and Renin-producing tumors.

Hypertension secondary to endocrine disorders include: Adrenocortical hyperfunction, Cushing's syndrome, Primary hyperaldosteronism (Conn's syndrome), Congenital or hereditary adrenogenital syndrome (17α - hydroxylase, 17β - hydroxylase defects), Pheochromocytoma, Acromegaly, Hyperparathyroidism, Thyroid dysfunction, Polycystic ovarian syndrome and Metabolic syndrome (Diabetes mellitus, dyslipidaemia, obesity)

Other secondary hypertension results from: Obstructive sleep apnea, Aortic coarctation, Pre-eclampsia, Increased intravascular volume, Takayasus disease, Lymphoedema, Pregnancy, Neurogenetic, Psychogenic, Diencephalic syndrome, Bulber poliomyelitis, Polyneuritis, Increased intracranial pressure, Spinal cord section, Fever Hyperkinetic heart disease, Aortic valve insufficiency, Patent ductus arteriosus, Drug-induced (Sympathomimetic amines, Monoamine oxidase inhibitors and Oral contraceptives).

Liquorice and carbenoxolone: Liquorice and Carbenoxolone sodium have mineralocorticoid-like effects and may produce sodium and water retention and hypokalaemia following ingestion. This may cause or exacerbate hypertension, heart

failure, oedema, alkalosis, and muscle weakness, congestive heart failure and arrhythmias (Fyfe, *et al*, 1969; Davies, *et al*. 1974; Chamberlain, 1970; Wash and Bernard, 1975).

-NSAID and steroids.

-Rebound hypertension

(Bowman and Rand, 1980; Raine *et al*., `2000; Ashley *et al*., 2004).

2.4.3 Consequences of untreated hypertension

Most complications from high blood pressure relate to the circulatory system, as a result of the added strain to the heart and blood vessels which can affect other major organs too, leading to potentially life-threatening complications. It is thus important to keep blood pressure at manageable levels, especially if hypertension is already been diagnosed.

The most common complications arising from high blood pressure include:

i) **Atherosclerosis**: By placing added stress on the blood vessels, hypertension can result in a build-up of plaque (cellular waste products, such as cholesterol, fatty substances, calcium, and fibrin) on the inside walls of the blood vessels. This can result in the condition known as atherosclerosis. In these cases, the narrowed arteries limit or block the flow of blood to the heart muscle, depriving it of oxygen. This is a serious condition which, if left untreated, can lead to a heart attack or stroke.

ii) **Stroke or Heart Attack**: If an atheroschlerotic plaque breaks off inside the artery, or the blood vessel ruptures, a blood clot can form within the

artery. If this blocks blood flow to the brain it can lead to a stroke. If it blocks blood flow to the heart it can result in a heart attack. The relationship between blood pressure and mortality from stroke or ischeamic heart disease is linear or continous: for every 20mmHg or 10mmHg increase in systolic blood pressure or diastolic blood pressure respectively, the risk of mortality from stroke or ischeamic heart disease doubles (Chobanian et al., 2003).

iii) **Aneurysm**: This occurs when the blood vessels have been weakened to such an extent that part of the blood vessel wall 'balloons' or bulges. The most common locations for an aneurysm include the main artery that carries blood from the heart, arteries in the brain, legs, intestines, and the arteries leading to the spleen.

iv) **Kidney disease**: High blood pressure can damage the small blood vessels in the kidneys, which can affect their ability to excrete waste properly. This can cause a number of symptoms, including tiredness, water retention, leading to swollen ankles, feet, or hands, shortness of breath, blood and/or protein in your urine, an increased urge to urinate, especially at night and an itchy skin.

v) **Vascular dementia**: High blood pressure can cause the blood vessels that supply the brain with blood to narrow or become damaged. If the brain is not supplied with enough oxygen due to stroke, cells in the brain may be damaged causing adverse effects on the person's memory, thinking, or language skills, a condition called vascular dementia.

vi) **Eye Disease**: Hypertension can damage the capillaries (small blood vessels) in the retina, leading to deprivation of oxygen in the eye tissue resulting in eye disease.

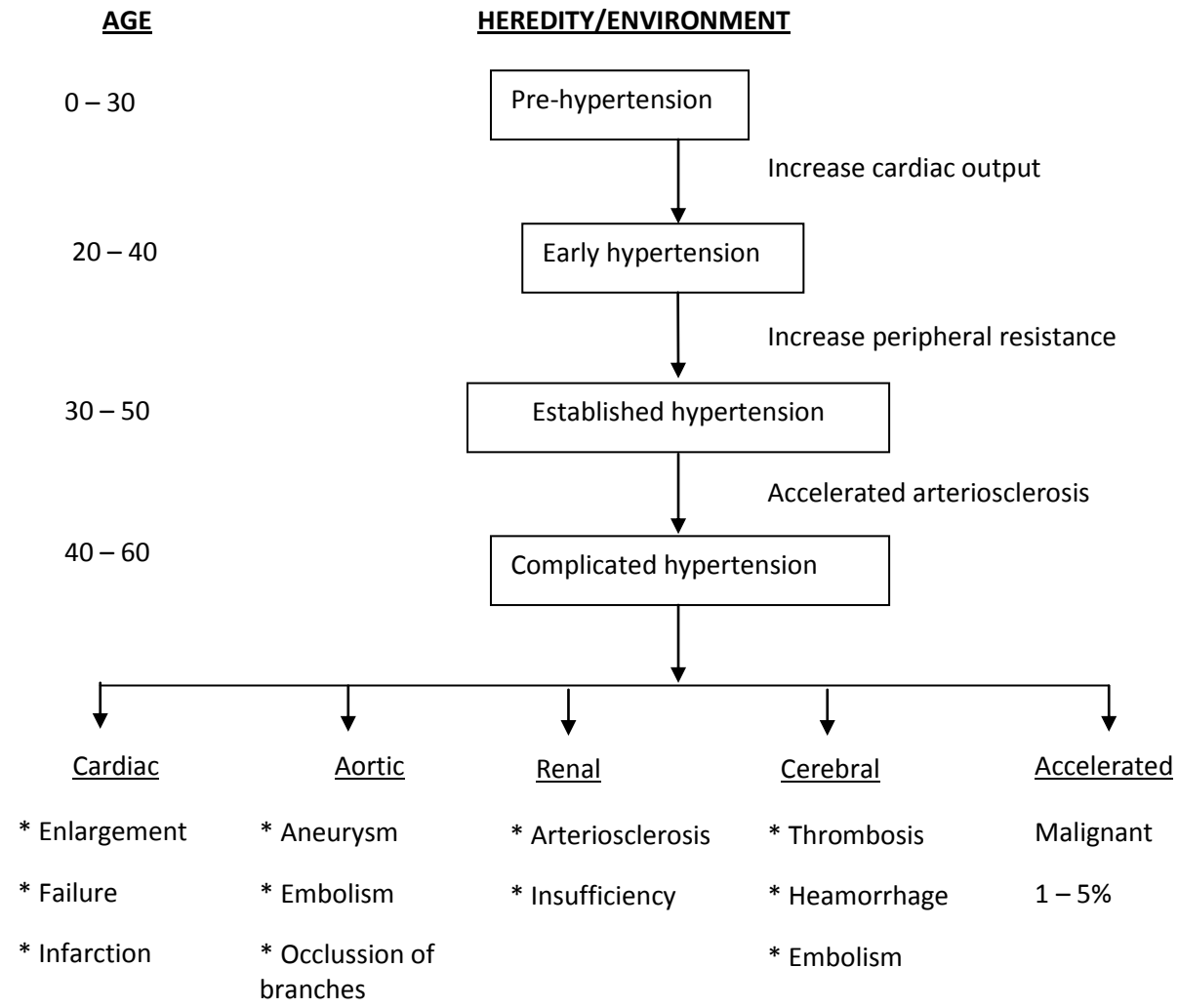


Fig. 2.21: Natural history of untreated essential hypertension (Kaplan, 1978)

2.5 Management of Hypertension

2.5.1 Diagnostic consideration

Baseline diagnostic information is required to search for potentially curable secondary hypertension and to provide a basic assessment of the patients underlying clinical status (including evidences for any target organ damage induced by the hypertension) and to establish fundamental laboratory information.

The hypertensive work up should be initiated with a complete history and physical examination. The history provides silent clues to the aetiology of hypertension. The physical examination can also provide clues to the underlying hypertensive disorder as well as supportive evidence of target organ damage secondary to blood pressure elevation. End organ damage can be detected by the palpation of the apex beat, bibasal crackles and examination of the fundi. Diagnosis of hypertensive retinopathy is based on four stage grading system (Table 2.4).

Table 2.4: Stages of hypertensive retinopathy

Stage	Signs
I	Arteriolar narrowing and tortuosity Increased silver reflex (‘silver wiring’)
II	Arteriovenous nipping
III	Cotton-wool ‘exudates’ Flame and blot haemorrhage
IV	Papilleodema

The physical examination involves the documentation of accurate blood pressure. Improper blood pressure measurement can lead to a diagnosis of hypertension in normotensive individuals. A 24-hour ambulatory blood pressure recording is particularly useful when clinic measurements show variability with resistant hypertension or to diagnose ‘white coat’ hypertension. Those who are already at high risk, either because of target organ damage or cardiovascular complications do not require ambulatory blood pressure measurement. Such patients can be treated on the basis of clinic measurement (Ashley *et al*., 2004).

Laboratory investigations are aimed at diagnosing rare secondary causes and quantifying end organ damage. Laboratory investigations can meaningfully be divided into three categories, i.e. routine diagnostic, prognostic and special diagnostic studies (Table 2.5).

Table 2.5: Diagnostic laboratory work up for patients with hypertension

1.	Routine diagnostic studies	a.	Urinalysis
		b.	Serum potassium
		c.	Intravenous pylogram
2.	Prognostic test	a.	Electrocardiogram
		b.	Chest x-ray
		c.	Blood urea nitrogen (BUN)
		d.	Creatinine
		e.	Blood glucose
		f.	Serum cholesterol
		g.	Electrolytes
3.	Special diagnostic	a.	Percutaneous renal biopsy studies
		b.	Renal scan
		c.	Isotope renography
		d.	Renal and adrenal arteriography
		e.	Renal function studies
		f.	Catecholamine excretion
		g.	Glucocorticoid excretion
		h.	Aldosterone secretion or excretion
		i.	Plasma renin activity
		j.	Adrenal venography
		k.	Skull X-ray, EEG.

2.5.2 Therapy of hypertension

The objective of the treatment is to reduce morbidity and mortality by reducing blood pressure and thus preventing cardiovascular and end-organ damage. Therapy with antihypertensive drugs reduces the incidence of all strokes by 38% in women, by 34% in men, by 36% in older persons, and by 34% in persons older than 80 years (Aronow and Frishman, 2004). The presence of complications is the strongest indication for instituting vigorous treatment immediately, with the exception that acute phase of myocardial or cerebral infarctions call for careful treatment to avoid worsening of the conditions by an excessive fall in blood pressure. The progression of events in malignant hypertension is slowed or halted by lowering the blood pressure.

The principles of treatment are to bring the blood pressure to as near the normotensive range as possible without undue side effects. The target blood pressure is normally set at a systolic blood pressure of less than 140mmHg and a diastolic blood pressure of less than 90mmHg for patients without compelling indications. Patients with compelling indications, such as diabetes or chronic kidney disease are at risk for cardiovascular events and therefore require lower target blood pressures for cardiovascular protection.

Reaching blood pressure goals may require several steps. The initial recommendation in the treatment algorithm for hypertension from JNC-7 (fig 2.22) involves lifestyle modifications. If the blood pressure goal is not achieved, an initial drug should be prescribed. Different classes of drugs for the management of hypertension exist. The major classes are diuretics, beta-blockers, angiotensin converting enzyme (ACE) inhibitors, angiotension – II receptor blockers (ARB) and calcium channel blockers

(CCB). Those used to a lesser extent include: centrally acting agents, aldosterone antagonists and direct vasodilators.

The recommended initial therapy for most patients with uncomplicated stage I hypertension is diuretics. Other drug classes recommended for mono therapy include: β-blockers, angiotensin converting enzymes (ACE) inhibitors, angiotensin receptor blockers (ARB) and calcium channel blockers (CCB). Uncomplicated stage II hypertension will likely require dual therapy using a diuretic or β-blocker and another drug from a different class. Doses can subsequently be titrated upwards and additional drugs added to form triple or quadruple therapy to achieve goal blood pressures (blood pressure <140/90mmHg in uncomplicated patients). In Ilorin, Nigeria, like in some other parts of the country, antihypertensive utilization conforms to the guidelines for the management of hypertension in blacks with majority of patients on diuretics particularly in combination with other agents. Angiotensin converting enzyme inhibitors are increasingly being used whereas beta-blockers and angiotensin receptor antagonists are still less utilized (Olanrewaju *et al.*, 2010). In an earlier study on pattern of drug utilization among hypertensives in a South Western Nigeria teaching hospital, diuretics and centrally acting agents were the most frequently prescribed anti-hypertensive drugs as physicians' prescribing decisions appeared to be significantly influenced by cost consideration (Yusuff and Balogun, 2005).

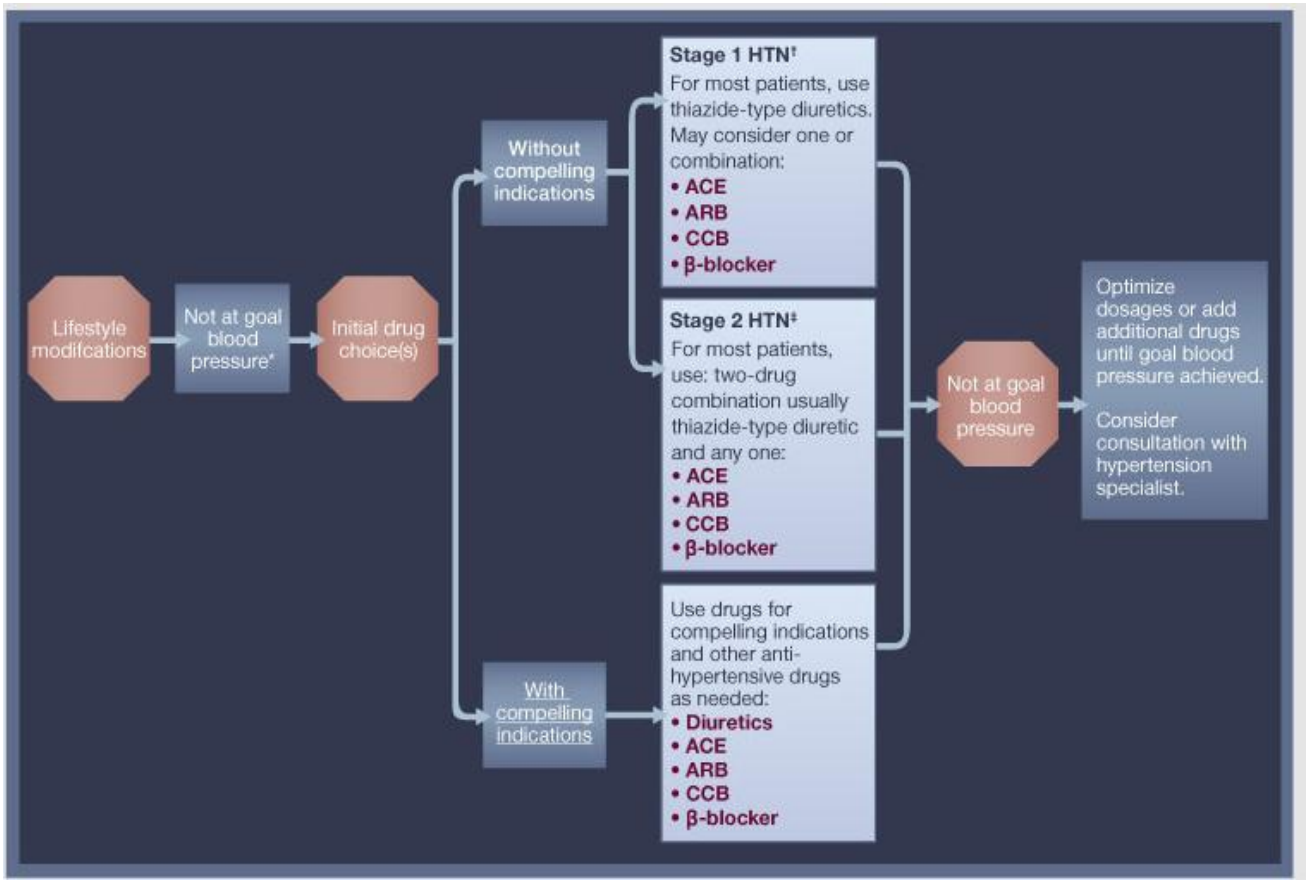


Fig. 2.22: JNC7 Hypertension Treatment Algorithm (Chobanian *et al*., 2003)

Key

* Goal blood pressure < $\frac{140}{90} mmHg$

* Stage 1 HTN = SBP 140-159 or DBP 90 – 99mmHg

* Stage 2 HTN = SBP >160 or DBP $\geq$100mmHg

The initial recommendation is lifestyle modifications. If the blood pressure goal is not achieved by recommended lifestyle modifications, an initial drug should be prescribed based upon guideline recommendations.

2.5.2.1 Non-drug therapy of hypertension

Non-drug approaches to the reduction of blood pressure are important and advisable as the initial approach in the management of hypertension and can be used alone during an initial period for those with mild hypertension and low risk. Whatever the

degree of hypertension, lifestyle modifications can have a positive impact on cardiovascular risk profile. These approaches can also augment the efficacy of antihypertensive drug and lower overall cardiovascular disease risk in patients with high level of blood pressure.

Lifestyle modifications involve dietary and behavioural methods and include:-

Weight reduction: Obesity and hypertension are closely associated and the degree of obesity is positively correlated with the incidence of hypertension. Hypertension in obese can be lowered by losing weight regardless of change in salt consumption (Hoffman, 2006). Dietary weight reduction in obese subjects produces a useful fall in arterial pressure. A weight loss of 3kg on average can produce a fall in blood pressure of 7mmHg systolic and 4mmHg diastolic (Swales, 2000).

Sodium restriction: The blood pressure in most hospitalized hypertensives can be lowered with severe restriction of salt, but there is poor compliance. Moderate restriction will on average lower blood pressure by 12mmHg systolic and 6mmHg diastolic; the higher the initial pressure, the greater the response. Subjects over 40 years of age are more responsive to the hypotensive effects of moderate restriction of salt (Grobbee *et al* 1986). Moderate salt restriction also enhances the effects of angiotensin-converting enzyme inhibitors, β-blockers and diuretics (Swales, 2000).

Alcohol restriction: Consumption of alcohol can raise blood pressure. Heavy consumption of alcohol increases the risk of cerebrovascular accidents but not coronary heart disease. The elevated blood pressures in heavy drinkers are lowered by withdrawal (Hoffman, 2006).

Potassium supplementation: Doubling potassium intake has a modest blood pressure lowering action of the same order of magnitude as moderate sodium restriction. Increased intake of potassium might reduce blood pressure by increasing excretion of Na^+, suppressing rennin secretion, causing arteriolar dilatation (possibly by stimulating Na^+, K^+ ATPase activity, and decreasing intracellular concentrations of Ca^{2+}), and impairing responsiveness to endogenous vasoconstrictors. A high-K^+ diet should however, not be recommended for patients on angiotensin-converting enzymes inhibitors (Hoffman, 2006).

Physical exercise: Increased physical activity lowers rate of cardiovascular disease in men. Lack of physical activity is associated with higher incidence of hypertension. Repetitive isotonic exercise produces a useful fall in blood pressure independently of any fall in weight. It also reduces blood volume and plasma catecholamines and elevates plasma concentrations of artrial natriuretic peptide (Hoffman, 2006). Moderate exercise in the form of daily jogging or brisk walking is probably sufficient to have a useful effect in lowering blood pressure, as well as improving well-being (Swales, 2000).

Relaxation and biofeedback therapy: Long-term successful stimuli can cause sustained hypertension; and this indicates that relaxation therapy will lower blood pressure in some hypertensive patients. Biofeedback and relaxation exercise can lower blood pressure in some hypertensive patients but the long-term efficacy of such treatment has been difficult to demonstrate because patients must be highly motivated. Only patients with mild hypertension who wish to use this method should be encouraged to try it (Hoffman 2006).

Quitting smoking: Cigarette smoking acutely raises blood pressure due to action of nicotine. This action is due to stimulation of sympathetic ganglia and the adrenal medulla, together with the discharge of catecholamines from sympathetic nerve endings. Contributing to this sympathomimetic response is also the activation of chemoreceptors of the aortic and carotid bodies, which reflexly results in vasoconstriction, tachycardia, and elevated blood pressure (Taylor, 2006).

Smoking is a major risk factor for coronary heart disease. Hypertensive patients should be encouraged to stop smoking (Hoffman, 2006).

Coffee: Consumption of caffeine can raise blood pressure and elevate plasma concentrations of epinephrine. Hypertensive patients should be encouraged to avoid coffee.

Other dietary manoeuvres: Fish oil (omega-3 fatty acids), olive oil and diet high in fruit and vegetables have blood pressure lowering effects (Swales, 2000).

Efforts to apply non-drug methods to therapeutic blood pressure reduction have concentrated upon some forms of blood pressure like mild hypertension. Man can be trained to acquire some control over autonomic functions, including blood pressure (Shapiro *et al*., 1969). Such learning can be facilitated by providing information about the level of blood pressure at that time, a technique known as biofeedback (Elder *et al*., 1977; Messerli *et al*., 1979).

Combinations of non-drug methods might be of considerable interest. Taking half daily measure of sodium, doubling potassium intake, 20 minutes of meditation twice a day, would certainly have a beneficial effect. Such measures would be impracticable in the short term but might on a long time scale be feasible (Dollery, 1982).

Atherosclerosis, especially of coronary arteries is associated with hypercholesterolemia and to a lesser extent hypertriglyceridemia, diabetes mellitus and cigarette smoking. Reduction in fat intake and no smoking would have a beneficial effect.

2.5.2.2 Anti hypertensive drug therapy

The treatment of hypertension with drugs is reserved for patients who do not achieve blood pressure goals in an acceptable range by non-drug means. The initial goal of antihypertensive therapy is to achieve and maintain the diastolic blood pressure below 90mmHg without compromising renal, cerebral or myocardial function or producing intolerable symptoms.

The specific drug or drugs chosen will depend on the clinical profile of the patient. The choice should be driven by likely benefit in an individual patient, taking into account concomitant diseases such as diabetes mellitus, problematic adverse effects of specific drugs and cost (Hoffman, 2006).

The principle of drug therapy in hypertension indicates the use of few drugs and low dose, starting with the least toxic agent. The dose can be gradually increased until maximal effect is achieved. Another agent which acts by different mechanism and which may also reduce the adverse effect of the other can be added. Rational combinations to be choosen should be based on complimentary mechanisms of action. If stability is attained for several years cautious withdrawal can be instituted to prevent rebound effect (Green andHarris, 1993).

In the medical outpatient clinic of the University of Port-Harcourt Teaching Hospital, Nigeria, Akpa et al (2008) reported that the percentage of hypertensive patients who

achieved adequate blood pressure control is low. They stressed the need to identify impediments to good blood pressure control and deal with them so as to optimize the benefit of antihypertensive treatment. Thiazide diuretics are reported to be the backbone of hypertension treatment especially in low resource settings even as mono-therapy in a Nigerian town by Ilesanmi et al., 2012. In their opinion, hypertensive management requires a response adapted to the local context and that proven solutions are needed to optimize the application of treatment guidelines in less developed societies with limited resources and to explore cost reduction strategies in the management of hypertensives in a poor resource setting. Alpha methyl dopa, a useful drug in the management of hypertension was not included in the guideline.

Patients are likely to be taking drugs for the rest of their lives; it is important to use the least toxic agents first, at a minimum effective dose and to monitor therapy regularly. Minimising the number of daily doses encourages compliance and reduces adverse effects.

High-risk hypertensive patients are those with compelling indications for specific drugs on account of other underlying serious cardiovascular diseases (heart failure, post-myocardial infarction or high coronary artery disease risk), chronic kidney disease or diabetes (Chobanian *et al*., 2003).

Diuretics, ACE inhibitors, angiotensin II receptor antagonists, and spironolactone should ideally be indicated for hypertensive with congestive heart failure, while ACE inhibitors and angiotensin II receptor antagonists should be first-line drugs in treatment of hypertension in diabetics because of their benefit in diabetic nephropathy. Nephropathic changes associated with microvoscular diseases and albuminuria develop in types I and II diabetes mellitus. ACE and angiotensin II

receptor antagonists reduce the progression of the nephropathy; they improve renal haemodynamics and have antiproteinuric effect that is independent of their antihypertensive action (Kasiske *et al*., 1993, Strippoli *et al*., 2004).

Isolated systolic hypertension (systolic blood pressure >160mmHg and diastolic blood pressure <90mmHg) can be managed with diuretics and Ca^{2+} channel blockers (Hoffman, 2003). Hypertensive emergencies are clinical syndromes that result from, or are complication of sustained elevation of blood pressure, usually exceeding 200mmHg systolic and /or 120mmHg diastolic. Hypertensive emergencies include hypertension with vascular damage (termed malignant hypertension) and hypertension associated with haemodynamic complications such as heart failure or stroke. The evidence of new or progressive damage to end organs caused by elevation of blood pressure determines the seriousness of the clinical situation.

Hypertensive emergencies in which the blood pressure must be immediately lowered include: hypertensive encephalopathy, intracranial haemorrhage, and acute congestive heart failure, dissecting aortic aneurysm, severe hypertension associated with toxaemia of pregnancy, unstable angina pectoris, acute myocardial infarction, head injury or excessive burns.

The blood pressure, however, must not be precipitously reduced as it can lead to poor perfusion of critical organs or exacerbation of cerebrovascular insufficiency. The goal of treatment in the first few hours or days is not complete normalisation of blood pressure because chronic hypertension is associated with autoregulatory changes in cerebral blood flow. Blood pressure should be lowered by about 25%, maintaining diastolic blood pressure at no less than 100-110 mmHg, subsequently, blood pressure can be reduced to normal levels using oral medications over several weeks.

Controlled reductions of blood pressure in hypertensive emergency are most easily achieved with titrable infusions, such as with vasodilators (sodium nitroprusside, diazoxide or hydralazine). Oral antihypertensive medication with minoxidil, clonidine or β-blocker should be started as soon as possible. This allows smoother long-term management as the goal of treatment in the first few hours or days is not complete normalisation of blood pressure because chronic hypertension is associated with autoregulatory changes in cerebral blood flows and rapid normalisation may lead to cerebral hypoperfussion and brain injury. Diuretics are administered to prevent volume expansion that occurs during powerful vasodilator administration (Benowitz, 2007).

If pheochromocytoma is suspected, α-adrenergic blockers (phenoxybenzamine and phentolamine) should be used first (Ashley *et al*., 2004). These irreversible α-blockers, destroy the α-receptors by alkylation; unlike prazosin, doxazosin, labetolol, indoramin, which cause competitive blockade, and can be overcome by a surge of noradrenaline release from the tumor (Brown, 2000).

2.5.3 The pharmacology of antihypertensive agents

Antihypertensives can, based on their primary sites or mechanisms of action (Hoffman, 2006), be classified as follows:

a. Diuretics

I. Thiazides and related agents: Bendroflumethiazide

Hydrochlorothiazide [Esidrex]

Hydroflumethiazide [Hydrenox]

Polythiazide [Nephril]

Chlorthalidone[Hygroton]

Indapamide [Natrilix]

II. Loop diuretics: Frusemide [Lasix$^{(R)}$]

Bumetanide [Burinex]

Ethacrynic acid [Edecrin]

Torsemide

III. K^+-sparing diuretics: Amiloride [Midamor]

Triamterene [Dytac]

Spironolactone [Aldactone]

Canrenone

b. Sympatholytic Drugs

i. β - Adrenergic antagonists: Propranolol [Inderal]

Atenolol [Tenormin]

Metoprolol

Betaxolol

Timolol [Blocadren, Timoptol]

ii. α - Adrenergic antagonists: Prazocin [Minizide]

Terazocin

Doxazocin

Indoramin

Phenoxybenzamine [Dibenyline]

Phentolamine [Rogitine]

iii. Mixed adrenergic antagonists: Carvedilol

Labetolol

iv. Centrally acting agents: Methyldopa [Aldomet]

Clonidine [Catapress]

Guanabenz

Guanfacine

v. Adrenergic neurone blocking agents: Guanadrel

Guanethidine [Ismelin]

Reserpine [Serpasil]

C Ca^{2+} Channel blockers: Nifedipine [Adalat]

Amlodipine [Amlovar]

Diltiazem [Univer]

Verapamil

Felodipine

Isradipine

Nicardipin

Nisoldipine

D Vasodilators

i. Arterial: Hydrallazine [Apresoline]

Minoxidil

Diazoxide

Fenoldepam

ii. Arterial and venous: Nitroprusside

E. Angiotensin Converting Enzymes Inhibitors: Captopril [Capozide]

Enalapril

Lisinopril [Zestril]

Fosinopril

Quinapril

F. Angiotensin – II Receptor Antagonists: Losartan

Candesartan

Eprosartan

Valsartan

Irbesartan

Olmesartan

Telmisartan

(Hoffman, 2006).

2.5.3.1 The pharmacology of diuretics

Diuretics lower blood pressure primarily by depleting sodium stores. They reduce blood pressure by reducing blood volume and cardiac output. Thiazide diuretics are appropriate for most patients with mild or moderate hypertension and normal renal and cardiac function. Loop diuretics are employed in severe hypertension when multiple drugs with sodium-retaining-properties are used. Potassium sparing diuretics

conserve potassium and enhance the natriuretic effects of other diuretics (Ives,2007; Benowitz, 2007).

Mechanisms of Action: Diuretics lower blood pressure primarily by reducing blood volume and cardiac output. Some diuretic like indapamide have direct vasodilating effect in addition to diuretic action. Thiazides and related diuretics are the most frequently used classes of antihypertensives. They act on the luminal side of proximal part of the distal convoluted tubule in the nephron where it inhibits Na^+ and Cl^- reabsorption by inhibiting Na^+ - Cl^- symporter. This leads to increased excretion of Na^+, Cl^- and water which stimulates potassium excretion further down in the distal tubule. Some thiazide diuretics are weak inhibitors of carbonic anhydrase an effect that increase HCO_3^- and phosphate excretion (Jackson, 2006).

Pharmacokinetics: All of the thiazides are orally active. Chlorothiazide can be parentrally administered. They have wide range of half-lives and they differ in metabolism. They are also secreted by the organic acid secretory system in the proximal tubule and compete with the secretion of uric acid by that system. The use of thiazides may blunt uric acid secretion and elevate serum uric acid level (Benowitz, 2007). Also drugs such as probenecid can attenuate the diuretic response to thiazides by competing for transport into the proximal tubule. The plasma protein binding of thiazides vary considerably and this determines the contribution that filtration makes to tubular delivery of specific thiazides (Jackson, 2006).

Adverse effects: The administration of thiazide diuretics is associated with the incidence of erectile dysfunction. Most serious adverse effects of Na^+, Cl^- and also Na^+ - K^+ - $2Cl^-$ symports inhibitors are abnormalities of fluid and electrolyte balance; which include extracellular volume depletion, hypotension, hypokalemia,

hypochloremia, metabolic alkalosis, hypomagnesemia, hypercalcemia, hyperurecemia and hypercalceamia. Thiazide diuretics produce dose dependent K^+ depletions which vary with individuals. This depletion can enhance two types of ventricular arrhythmias: polymorphic ventricular tachycardia (torsades de pointes) and ischemic ventricular fibrillation, the leading cause of sudden cardiac death and a major contributor to cardiovascular mortality in treated hypertensive patients. Thiazide diuretics decrease glucose tolerance which may unmask latent diabetes mellitus. They increase plasma level of lipids (Jackson, 2006).

Drug Interaction: Thiazide diuretics may diminish the effect of anticoagulants, uricosuric agents, sulphonyl ureas and insulin; they may increase the effects of anaesthetics, diazoxide, digitalis glycosides, lithium, loop diuretics and vit. D. Non steroidal anti inflammatory drugs reduce effectiveness while bile acid sequestrants reduce absorption of thiazides. The risk of hypokalaemia induced by thiazides is increased by amphotericin B and corticosteroids. Thiazide diuretic-induced K^+ depletion may account for many cases of quinidine induced torsade de pointes, a lethal drug interaction (Jackson, 2006).

2.5.3.2 The pharmacology of β-adrenergic antagonists

β-adrenoceptor antagonists are efficacious in the treatment of ischaemic heart disease, congestive heart failure and certain arrhythmias. They are useful in preventing tachycardia that often results from treatment with direct vasodilators.

Propranolol, a competitive β-receptor antagonists, is a useful prototype to which other β-antagonists are compared. It interacts with β_1, and β_2 receptors with equal affinity, it lacks intrinsic activity and does not block α receptors. The β-antagonists can be

distinguished by the following properties which may have clinical significance: relative affinity for β_1 and β_2 receptors; intrinsic sympathomimetic activity, blockade of α receptors, differences in lipid solubility, capacity to induce vasodilatation, and pharmacokinetic properties (Westfall *et al*., 2006). Two newer drugs are Carvedilol, a non-selective β-adrenoceptor antagonist with α_1-blocking activity and nevibolol, a β_1-selective antagonist that also causes vasodilatation through endothelium dependant mechanism (Rang *et al*., 2007).

Mechanism of action: β-adrenoceptor blockade affects the regulation of circulation through a number of mechanisms which include the following:

- Reduction in myocardial contractility, heart rate and cardiac output.
- Blockade of the β-receptors of juxtaglomerular complex, reducing renin secretion and thereby diminishing production of the circulating angiotensin II.
- Alteration of the control of sympathetic nervous system at the level of the central nervous system (CNS), altered baroreceptor sensitivity, altered peripheral adrenergic neuron function, and increased prostacyclin biosynthesis.

Peripheral vasodilatation is produced by some β-receptor antagonists through one or more of the following activities: production of nitric oxide, activation of β_2 receptors, blockade of α_1 receptors, blockade of Ca^{2+} entry, opening of K^+ channels and antioxidant activity. They contribute to the hypotensive effects by enhancing hypotension, increasing peripheral blood flow, and decreasing after load (Westfall *et al*., 2006).

Pharmacokinetics: Propranolol is highly lipophilic and is absorbed almost completely after oral administration. Much of it is however subjected to first pass metabolism variation in presystemic clearance in the liver, and this contributes to enormous variability in plasma concentration (approximately twenty fold) after oral administration. The degree of hepatic extraction of propranolol declines as the dose is increased. Concomitant ingestion of food and also long term administration of propranolol increases its bioavailability.

The volume of distribution of propranolol is large (4 litres/Kg). It is approximately 90% bound to plasma proteins. The metabolism is extensive with most of the metabolites like 4-hydroxypropranolol appearing in the urine (Westfall et al., 2006).

Adverse effects: The main adverse effects of β-receptor antagonists result as pharmacological consequences of blockade of β-receptors.

- Bronchoconstriction caused by β_2 receptor blockade in bronchial smooth muscles may cause life threatening increase in airway resistance in patients with bronchospastic disease (e.g. bronchial asthmatics) or patients with obstructive lung disease (e.g. chronic bronchitis or emphysema).

- Cardiac depression can occur and lead to heart failure, myocardial infarction or cardiomegaly. β-blockade may exacerbate heart failure in patients with compensated heart failure.

- Bradycardia is a normal response of β-blockade but can lead to life threatening bradyarrhythmias in patients with partial or complete atrioventricular conduction defects particularly if treated with antiarrhythmic drugs that impair cardiac conduction.

- Metabolism. β-adrenergic blockade may blunt recognition of hypoglycaemia in patients and may delay recovery from insulin-induced hypoglycaemia. The use of β-receptor antagonists in patients with diabetes who are prone to hypoglycaemia should be with caution. β_1- selective agents may be preferable for these patients.

- Fatigue is probably due to reduced cardiac output and reduced muscle perfusion in exercise.

- Cold extremities are presumably due to a loss of β-receptor mediated vasodilatation in cutaneous vessels.

- Others include sleep disturbance, depression and incidence of sexual dysfunction. (Rang, 2007; Westfall *et al*., 2006).

Interaction: Pharmacokinetic and pharmacodynamic interactions occur between β-receptor antagonists and other drugs. The absorption of β-blockers may be decreased by aluminium salts, cholestyramine and colestipol. Phenytoin, rifampicin, phenobarbitone and cigarette smoking reduce plasma concentration of propranolol through its extensive metabolism by their induction of hepatic biotransformation enzymes. Cimetidine and hydralazine may increase the bioavailability of agents such as propranolol and metoprolol by affecting hepatic blood flow. They decrease hepatic blood flow and this contributes to the decrease hepatic clearance seen with these drugs (McDevitt, 1988; Blautarb. 1995). Clearance of lidocaine can be impaired by β-receptor antagonists.

Pharmacodynamically, β-receptor antagonists and Ca^{2+} channel blockers have additive effects on the cardiac conducting system. Indomethacin and other non-

steroidal anti inflammatory drugs may however, oppose the antihypertensive effects of β-receptor antagonists (Westfall, 2006). This possibly happens due to the inhibition of renal synthesis of vasodilating prostaglandins, PG E_2 and PG I_2 which modulate the effect of angiotensin and norodrenaline on the kidney by causing compensatory vasodilatation (Lam and Shepherd, 1990; Rang *et al*., 2007).

2.5.3.3 The pharmacology of α-adrenergic antagonists

The α-adrenoceptor antagonists consist of the following main groups:

- non-selective α-receptor antagonists (e.g. phenoxybenzamine, phentolamine)
- α_1- receptor selective antagonists (e.g prazocin, doxazocin, terazocin)
- α_2- receptor selective antagonists (e.g yohimbine, idazoxan)
- ergot derivatives (e.g ergotamine, dihydroergotamine) (Rang *et al*., 2007).

The α- adrenergic receptors mediate many of the important actions of endogenous catecholamines. α_1- receptor mediation involve contraction of arterial and venous smooth musles, while α_2-rectpor mediation are involved in suppressing sympathetic output, increasing vagal tone, facilitating platelets aggregation, inhibiting the release of noradrenaline and acetylcholine from nerve endings, and regulating metabolic effects.

α_1 -selective adrenergic receptor blockade without affecting α_2- adrenergic receptors is the most employed of this group of antihypertensive agents; Prazocin, terazocin and doxazoxin are the available agents for the treatment. Prazocin is the prototype of the family.

Mechanism of action: Prazocin causes blockade of α_1 adrenergic receptors. This inhibits vasoconstriction induced by endogenous catecholamines. Vasodilatation occur in arteriolar resistance vessels and veins. The result is a fall in blood pressure due to decreased peripheral resistance. The fall in blood pressure is opposed by baroreceptor reflexes that cause increases in heart rate and cardiac output as well as fluid retention (Westfall *et al*., 2006).

Pharmacokinetics: After oral administration, prazocin is well absorbed. The bioavailability is about 50% to 70%; and peak concentrations are generally attained within 1-3 hours after an oral dose. It is tightly bound to plasma proteins (primarily α_1-acid glycoprotein) and only 5% of the drug is free in the plasma. The plasma half-life is 2-3 hours. It is extensively metabolized in the liver and excreted in the kidney (Westfall *et al*., 2006).

Adverse effect: First-dose effect is the major adverse effect of prazocin and its congeners. Marked postural hypotension and syncope are seen with first dose. The syncope may be characterized by drowsiness, weakness, dizziness, headache, lack of energy, nausea, palpitation. There may be urinary frequency, incontinence and priapism.

Interaction: Prazocin enhances the hypotensive effects of antidepressants (Westfall *et al*., 2006 which happens by potentiation of action. Prazocin, an α- adrenoceptor antagonist, causes vasodilatation and fall in arterial blood pressure. It relaxes both arterial and venous vascular smooth muscles. It may cause orthostatic hypotension, which also occurs with Tricyclic anti depressants (TCA) an action that seems anomalous for drugs that enhance noradrenergic transmission; this possibly results from an effect on adrenergic transmission in the medullary vasomotor centre (Rang *et*

al., 2007). It enhances by potentiative action the hypotensive effect of calcium channel blockers, which also lower blood pressure by relaxing arteriolar smoothmuscle and decreasing peripheral vascular resistance through the decrease in free intracellular concentration of Ca^{2+} by inhibiting transmembrane movement of Ca^{2+} through voltage sensitive Ca^{2+} channels (Weber, 2002). β-blockers that cause reduction in myocardial contractility, heart rate and cardiac output by antagonising, βadrenergic receptors including those of the juxtaglomerular complex, reduce renin secretion and diminish circulating angiotensin II. Diuretics which cause salt and water excretion, decrease plasma and extracellular volume. This leads to reduction in vascular resistance and cardiac output (Westfall *et al*., 2006).

2.5.3.4 The pharmacology of mixed adrenergic antagonists

The drugs in this group combine the actions of non selective β (i.e. β_1 and β_2) adrenoceptor antagonists and α- adrenoceptor antagonists. Labetolol and carvedilol are most employed drugs in the group for the treatment of hypertension. Labetolol is the prototype of the group and is an equimolar mixture of four stereoisomers. Two isomers are relatively inactive, a third is a potent α blocker and the last is a potent β blocker. Labetolol has 3:1 ratio of β:α antagonism after oral dosing. Carvedilol is also a racemic mixture; the S(-) and R(+) isomers with equal α-blocking potency. The ratio of α_1:β receptor antagonist potency for carvedilol is approximately 1:10 (Hoffman, 2006; Benowitz, 2007).

Mechanism of action: Labetolol is a competitive antagonist at both α_1 and β receptors; action on these receptors contribute to the fall in blood pressure in patients with hypertension. α_1 receptor blockade leads to relaxation of arterial smooth muscles and

vasodilatation particularly in the upright position. The blockade of β_1 receptors also contributes to a fall in blood pressure, in part by blocking reflex sympathetic stimulation of the heart. Labetolol also has intrinsic sympathomimetic activity on β_2 receptors which contributes to vasodilatation.

Pharmacokinetics: Labetolol is extensively absorbed after oral administration. It is subjected to extensive first-pass clearance; bioavailability is about 20% to 40% and is highly variable. Food intake increases its bioavailability. Labetolol is extensively metabolised in the liver by oxidative transformation and glucuronidation; the unchanged drug found in the urine is very little. Hepatic blood flow determines the rate of metabolism. Its elimination half life is about 8 hours (Westfall *et al*., 2006).

Adverse effects: Labetolol commonly causes postural hypotension. Other adverse effects include tiredness, weakness, headache, scalp tingling, lichenoid skin rash, epigastric pain, nausea, vomiting, liver damage, and difficulty in micturition and ejaculation (Grahame-Smith et al., 1984).

Interactions: Labetolol is best used in combination therapy. Its effects are enhanced by other antihypertensives like diuretics. Cimetidine increases the systemic availability of labetolol after oral administration by retarding its hepatic metabolism due to inhibition of microsomal cytochrome P450 (Grahame-Smith *et al*., 1984).

2.5.3.5 The pharmacology of central acting agents

These agents reduce sympathetic outflow from vasopressor centres in the brain stem but the sensitivity to baroreceptor control is retained. Methyldopa is the prototype of the class. It is useful in the treatment of mild to moderately severe hypertension. It

lowers blood pressure by reducing peripheral vascular resistance, with a variable reduction in heart rate and cardiac output.

Mechanism of action: Methyldopa, a prodrug, affects nor adrenaline synthesis. It enters the brain and is taken up by nor adrenergic neurones. In nor adrenergic neurones it is decarboxylated to α-methyldopamine which is then β-hydroxylated to α-methylnoradrenaline. The α-methylnoradrenaline is stored in adrenergic nerve vesicles as false transmitter. When the adrenergic neurone discharges its neurotransmitter, α-methyl noradrenaline is released instead of noradrenaline; α-methylnoradrenaline acts in the central nervous system to inhibit adrenergic neuronal outflow from the brain stem. It acts as an agonist at presynaptic α_2- adrenergic receptors in the brain stem, attenuating noradrenaline release and thereby reducing the output of vasoconstrictor adrenergic signals to peripheral sympathetic nervous system (Benowitz, 2007; Hoffman, 2006).

Pharmacokinetics: Methyldopa is orally active. It is absorbed by an active aminoacid transporter. Peak plasma concentrations occur after 2 to 3 hours. The apparent volume of distribution is 0.4 litre/kg and its half-life of elimination is about 2 hours. Methlodopa is transported into the central nervous system through active process. It is excreted in the urine as sulphate conjugate and as parent drug. The half-life of methyldopa is prolonged in patients with renal failure to 4 or 6 hours (Hoffman, 2006).

Adverse Effects: Active metabolite of methyldopa acts on α_2-adrenergic receptors in the brain stem to inhibit the reticular activating system; sedation is thus produced. Depression may occasionally occur. Methyldopa produces dryness of the mouth, reduction in libido, parkinsonian signs and hyperprolactineamia that may cause

gyneacomastia and galactorrhea. Bradycardia and sinus arrest may be precipitated by methyldopa in individuals with sinoatrial node dysfunction.

Adverse effects unrelated to the pharmacological actions of methyldopa include hepatotoxicity, haemolytic anaemia, and development of positive coombs test (antiglobulin test). Rare adverse effects include leucopenia, thrombocytopenia, redcall aplasia, lupus erythematosus-like syndrome, lichenoid and granulomatous skin eruption, myocarditis, retroperitoneal fibrosis, pancreatitis, diarrhoea and malabsorption (Hoffman, 2006).

Interactions: The hypotensive effect of methyldopa is antagonised due to fluid retention by corticosteroids, oral contraceptives and non-steroidal anti inflammatory drugs. The antihypertensive effect is also antagonised by Tricyclic antidepressants and phenothiazines (Easterbrook, 1999).

2.5.3.6 The pharmacology of adrenergic neurone blocking agents

These agents essentially Guanadrel and Guanethidine lower blood pressure by preventing normal physiologic release of noradrenalin from postganglionic sympathetic neurones. All the therapeutic and adverse effects of these drugs result from functional sympathetic blockade. The antihypertensive effect is achieved by a reduction in peripheral vascular resistance that results from inhibition of α receptor-mediated vasoconstriction.

Reserpine, another member of the class, is an alkaloid from the root of *Rauwolfia serpentine*. It reduces both cardiac output and peripheral vascular resistance (Hoffman, 2006).

Mechanism of action: Guanadrel is considered the representative of the class. It specifically inhibits the function of peripheral postganglionic adrenergic neurones. It is an exogenous false transmitter that is accumulated, stored and released like noradrenaline. It is however, inactive at adrenergic receptors. The drug is actively transported into the neurone, its site of action, by the same transporters that are responsible for the reuptake of noradrenaline. It is concentrated within the adrenergic storage vesicles where it replaces noradrenaline. Its presence in the storage vesicles depletes the normal transmitter, and can be released by stimuli that normally release noradrenaline but is inactive at adrenergic receptors. The replacement of noradrenaline with an inactive transmitter is probably the principal mechanism of action of guanadrel (Hoffman, 2006).

Pharmacokinetics: The absorption of guanadrel after oral administration is rapid; peak plasma level is reached in 1-2 hours. Maximum effect on blood pressure is not seen until 4-5 hours after oral administration; this is because it has to be transported into and accumulated in adrenergic neurones. The elimination half life is 5-10 hours. The half life of pharmacological effect of guanadrel is determined by the drugs persistence in neuronal pool and is at least 10 hours. Its clearance from the body is by both renal and nonrenal disposition (Hoffman, 2006).

Adverse effects: Adverse effects of guanadrel are related to sympathetic blockade. Symptomatic hypotension during standing, exercise, ingestion of alcohol, or hot weather is the result of the lack of sympathetic compensation for these stresses. There is associated fatigue, lassitude, sexual dysfunction and diarrhoea (Hoffman, 2006).

2.5.3.7 The pharmacology of calcium channel blockers

Ca^{2+} channel blockers are important group of drugs for the treatment of hypertension. Voltage sensitive calcium channels mediate the entry of Ca^{2+} into smooth muscles, cardiac myocytes, sinoatrial node and artrioventricular nodal cells in response to electrical depolarization. Ca^{2+} is a trigger for contraction in albeit by different mechanisms in both smooth muscle and cardiac myocytes. The subclasses of Ca^{2+} blockers with clinical application include verapamil (a phenylalkylamine); diltiazem (a benzothiazepine); nifedipine, amlodipine, isradipine (dihydropyridines); and bepridil (a diarylaminopropylamine ether). All these work by inhibiting calcium ion channel function resulting in relaxation of vascular smooth muscles especially in arterial beds and consequently vasodilatation (Michel, 2003).

Mechanism of Action: Intracellular calcium flux is a final common path for a spectrum of cellular responses to a wide variety of stimuli. An increased concentration of cytosolic Ca^{2+} causes increased contraction in cardiac and vascular smooth muscle cells. The contraction of cardiac myocytes (Ca^{2+} -induced Ca^{2+} release) is initiated by the entry of extracellular Ca^{2+}. The release of Ca^{2+} from intracellular storage sites also contributes to contraction of vascular smooth muscles particularly in some vascular beds. Cytosolic Ca^{2+} concentrations may be increased by various contractile stimuli. Many hormones and neurohormones increase Ca^{2+} influx through receptor-operated channels. High external concentrations of K^{+} and depolarizing electrical stimuli increase Ca^{2+} influx through voltage-sensitive or "potential operated", channels. Contraction of vascular smooth muscles is dependent on the free intracellular concentration of Ca^{2+}; while inhibition of transmembrane movement of Ca^{2+} through voltage sensitive Ca^{2+} channels can decrease the total amount of Ca^{2+} that reaches intracellular sites. Ca^{2+} - calmodulin-dependent activation

of myosin light chain kinase, resulting in phosphorylation of myosin light chains, causes an increase in actin-myosin ATpase activity and contraction. The Ca^{2+} channel blockers produce their effects by binding to the α_1 subunit of the L-type Ca^{2+} channels, reducing Ca^{2+} flux through the channels. Blood pressure is lowered by relaxing arteriolar smooth muscle and decreasing peripheral vascular resistance (Michel, 2006).

Pharmacokinetics: Ca^{2+} blockers are orally active; their absorption is nearly complete after oral administration. Their bioavailability is reduced in some cases by first-pass hepatic metabolism. They are extensively bound to plasma protein (70% - 98%). Their elimination half-lives vary widely between 1.3 to 64 hours. Their bioavailability and half life may increase during repeated oral administration because of saturation of hepatic metabolism.

Adverse Effects: The most common side effects of Ca^{2+} channel blockers are due to excessive vasodilatation; these include dizziness, hypotension, flushing, digital dysesthesia and nausea. Patients may experience constipation, peripheral edema, coughing, wheezing and pulmonary edema. Myocardial ischemia and worsening of angina may occur. Bradycardia, asystole and exacerbation of heart failure may occur with verapamil (Michel, 2006).

Interaction: Verapamil blocks the P-glycoprotein drug transporters. The elimination of digoxin and other drugs that are cleared by these transporters is inhibited by verapamil and these increase their plasma concentration. Concurrent administration of Ca^{2+} channel blockers with quinidine may cause excessive hypotension particularly in patients with idiopathic hypertrophic subaortic stenosis (Hoffman, 2006). Also, concomitant use of verapamil with β-blockers or Amiodarone increases the risk of

AV node block which may be complete and result in asystole, heart failure and severe hypotension. Rifampicin increases the metabolism of nifedipine; while grape fruit juice raises the plasma concentration of nifedipine and other dihydropyridines (except amlodipine). Nifedipine increases the plasma concentration of phenytoin (Chatu, et al., 2000).

2.5.3.8 The pharmacology of vasodilators

This class of antihypertensives consists of oral vasodilators, hydralazine and minoxidil, which are used for long-term outpatient therapy of hypertension; and parenteral vasodilators like nitroprusside, diazoxide and fenoldopam which are used to treat hypertensive emergencies. All of the vasodilators useful in hypertension relax smooth muscles of arterioles, thereby decreasing systemic vascular resistance. Sodium nitroprusside also relaxes veins.

Mechanism of Action: Hydralazine causes direct relaxation of arteriolar smooth muscles. The molecular mechanism mediating the action may involve a fall in intracellular calcium concentrations. A variety of changes in cellular signalling pathways are influenced by hydralazine, although precise molecular targets that explain its capacity to dilate arteries remain uncertain. Decreased arterial resistance and decreased mean arterial blood pressure elicit compensatory responses, mediated by baroreceptors, sympathetic nervous system, renin, angiotensin and aldosterone (Hoffman, 2006).

Pharmacokinetics: Hydralazine is rapidly absorbed through the gastrointestinal tract. It is rapidly metabolized by the liver during the first pass so that bioavailability is low (average 25%) and variable among individuals. It is metabolized in part by

acetylation. The rate of acetylation is an important determinant of the bioavailability. The peak concentration of hydralazine in plasma and the peak hypotensive effect of the drug occur within 30 – 120 minutes of ingestion. Its plasma half-life ranges from 1.5-3 hours, but the hypotensive effect lasts longer than do the plasma concentration. The systemic clearance of hydralazine is about 50 ml/Kg per minute (Hoffman, 2006).

Adverse Effect: The common adverse effects of hydralazine are headache, nausea, flushing, hypotension, palpitation, tachycardia, dizziness and angina pectoris. Myocardial ischemia due to the increased O_2 demand induced by the baroreceptor reflex-induced stimulation of the sympathetic nervous system and also because hydralazine does not dilate the epicardial coronary arteries.

Other adverse effects are caused by immunological reactions, of which drug-induced lupus erythematosus is the most common. Administration of hydralazine can result in an illness that resembles serum sickness, heamolytic anaemia, vasculitis, rapidly progressive glomerulonephritis (Hoffman, 2006), bone marrow depression and peripheral neuropathy (Easterbrook, 1999).

Interactions: The hypotensive effect of hydralazine, minoxidil and sodium nitroprusside is enhanced by other antihypertensives.

2.5.3.9 The pharmacology of angiotensin converting enzymes inhibitors

The renin-angiotensin system plays a significant role in the pathophysiology of hypertension, congestive heart failure, myocardial infarction and diabetic nephropathy. The renin-angiotensin system plays a major role in both the short- and long-term regulation of arterial blood pressure. Modest increases in plasma concentrations of angiotensin II acutely raise blood pressure. The ability to reduce the

levels of angiotensin II with orally effective inhibitors of angiotensin-converting enzyme represents an important advancement in the treatment of hypertension (Jakson, 2006).

Mechanism of Action: The essential effect of angiotensin – converting enzyme inhibitors, such as captopril, lisinopril or enalapril on the renin-angiotensin system is to inhibit the conversion of the relatively in-active angiotensin I to the active angiotensin II; or angiotensin I to angiotensin III. Angiotensin converting enzyme inhibitors are highly selective, attenuate or abolish responses to angiotensin I and not angiotensin II and increase bradykinin levels which stimulates prostaglandin biosynthesis. Bradykinin and /or prostaglandin may contribute to the pharmacological effects of angiotensin-converting enzyme inhibitors. The ACE inhibitors blunt the rise in aldosterone concentrations in response to Na^+ loss; thus enhancing the efficacy of diuretics (Hoffman, 2006).

Pharmacokinetics: Many angiotensin-converting enzymes are orally active. Many are ester-containing prodrugs that are 100 to 1000 times less potent but have a much better oral bioavailability than the active metabolite. Angiotensin-converting enzyme inhibitors differ with regard to pharmacokinetics. They differ in tissue distribution and are cleared predominantly by the kidneys. Captopril, the prototype of the group, has bioavailability of 75% after oral administration. Peak plasma concentration occurs within an hour, and is rapidly cleared with a half-life of about two hours. Most of the drug is eliminated in the urine (Jackson, 2006).

Adverse Effect: Hypotension may occur after initial doses of any angiotensin converting enzyme inhibitor in patients who are hypovolemic due to diuretics, salt restriction, or gastrointestinal fluid loss. Other adverse effects include hyperkalaemia,

dry cough, skin rash, angioedema, proteinuria, glycosuria and acute renal failure. Neutropenia and hepatotoxicity are rare but serious side effects. An alteration in or loss of taste, dysgeusia, is a reversible adverse effect and occurs more frequently with captopril (Jakson, 2006).

Interactions: Important drug interactions include those with K^+-sparing diuretics and K^+ supplements which may exacerbate Angiotensin converting enzyme inhibitor-induced hyperkalamia. Non steroidal anti-inflammatory drugs may impair the hypotensive effects of angiotensin-converting enzymes inhibitors by blocking bradykinin-mediated vasodilatation, which is in part, prostaglandin mediated. Angiotensin-converting enzyme inhibitors may increase plasma levels of digoxin and lithium and may increase hypersensitivity reactions to allopurinol (Jackson, 2006).

2.5.3.10 The pharmacology of angiotensin-ii receptor blockers

Angiotensin II receptor blockers available for clinical application bind to the angiotensin II type 1 (AT_1) receptors with high affinity and more selectivity for AT_1, than AT_2. The binding of angiotensin receptor blockers to the AT_1 receptors is competitive and the inhibition of the biological response to angiotensin II is often insurmountable and this may be due to slow dissociation kinetics of the compounds from the AT_1 receptor; other factor that may contribute to unsurmountable blockade include angiotensin receptor blockade-induced receptor internalization and alternative binding sites for angiotensin receptor blockers on the AT_1 receptors (Jackson, 2006).

Mechanism of Action: Angiotensin receptor blockers potently and selectively inhibit the biological effects of angiotensin II by blocking AT_1 receptor; the actions of angiotensin II via the AT_1 receptor are inhibited regardless of the biochemical

pathway leading to angiotensin II formation. The biological effects of angiotensin II blocked by angiotensin-receptor blockers include angiotensin II-induced (1) contraction of vascular smooth muscles, (2) rapid pressor responses, (3) slow pressor responses, (4) thirst, (5) vasopressin release, (6) aldosterone secretion, (7) release of adrenal catecolamines, (8) enhancement of noradrenergic neurotransmission, (9) increase in sympathetic tone (10) changes in renal function, and cellular hypertrophy and hyperplasia (Jackson, 2006). By antagonizing the effects of angiotensin II, angiotensin-receptor blockers relax smooth muscle and thereby promote vasodilatation, increase in renal salt and water excretion, reduce plasma volume, and decreased cellular hypertrophy (Hoffman, 2006).

Pharmacokinetics: Angiotensin receptor blockers are orally active and bioavailability is generally low (less than 50%). They are highly protein bound (more than 90%). Peak plasma concentration of losartan after oral administration is 1-3 hours. The plasma half life is 2.5 hrs. The plasma clearance of losartan, (600ml/min), is due to renal and hepatic clearances (Jackson, 2006).

Adverse Effects: The adverse effects are usually mild. Symptomatic hypotension including dizziness, occur in patients with intravascular volume depletion. Hypokalaemia and angioedema may occur. Angiotensin-receptor blockers have teratogenic potential and should be discontinued before the second trimester of pregnancy (Jackson, 2006).

Interactions: Blood pressure lowering effects of other antihypertensive drugs is enhanced by angiotensin-receptor blockers. Alcohol, anaesthetics and diuretics enhance hypotensive effects of angiotensin-receptor blockers. Concomittant administration of nonsteroidal anti-inflammatory drugs with angiotensin receptor

blockers increases the risk of renal impairment. Excretion of lithium is reduced by angiotensin II receptor antagonists (British National Formulary, 2006).

2.5.4 Pharmacotherapy for hypertension in geriatric

Older patients may suffer from multiple concurrent illnesses which are best treated with different medications and the care is therapy targeted to a specific illness for which a clear likelihood of patient benefit exists. Aging is a universal process whose manifestations are familiar and unambiguous; it has been defined as progressive loss of function accompanied by decreasing fertility and increasing mortality and disability. Aging has dramatic effect on response to pharmacological, surgical and rehabilitative interventions.

The prevalence of markers of disease, diseases per se, disability consequent on disease and mortality rate increases exponentially in old age. The high prevalence of disease promotes high use of medication in older people. Older people use on average two to five prescription medications on a regular basis, and polypharmacy defined as the use of five or more medications, occurs in 20 to 40 of this age group (McLean et al., 2004). Elderly persons have higher prevalence of hypertension than younger and middle aged persons and elders with hypertension have a much higher risk of cardiovascular morbidity and mortality than middle aged persons with hypertension. Several medications are used to try to lower blood pressure and reduce or prevent complications such as stroke or heart attack. The use of pharmacologic agents in elderly patients is one of the most difficult aspects of patient care. There is a clear indication of relationship between occurrence of adverse drug reaction and number of medications to which the patient is concurrently exposed. This leads to the concept of

"therapeutic burden" which co-varies with "disease burden". Drug therapy in geriatric can generally be summarized in the phrase 'start low and go slow' (Abernethy, 1999). The incidence of adverse drug reaction correlates with age. Old age in most species is associated with impaired adaptive and homeostatic mechanisms leading to susceptibility to environmental or internal stresses with increasing rate of disease and death (Grimley, 2000). From cellular perspective, several mechanisms are considered to underlie the primary aging process and probably contribute to age related changes in adaptive responses including pharmacological responses. These include oxidative stress, mitochondrial dysfunction, telomere shortening, and various genetic mechanisms (McLean et al., 2004).

The basis for increased incidence of adverse drug reactions may be attributed in some cases to increased drug exposure (pharmacokinetic), in other cases to altered drug effect at a fixed drug exposure (pharmacodynamic), and more commonly a combination of the two. The age-related pathological changes which result in increased drug exposure are decreased renal glomerular filtration with decreased drug renal clearance and decreases in hepatic drug oxidation. The oxidative pathways most involved are the cytochrome P450 (CYP) 3A isoenzymes. The most clearly defined pharmacodynamic changes are the β-adrenoceptor dysfunction, baroreflex dysfunction, and impaired vascular endothelial function (Abernethy, 1999).

The relationship between the risks of adverse drug reactions and old age is well established; and when the adverse drug reactions occur they are more likely to be severe (Walker and Wynne, 1994). Old age is a risk factor for adverse drug reactions, a marker for comorbidity, altered pharmacokinetics, and polypharmacy. The consistent association of age-related changes in pharmacodynamics, pharmacokinetics and polypharmacy leads to a strong recommendation for minimizing therapeutic

burden with respect to both dose and number of drugs administered (Abernethy, 1999).

2.5.5 Role of medicinal plants in the treatment of cardiovascular diseases

In the developing countries, remedies from plants play an important role in the healthcare of millions of people (Ernest, 2001). According to the World Health Organization (WHO), about 80% of people in developing countries rely primarily on medicinal plants for their primary health care (Wood-Sheldon *et al*., 1997).

.Attempts by the low-income group, particularly the rural dwellers in the developing countries, to control HTN and its attendant complications in the face of the scarce socioeconomic resources, have led more people opting for herbal remedy (Farnwort *et al*., 1985). The hypotensive and antihypertensive effects of some of these medicinal plants have been validated while claims have also been disproved through evidences obtained from rigorous scientific evaluations of acclaimed hypotensive effect. However, more scientific research needs to be done to verify the effectiveness and elucidate the safety profile of such herbal remedies. Examples of naturally occurring medicinal plants that have so far been scientifically studied and reported to have hypotensive or antihypertensive effects include:

Allum sativum (Family: Alliaceae or Liliaceae; Common name: Garlic): Garlic has been used over centuries for a variety of cardiovascular conditions, and has been shown to have antihypertensive action. One of the primary active compounds that gives garlic its characteristic odor and many of its healing benefits is called allicin which is thought to increase nitric oxide (NO) production, resulting in smooth muscle relaxation and vasodilatation. Various studies showed that garlic consumption is

related to decrease of BP in patients with increased systolic pressure, but not in patients without increased systolic pressure. Garlic preparations have been also been found to be superior to placebo in reducing BP in individuals with hypertension (Ried *et al*., 2008). The antioxidative and antihypertensive effect of garlic has been observed in 20 patients with hypertension compared to 20 patients with normal pressure, who have been receiving garlic pearls preparation for a period of two months. The results have revealed decreased BP, significant reduction of 8-hydroxy-2-deoxyguanosin, level of nitric oxide, and lipid peroxidation, and an increased level of antioxidative vitamins C and E (Qidwai & Ashfaq, 2013). Similar cardioprotective effects were observed in the study on hypertensive rats by Harauma & Moriguchi, 2006. Garlic's antihypertensive mechanism probably involves its prostaglandin-like effects, which decrease peripheral vascular resistance. Garlic compounds were observed to inhibit the angiotensin-converting enzyme in vitro, while aged garlic extract (AGE) enhanced NO production and activates nitric oxide synthase (NOS) (Hara *et al*., 2013)

Avena sativa (Family: Poaceae/Gramineae; Common names: Dietary Fibber, Green Oat): A diet containing soluble fiber-rich whole oats can significantly reduce the need for antihypertensive medication and improve BP control. The inclusion of oats to the normal diet of patients with hypertension significantly reduced both systolic and diastolic BP (Tabassum & Ahmad, 2011). Other studies similarly suggest that intake of grains rich in water-soluble fibre in the presence of reasonable amounts of total fibre, like oats and buckwheat, are associated with lower serum cholesterol and BP values. These results imply that a high-fibre diet may be a useful part of a lifestyle modification to prevent cardiovascular diseases. (He *et al*., 1995; Davy *et al*., 2002).

Camellia sinensis (Family: Theaceae; Common name: Tea): There are many potential health benefits from drinking tea which sparked research for cardiovascular benefits. A study reported that nonhabitual tea drinkers were at higher risk of hypertension than habitual tea drinkers, and there was a progressive reduction in risk associated with higher levels of tea consumption in daily intake. From the several models launched in this study, it was hypothesized that the threshold of tea consumption likely to be beneficial for reducing hypertensive risk is to consume green or oolong tea, 120 mL/day or more, for at least 1 year (Yang *et al*., 2004). Black and green tea polyphenols also attenuated the development of hypertension through their antioxidant properties in male stroke-prone spontaneously hypertensive rats because of observed decreased myosin light chain (MLC) phosphorylation related to NO bioavailability and an increase in catalase, a scavenger of hydrogen peroxide (H_2O_2) in rat aorta (Negishi *et al*., 2004).

Glycine max (Family: Fabaceae Common name: Soyabean): Soybean has been found to be effective as a hypotensive agent. Blood pressure decreased significantly in one study, but not in the other five. The average change is in systolic BP and it is 1 mm Hg. Several studies that evaluated the effect of soy isoflavones also did not find a significant effect on blood pressure (Sacks *et al*., 2006).

Hibiscus sabdariffa (Family: Malvaceae; Common name: Roselle): The leaves, calyx, and corolla of this plant are edible and are used traditionally in many West African countries for various medicinal purposes. The antihypertensive effects of the crude extract of the plant have been attributed to mediation through acetylcholine - and histamine - like dependent mechanisms through direct vasorelaxant effects. Earlier report showed that the petal crude extract of the plant had a direct relaxant effect on the aortic smooth muscle of rats. The chronic administration of aqueous extract of *H.*

sabdariffa has been reported to reverse cardiac hypertrophy in renovascular hypertensive rats (Tabassum & Ahmad, 2011). A randomized, double-blind, placebo-controlled clinical trial was conducted in 65 pre- and mildly hypertensive adults, age 30–70 yrs, not taking blood pressure (BP)-lowering medications, with either three 240-mL servings/day of brewed hibiscus tea or placebo beverage for 6 weeks. The hibiscus tea effectively lowered BP in pre- and mildly hypertensive adults. Dietary intake of vitamin C, sodium, potassium, calcium, magnesium, and other dietary factors known to have an effect on BP, i.e. alcohol and caffeine, did not differ between the placebo and treatment groups during the intervention and thus, were not responsible for the observed change in BP (McKay *et al*., 2010).

Linum usitatissimum (Family: Linaceae; Common name: Linseed, Flaxseed): It is an annual herb believed to have originated in Egypt. Linseed and its oil are rich in α-linolenic acid, an essential fatty acid that appears to be beneficial for the heart diseases, inflammatory bowel disease, arthritis, and other health problems. α-linolenic acid belongs to a group of substances called omega-3 fatty acids. Several studies suggest that diets rich in omega-3 fatty acids lower BP significantly in people with hypertension. Flaxseed may protect against atherosclerotic cardiovascular disease through a number of mechanisms, including reducing serum cholesterol, platelet aggregation, and inflammatory markers; improving glucose tolerance; and acting as an antioxidant. Daily consumption of 15 to 50 g/day of ground flaxseed can modestly reduce total cholesterol and low-density lipoprotein concentrations without altering triglycerides or high-density lipoprotein cholesterol. However, the exact mechanism as an antihypertensive is unclear (Al-Bishri, 2013).

Moringa oleifera (Family: Moringaceae; Common name: Murungai): The leaves of M. oleifera caused a fall in systolic, diastolic, and mean BP in anaesthetized rats in a

dose-dependent manner. The antihypertensive effect was brief, returning to normal within two minutes. It was also established that thiocarbamate and isothiocyanate fractions of the crude extract were responsible for the antihypertensive activity (Tabassum & Ahmad, 2011). Mengistu *et al*, (2012) demonstrated that the intravenous administration of the fresh leaf extract exhibited blood pressure lowering effect in normotensive anaesthetized guinea pigs. The mode of action is neither cholinergic nor adrenergic pathway because its effect is not altered by atropine which is a muscarinic receptor blocker of acetylcholine and propranolol which is a non-selective blocker of adrenergic receptors. In tissue preparation however, the crude leaf extract exhibited vasodilatation effect on high K+-induced contraction of isolated aorta thus may be acting through blockade of the voltage sensitive Ca^{2+} channels.

Punica granatum (Family: Lythraceae; Common name: Pomegranate): As some antioxidants were recently shown to reduce blood pressure (BP), the effect of pomegranate juice consumption (50 mL, 1.5 mmol of total polyphenols per day, for 2 weeks) by hypertensive patients on their BP and on serum angiotensin-converting enzyme (ACE) activity was studied. A 36% decrease in serum ACE activity and a 5% reduction in systolic BP were noted (Tabassum & Ahmad, 2011). A similar dose-dependent inhibitory effect (31%) of pomegranate juice on serum ACE activity was observed *in vitro*. A reduction in serum ACE activity, without a decrease in blood pressure BP, was previously shown to attenuate atherosclerosis. Pomegranate juice can offer a wide protection against cardiovascular diseases which could be related to its inhibitory effect on oxidative stress and on serum ACE activity (Aviram & Rosenblat, 2012).

Sesamum indicum (Family: Pedaliaceae; Common name: Sesame): After a 5-week treatment (feeding) period, Sesamin dose dependently attenuated the increase in

systolic BP in deoxycorticosterone acetate (DOCA) - salt model of hypertension in rats. (Nakano *et al*.,2006). In another study 60mg/kg sesamin and a placebo were administered over a 4 week period to hypertensive individuals. After the 4 week treatment period, it was observed that systolic BP had a decreased mean of 3.5mmHg and diastolic BP, 1.9mmHg. The placebo group had no significant decrease (Miyawaki *et al*., 2009).

Viscum album (Family: Santalaceae; Common name: Mistletoe): The aqueous extract of V. album contains some biologically active principles that may act as inducer of the nitric oxide/soluble guanylate cyclase pathway producing vasorelaxation (Iwalokun *et al*., 2011).The blood pressure was reduced by administration of chloroform methanol fraction (CMF) at a dose of 0.5 ml/kg and 1ml/kg to a certain degree. In a study on the effect of consecutive 14-days administration of CMF, it was reported that the mistletoe extract produced antihypertensive effect without alteration in HR (Ye *et al*., 2009).

The plants used in South Africa for the treatment of cardiovascular diseases are as follows:

Plant	**Family**	**Parts used**
• *Agathosma betulina*	*Rutaceae*	Leaves
• *Cannavis Sativa*	*Cannabaceae*	Leaves
• *Cissampelos Capensis*	*Minespermaceae*	Roots
• *Dodonaea Angustifolia*	*Sapindaceae*	Leaves
• *Gunnera Perpensa*	*Gunneracea*	Bulb and leaves
• *Geranium in Canum*	*Geranaiceae*	Leaves & stem

- *Helicrysum Odoratissimum* *Asteraceae* Leaves & roots
- *Hypoxishemerocallidea* *Hypoxidaceae* Corm & root
- *Rhoicissus digitata* *Vitaceae* Bulb
- *Sutherlandia Frutescesnce* *Fabaceae* Leaves

Olorunnisola et al (2011).

2.6 The Genus Syzygium

This is a genus of flowering plants. It belongs to the order *myrtales* and family, *myrtaceae*. It has a native range that extends from Africa through Southern Asia to pacific. Most species are ever green trees and shrubs. Several species are ornamental and some produce edible fruits. The genus has over1000 species. Many members have folkloric uses.

Syzygium aromaticum (L) (*Myrtaceace)* is used as remedy against 'weakness of the brain,' stroke and loss of memory (Tabernarmontanus, 1987). *Syzygium Pondoense, (Myrtaceae*), has many medicinal properties. *Eugeniin* extracted from bulbs has antiviral activity against *Herpes simplex*. Bark infusions of the plant are said to ease pain and cough (Mhlonishwa, 2002). *Syzygium jambos* have been used in Indian traditional medicine for the treatment of numerous ailments. The flowers are believed to reduce fever; the seeds were used for dysentery, diarrhoea and Catarrh. Other indications include diuresis, antihyperglycemia, asthma and bronchitis. Like *Syzygium aromatium* (clove), *Syzygium lineare, Syzygium cuminii* and S*yzygium travoncoricum, Syzygium jambos* have antibacterial and antifungal activities (Mohanty and Cock, 2012). *Syzygium jambolana* is used in traditional medicine for treatment of diabetes mellitus (www.liebertpub.com).

Syzygium aromaticum (*myrtaceae)* is also indicated for digestive tract relaxation, toothache, impotence, hiccups, nausea, vomiting and treatment of infestations by intestinal parasites and infections by bacteria and fungi. It has analgesic and antinflammatory effects.

Syzygium lineae was evaluated for antimicrobial activity; it is used as antibacterial agent (Veeramuthu et al, 2006). The bark, leaves, roots and fruits of *Syzygium malaceense* (*Myrtaceae*) are used medicinally in Malasiya, Cambodia and Brazil. Indications include cough, hypertension, diuresis, diabetes, sore throat, buccal ulcers, skin diseases, and dysentery. It is also used as emmenagogue and abortifacient. A decoction of the bark is used as antidiarrhoeal. The poisonous back has been reported to cause death .

A decoction of the *Syzygium guineense* (myrtaceae) leaves has been used traditionally in the treatment of wound ulcers and infections. The stem is boiled and the decoction drunk for treatment of hypertension.

2.6.1 *Syzygium guineense*. (Willd.) DC.

Syzygium guineense is a medium – sized or tall evergreen tree. It is a leafy forest tree of the order myrtales and *myrtaceae* family. It is found in many parts of Africa, wild and domesticated. Its synonym is *Calyptonthes guineensis*. Its local names are: English – Waterpear, waterberry, waterboom, snake bean tree, wood land water berry, mountain water berry, bi-coloured water berry and rose apple.

Swahili – Mzumbarau, msanbran, mzambararani, myzaurin, mzambrau mwitu, mzambarai, Ambaric – dokma Tigrigna – liham, Lugunda – kalungisanvu, mizuti, Ndebele – Umdoni, Shona – Mukule and Hausa – Malmo.

Fulfude – Asurahi

Yoruba – Adere, Igi-aro

The bark varies in subspecies. It is greyish – white or silver mottled and smooth in young trees. In older trees it turns rough flaky, creamy, light grey, dark brown or black. The bark scales in rectangular flakes and produces red, watery sap if cut. Branches are drooping; the stems are thick and angular.

The leaves narrow at both ends. They are ovate – elliptic with margins that are un-toothed. They measures 5 – 17cm in length and 1.3 – 7.5cm in width. They are glabrous, grey – green, tough, shiny and fragrant when crushed.

Flowers are 1.5cm in diameter, sessile, fragrant, creamy white borne in terminal panicles forming heads up to 10 x 10cm. Fruits are ovoid or ellipsoid drupes measuring 1.2 – 3.5cm x 1 – 2.5cm. They are whitish green when immature and turned shiny purplish – black and juicy when riped. They are also one seeded.



Plate I: ***Syzygium guineense*** **foliage**



Plate II: ***Syzygium guineense*** **bark**

2.6.2 Medicinal and therapeutic uses of *Syzygium guineense*

Ripe pleasant fruits are eaten; they are also used as remedy for dysentery. Liquid from pounded bark and roots mixed with water is used as purgative. Decoction of the bark has been reported to cause death. The species is highly valued in Uganda for treatment of malaria (Ssegawa and Kasenene, 2007). The stem is boiled and the decoction drunk for treatment of hypertension.

In Sango Bay Forest Reserve in Southern Uganda, Hallea rubrostipulata, Warbugia Ugandensis and *Syzygium guineense* have been used to treat malaria (http://www.mariajournal.com).

The seed of the plant is used to treat diabetes mellitus in Lagos State, Nigeria (Adebayo, 2009).

Herpes Zoster was generally treated with several plants including *Rhus natalensis*, *Anona senegalensia*, *Capparis tomentosa*, *Garcinia buchananii and Syzygium guineense* (Kisangau, *et al*, 2007; Chinsembu and Hedimbi, 2010).

2.6.3 Research work done on *Syzygium guineense*

The extract of *Syzygium guineense* has been reported to possess antibacterial activity against Shigella, D., E. coli and Salmonella and this justifies its use as antidiarrhoeal (Tsakala, 1997). Malele *et al*., (1997) have also reported the inhibitory effect of methanolic extract of *Syzygium guineense* intrinsic contractions of isolated rabbit ileum. The inhibition was dose related but non-linear. The bath concentrations were 0.5 – 2.0gm/m/. It also produced a sustained hypotension in anaesthesized rats

(Malele *et al* 1997). The molluscicidal effects of the ethanolic stem bark extract of *S. guineense* has been demonstrated by Oketch – Rabah and Dossaji (1998).

The antidiabetic effect of hydroalcoholic extract *Syzygium guineese* and *Ajuga remota* was evaluated on alloxan induced diabetic and normoglyceamic mice (Tenmesgen, 2009). The antidiarrhoeal effect of the aqueous and hydroalcoholic extracts of dried twigs of stem bark and fruit of *Syzygium guineense* was demonstrated *in vitro* on guinea pig ileum, and *in vivo* using intestinal transit and castor oil induced diarrhea in mice Nigatu, 2004)

2.7 Models of experimental hypertension

An animal model is a living, non-human animal used during the research and investigation of human disease, for the purpose of better understanding the disease process without the added risk of harming an actual human. The animal chosen will usually meet a determined taxonomic equivalency to humans, so as to react to disease or its treatment in a way that resembles human physiology as needed. Many drugs, treatments and cures for human diseases have been developed with the use of animal models [Kari *et al*., (2007), Chakraborty *et al*., (2009)].

There are three main types of animal models: Homologous, Isomorphic and Predictive. Homologous animals have the same causes, symptoms and treatment options as would humans who have the same disease. Isomorphic animals share the same symptoms and treatments, only. Predictive models are similar to a particular human disease in only a couple of aspects. However, these are useful in isolating and making predictions about mechanisms of a set of disease features. Animal models can also be more broadly classified into four categories: 1) experimental, 2) spontaneous,

3) negative, 4) orphan (Hughes and Lang, 1978). Experimental models are most common and refer to models of disease that resemble human conditions in phenotype or response to treatment but are induced artificially in the laboratory. Animal models of diseases can be spontaneous, naturally occurring in animals or genetically engineered and cloned. They can also be induced artificially in the laboratory by chemical, physical or biological means,

Relevant models that mimic human cardiovascular disease may offer information on the cause and progression of the disease status as well as therapeutic intervension (Badyal *et al*., 2003). The most widely used models of experimental hypertension include the following (Dornas and Silva, 2011):

2.7.1 Genetic hypertension

2.7.1.1 Spontaneously hypertensive rats

Spontaneously hypertensive rats (SHRs) were inbred from Wister and their Wister-Kyoto (WKY) inbred non-hypertensive controls. They develop hypertension without physiological, pharmacological or surgical intervention at about 4-6 weeks (Zicha and Kunes, 1999). Environmental factors affect the development of the hypertension and the pathophysiology of this model is similar to that of essential hypertension in humans (Trippodo and Frohlic, 1981).

2.7.1.2 Dahl Salt-sensitive rats

This is a genetic model of hypertension with the feature of salt sensitivity. They are derived from Sprague- Dawley stock on the basis of developing hypertension with a high common salt diet (NaCl). Two strains of the animals exist on the basis of

pressure levels associated with high salt diet: Dahl sensitive rats (DS) and Dahl salt-resistant ores (DR). The DS animals develop systemic arterial hypertension following consumption of a high-salt diet, while the DR animals can maintain blood pressure within normal limits even with the same diet. The DS are prone to hypertensive nephropathy identified as glomerular lesions characterized by mesengial proliferation, matrix accumulation, glomerulosclerosis and endothelial dysfunction (Nagase *et al*., 2006).

2.7.1.3 Transgenic hypertension models

These can be generated by over-experession of a specific gene. The role of a specific gene in the pathogenesis of hypertension can be studied by this model. Mullins et al. (1990), developed TGR (mREN2)27 transgenic rat, a representative of this type of hypertension. Transgenic hypertension models (TGR) is not a representative of human hypertension; it allows in vivo analysis of the consequences of severe, monogenetic activation of R.A.A system and allows identification of the types of hypertensive damage that can be expected from activated R.A.A system (Dornas and Silva, 2011).

2.7.2 Renal hypertension

2.7.2.1 Renovascular hypertension

This involves the induction of elevated blood pressure through the technique of Goldblatt *et al*. (1934). This consists of constricting one or both renal arteries of animal models. The renal induced hypertension models include:

- Two kidney one-clip hypertension (1K1C; one renal artery is constricted and the contralateral kidney is left intact).
- One kidney one-clip hypertension (1K 1C; one renal artery is constricted and the contralateral kidney is removed).
- Two kidney two-clip hypertension (2K2C; constriction of aorta or both renal arteries).

In non-nephrectomised rats, constriction of the renal artery of the remaining kidney produces 1K1C Goldblatt hypertension. This model of hypertension is considered to be sodium-fluid volume-dependent and is an ideal model for studying the role of volume expansion in hypertension because water excretion does not occur due to the absence of the other normal kidney and hence fluid is retained.

2.7.3 Endocrine hypertension

2.7.3.1 Salt and mineralocorticoids

Sodium and water retension is caused by mineralocorticoids in the body until escape duresis occurs due to increased pressure on the kidneys and suppression of rennin secretion. The effects of this model on the kidneys are similar to hyper-aldosteronism in humans. A high salt diet and 11-desoxycorticosterone acetate (DOCA) cause increase in blood pressure within three weeks in isolated perfused cortical collecting ducts, and can cause a 30-fold increase in sodium absorption (Gartwitz and Jones, 1982).

DOCA salt models are not suited for long-term studies in chronic stable disease, because they progress quickly to severe hypertension and hypertrophy. It is,

moreover, not a very realistic model for many human hypertensive patients (Doggreell and Brown, 1998).

2.7.3.2 Psychosocial and environment- induced hypertension

Exposure to stressful situations can result in elevated blood pressure resulting in a state of persistent hypertension (Smith and Hutchins, 1979). Resetting of baroreceptor reflexes and structural auto-regulation in the peripheral vasculature may operate to sustain blood pressure at a high level once it is raised. An important risk factor for the development of cardiovascular disease in a modern world is chronic social stress, whose deleterious effects depend on the critical period of exposure, duration and type. These factors may alter functions of the basic regulatory stress response components in the hypothalamic pituitary-adrenal axis, sympatho-adrenal medullar system, renin-angiotensin- aldosterone system and sympathetic nervous system (Zimmerman and Frohlich, 1990; McCarty and Gold, 1996; Esch, *et al*., 2000).

2.7.4 Neurogenic Hypertension

Neurogenic hypertension can be defined as a permanent increase in blood pressure resulting from a primary neural change. Denervation of sinoaortic baroreceptors (SAD) is the neurogenic model of hypertension most often used. In rats, SAD leads to marked and sustained increase in BP. An increase in MAP stimulates baroreceptors and causes a reciprocal reduction in sympathetic outflow to resistance vessels and heart so as to restore MAP to the normal level and, therefore, cause neurogenic hypertension (Thrasher, 2002).

2.7.5 Hypertension by chronic inhibition of nitric oxide

Nitric oxide (NO) regulates systemic vascular resistance by exerting a tonic vasodilator effect (Török, 2008). Chronic administration of Nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase, promoted a persistent hypertension associated with renal injury, characterized by glomerulosclerosis, glomerular ischemia and interstitial infiltration of the kidney (Baylis, *et al*., 1992; Ribero *et al*., 1992). This hypertension is associated with intense peripheral vasoconstriction and consequent increase in peripheral vascular resistance. Biancardi, *et al*. (2007), proposed sympatho-excitatory action of central origin in the initiation and maintenance of hypertension as a result of vasoconstriction in response to L-NAME.

2.7.6 Hypertension induced by Angiotensin II (ANGII)

Angiotensin II is a potent vasoconstrictor of peripheral vasculature and induces growth of smooth muscle cells of blood vessels in the heart (Itoh *et al*., 1993). ANG II infusion causes increase in systemic BP. It leads to a slowly developing hypertension produces baroreflex-mediated sympatho-inhibition corresponding to the increased BP, but with added challenge of increased dietary salt (McBryde, *et al*., 2007).

2.7.7 Dietetically-induced Hypertension

Long term exposure to a special diet (high salt, fat and sugar) results in dietary hypertension in some animals or humans (Navarro-Cid, *et al*., 1995; Kang, *et al*., 2004; Giani, *et al*., 2009). High salt intake decreases both plasma levels and urinary excretion of nitrates (Fujiwara et al., 2000) and increased superoxide production in both vasculature and kidney. Rats maintained on high fat or high sugar diet

experience oxidative stress and inactivation of NO; this, enhanced by generation of reactive oxygen species (ROS) contribute to the development of hypertension (Roberts, *et al*., 2000). The reduction in NO availability in the high-fat and high sugar diet-fed animals was associated with marked salt sensitivity evidenced by significant rise in blood pressure on high salt diet (Roberts *et al*., 2003).

High fructose consumption by animals produces a model of the metabolic syndrome with hypertension, hyperlipidaemia and insulin resistance, and this accelerates progression of chronic kidney disease (Gersch *et al*., 2007).

CHAPTER 3.0 MATERIALS AND METHODS

3.1 Plant material

The fresh stem bark *of Syzygium guineense* was collected from the evergreen thick forest of Kabba, Kwara State. The plant was collected and identified by Mallam Mu'azzam Ibrahim an Ethnobotanist with the Department of Medicinal Plant Research and Traditional Medicine (MPR&TM) of National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja. The plant was identified and authenticated by Dr. Jemilat Ibrahim of the same institution, where a voucher sample (NIPRD/H/6595) was prepared and deposited for future references.

3.2 Chemicals and Drugs

(a) Methanol (Hopkins and Williams England), ethyl acetate (Hopkins and Williams England), n-Hexane (Hopkins and Williams England).

(b) Acetylcholine (ACH) (Sigma chemical Co., USA), Adrenaline (ADR) (Sigma chemical Co., USA), Atropine (Halewood chemical, England. B/N 69524), Phenoxybenzamine, Heparin Sodium (Biological E.LTD BATCH No: 340), Thiopentone Sodium (LD international, BATCH No: N1030).

(c) Oxytocin (Pemason Pharmaceutical LTD BATCH No: 120601), Phentolamine

Salbutamol, Propranolol (BATCH No: PB 766), Stilboestrol (M2B BATCH No: PL0012/4023) and Methylated Spirit.

(d) Sodium chloride, Potassium chloride, Sodium dihydrogen phosphate, D-Glucose, Sodium hydrogen carbonate, Calcium chloride, $MgCl_2$ $6H_2O$, Ca Cl_3 (10%), Ca Cl_3 (Molar), $CaCl_3$ (10%), Formaldehyde, Chloroform, Carbogen [95% O_2 + 5% CO_2], Tween 80, Deionised water, -n-Butanol, Ethylacetate (BDH, Germany).

The drug solutions were usually prepared fresh for each day's experiment to maintain stability of the drugs used. The solutions were kept in air tight, amber coloured bottles and stored in the refrigerator until ready for use.

3.2.1 Routes of drug administration

The methanolic stem bark extract of *syzygium guineense* and the most active fraction F3 were administered orally throughout the studies except for the intraperitonial administration in acute toxicity studies and also in the *in vitro* procedures where the drugs were applied directly to the tissues in the organ bath.

3.3 Animals

Wistar albino rats of both sexes (180-200g) were obtained from Animal Facility Centre of National Institute for Pharmaceutical Research and Development, Abuja, Nigeria while Cats (1.0 – 1.8kg), Rabbits (0.8 – 1.2kg) and Guinea pigs (300-350g) were obtained from the animal house of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The rats were fed with standard rodent diet (Top Feeds Limited Ibadan), given water *ad libitum* and maintained under laboratory conditions of temperature 22 ± 1°C, relative humidity 14 ± 1% determined by electrical impedance hygrometer, and 12 h light and 12 h dark cycle. All experiments

were performed according to the "Principles of Laboratory Animal Care" (NIH Publication No. 85; rev. 1985).

3.4 Extraction

The stem bark of *Syzygium guineense* was air-dried at room temperature for 2 weeks and then crushed with pestle and mortar. About 200 g of the dry plant material was macerated in 70% methanol for 48 hours. The resulting mixture was filtered using muslin cloth followed by Whatman filter paper (No. 1). The aliquots obtained was dried on water bath at 100 ^{0} C and stored at -4°C until required for use. The proportion of the yield obtained was calculated as: (The quantity of extract/ Quantity of plant material) X 100%.

3.5 Acute toxicity study of crude methanolic stem bark extract of *Syzygium guineense* in rat

The oral acute toxicity of stem bark extract of *Syzygium guineense* in rats was evaluated using modified Lorke's method (1983). The study was carried out in two phases. In the first phase, nine rats were randomized by weight into three groups of three rats each and were given 10, 100 and 1000 mg extract/kg body weight orally (via a cannula), respectively. The rats were observed for signs of adverse effects and pattern of mortality for the first four critical hours and thereafter daily for 7 days before the second phase. In the second phase of the study, another set of nine rats were randomized into three groups of three rats each and given 1600, 2900 and 5000 mg extract/kg body weight, respectively based on the result of the first phase. The experimental rats were kept under same conditions and observed for toxic signs and

mortality for the first critical four hours and thereafter daily for 7 days. A similar experiment was carried out in mice and in rat using intraperitoneal route of drug administration. The number of deaths in each group within 24h were recorded and the median lethal dose (LD_{50}) values were calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

LD_{50} = $\sqrt{MLD \times MTD}$

Where LD_{50} = median lethal dose

MLD = minimum lethal dose

MT = maximum tolerated dose

3.6 Anaesthetised Normotensive Cat Preparations

The cat was anaesthetised with phenobarbitone sodium at a dose of 40 mg/kg intraperitoneally. The trachea was exposed and cannulated for free flow of air. The femoral vein was exposed and isolated completely from all adjacent tissues and nerves, cannulated and tied. The carotid artery was also isolated and cannulated with a 1 mm diameter polyethylene tube which was connected to a pressure transducer (Statham Pressure Transducer P23 ID). The transducer was connected to a microdynamometer (7050) set at the speed of 95 mm/min with sensitivity of 2.0 μvolt. Heparin was then injected through the cannulated artery and vein to avoid formation of clots.

3.6.1 Hypotensive effect of crude methanolic stem bark extract of *Syzygium guineense* on the blood pressure of anaesthetised normotensive cat

Graded concentrations (5, 10, 20 and 40 mg/ml) of methanolic stem bark extract of *Syzygium guineense* were injected through the cannula into femoral vein dose dependently.

A solution of normal saline was used to flush the drug solution into the blood stream. The effects of the drug on the cat blood pressure was then recorded on tracing paper as determined by the microdynamometer. Blood pressure was calculated manually from the record (tracings) as: 1.0cm hight of contraction or relaxation = 10mmHg for systolic or diastolic blood pressure respectively.

3.6.2 Effects of standard drugs (adrenaline & acetylcholine) and methanolic stem bark extract of *Syzygium guineense* on the blood pressure of anaesthetised normotensive cat

The effects of methanolic stem bark extract of *Syzygium guineense* on the BP of anaesthetised normotensive cat were compared to those of Adrenaline and Acetylcholine.

Effects of intravenous injection of increasing doses of Adrenaline (1 -4µg/kg) and *Syzygium guineense* (5 - 20 mg/kg) washed in with 0.2ml normal saline were observed on the anaesthetised normotensive cat and recorded.

Graded doses (0.1 -0.8µg/kg) of acetylcholine were also administered intravenously and washed in with 0.2ml normal saline on the anaesthetised normotensive cat and

their effects on cat's blood pressure determined by microdynamometer and recorded on tracing paper.

3.6.3 Effect of interaction between atropine and acetylcholine and interaction between atropine and methanolic stem bark extract *of Syzygium guineense* and graded doses of methanolic stem bark extract of *syzygium guineense* on the blood pressure of anaesthetised normotensive cat

The effects of acetylcholine (0.1µg/kg), methanolic stem bark extract of *Syzygium guineense* (6.0 mg/kg) and interactions between atropine (2.5mg/kg) and acetylcholine (0.1µg/kg), atropine (2.5mg/kg) and methanolic stem bark extract of *Syzygium guineense* (6.0 mg/kg) and graded doses of the, methanolic stem bark extract of *Syzygium guineense* (1.0 – 4.0 mg/kg) on systolic and diastolic blood pressure of anaesthetised normotensive cat were determined.

3.6.4 Effect of interaction between methanolic stem bark extract of *Syzygium guineense* and adrenaline on the blood pressure of anaesthetised normotensive cat

Responses to interaction between doses of methanolic stem bark extract of *Syzygium guineense* (4 mg/kg-16mg/kg) and adrenaline (1µg/kg) on the blood pressure of anaesthetised normotensive cat were obtained and recorded.

3.7 Bioassy-guided Fractionation of crude extract of SG

Forty grams of methanolic stem bark extract of *Syzygium guineense* was subjected to column chromatography using Octadecylsilyl (ODS) silica gel stationary phase 100g.

Gradient elution was performed with 500 ml of each mobile phase mixture in series. The mobile phase consisted of Hexane: Ethyl acetate: Methanol, starting with hexane (100%) and 10% increment in Ethylacetate; this was followed by elution with Ethylacetate (80%) and 20% increment in methanol. Following fractionation of the crude extract, each of the *Syzygium guineense* fraction (SGF): SGF1, SGF2, SGF3, SGF4 and SGF5 were obtained.

3.7.1 Effects of SGF1, SGF2, SGF3, SGF4 and SGF5 on cat blood pressure

The five fractions (SGF1, SGF2, SGF3, SGF4 and SGF5) were evaluated separately for hypotensive activity in anaesthetized normotensive cat.

Graded doses (4-32 mg/kg) of each fraction were administered, the responses produced were recorded and the systolic and diastolic blood pressures calculated.

3.8 Isolated Guinea Pig Atria

A guinea- pig was sacrificed by a blow on the head and cutting the throat; the chest was opened and the heart removed as quickly as possible and placed in Ringer- Locke solution at room temperature. All other tissue was cut away until nothing was left except the atria. Threads were tied, one to the tip of each atria and stream of pure oxygen was blown. One thread was attached to a fixed pin in the bath and the other to a light- spring- loaded lever writing on a force- displacement transducer (Ugo basile) connected to microdynamometer (7050) set at sensitivity of 6.0 µvolt and speed of 95mm/min. The volume of the organ bath was 25ml.

6 minute cycle was adopted as follows:

0 min. The microdynamometer was started (thus recording the control rate and amplitude).

1 min. Drug was added.

3 min. The microdynamometer was stopped and the preparation washed.

6min. The microdynamometer was started for another cycle.

Adequate time with extra washing - were sometimes allowed between each dose to ensure recovery so that the rate and amplitude of the beats return to their original level.

3.8.1 Effect of methanolic stem bark extract of *Syzygium guineense* on guinea pig atria

Graded concentrations ($1x10^{-5}$ - $4x10^{-3}$ g/ml) of methanolic stem bark extract of *Syzygium guineense* were added to the guinea pig atria in the organ bath. The responses were determined by micodynamometer and recorded on tracing paper.

3.8.2 Effect of adrenaline alone and its interaction with methanolic stem bark extract of *Syzygium guineense* on guinea pig atria

The responses of guinea pig atria to graded concentrations ($1x10^{-5}$ – $4x10^{-5}$ g/ml) of methanolic stem bark extract of *Syzygium guineense* was determined. This was followed after series of washing and 5.0 minutes recovery by three different additions of ($1.5x10^{-9}$ g/ml) of adrenaline, added after another series of washing and the responses obtained were recorded. Responses to interactions between different concentrations ($1x10^{-3}$ – $4\ x10^{-3}$ g/ml) of methanolic stem bark extract of *Syzygium*

guineense and adrenaline after another series of washing were obtained were recorded.

3.8.3 Effect of fraction SGF3 on height of contraction in guinea pig atria

Graded concentrations ($4x10^{-3}$ -$32x10^{-3}$ g/ml) of fraction SGF3 were added to guinea pig atria in the organ bath. The responses obtained recorded.

3.8.4 Effect of SGF3 on height of contraction in atropinized guinea pig atria

Atropine ($0.125x10^{-3}$ g/ml) was interacted with graded concentrations ($4x10^{-3}$ -$32x10^{-3}$ g/ml) of fraction SGF3 on the height of contraction in guinea pig atria and the responses obtained were recorded.

3.9 Isolated Perfused Rabbit Heart

The isolated rabbit heart was set up based on the method of Langendorff -1895(Bell *et al*.,2011). A rabbit was sacrificed and the heart, with at least 1 cm of aorta attached, was removed as quickly as possible and placed in a dish of Ringer- Locke's solution at room temperature. Heparin, 1000 units, was injected into the ear vein before sacrificing the animal to prevent clot formation inside the heart. The heart was squeezed firmly several times when first placed in the Ringer- Locke solution to remove as much blood as possible from it. The aorta was cut just below the point where it divides and the heart was transferred to the perfusion apparatus where the aorta was tied unto the glass cannula. Care was taken to see that air bubbles did not enter the aorta and bubbles which have formed in the cannula were removed. The

perfusion fluid was oxygenated Ringer- Locke's solution, which was applied at a constant pressure from the reservoir and warmed to 37^{o}C by the water circulated from the thermostat. The pressure of the perfusion fluid closed the aortic valve, so the fluid passed only through the coronary vessels and escaped from the inferior vena cava. It was collected by a funnel placed beneath the lower chamber and the rate of flow was measured by a volume recorder.

Threads were attached to the ventricle by a hook. The thread passed over pulleys to connect to Ugo basile transducer which connected to microdynamometer (7050) set at sensitivity of 2.0 µvolt and speed of 95 mm/min. Readings of the rate of beating and of the coronary flow was taken over a period of 30 seconds. Drugs were added to the preparations by injection through the rubber cap into the perfusion fluid. Gas that collected below this cap was removed with a syringe from time to time, to prevent any entering the aorta. It took about 15 seconds before the drug reached the heart and, in most instances, the effects passed off in a few minutes as the drugs were washed through. The microdynanometer ran continuously and readings of the rate of beating and of the flow were taken about once every two minutes. Further doses were not given until the preparation had recovered and the rates have settled down steadily at new control levels.

3.9.1 Effect of methanolic stem bark extract of *Syzygium guineense* on isolated perfused rabbit heart

Graded concentrations (1-4mg/ml) of methanolic stem bark extract of *Syzygium guineense* were intermittently added to the isolated perfused rabbit heart and the effect on the myocardial contractions observed and recorded.

3.9.2 Effect of Adrenaline alone and interaction between adrenaline and methanolic stem bark extract of *Syzygium guineense* on isolated perfused rabbit heart

Effect of addition of graded concentration (1-4µg/ml) of adrenaline on isolated perfused rabbit heart and the effect of interaction of adrenaline (1µg/ml) and (1-4 mg/ml) of methanolic stem bark extract of *Syzygium guineense* were observed and recorded.

3.9.3 Effect of SGF3 fraction on isolated perfused rabbit heart

Graded concentrations (1-4µg/ml) of fraction SGF3 were added to the isolated perfused rabbit heart at intermittent intervals at steady control levels. The effects on the contraction of the heart were observed and recorded.

3.9.4 Effect of interaction of fraction SGF3 and adrenaline on isolated perfused rabbit heart

Effects of interaction of graded concentrations (1-4µg/ml) of fraction SGF3 and1.5ng adrenaline on the contraction of isolated perfused rabbit heart were observed and recorded.

3.10 Rabbit Ileum Preparation

Ileum was obtained from a freshly sacrificed rabbit. The intestine was cut at a point 5 to 10 cm below the stomach and a length from there downwards towards the caecum. It did not require thorough washing, because it was wider than guinea- pig intestine

and its spontaneous activity caused it to clear itself. The 2-3 cm length pieces of the ileum in carbogen aerated Tyroid's solution were tied at both ends with a nylon thread. One end of each was tied to the base of an organ bath and the other to Ugo basile transducer which was connected to microdynamometer (7050) set at sensitivity of 3.0 µvolt and speed of 24 mm/min. Each tissue bathed in carbogen aerated Tyroide's solution was allowed 45 minutes to stabilize before the initial challenge with a standard drug or test drug. The bath solution was changed after every 15 minutes during the stabilisation period.

3.10.1 Effect of acetylcholine and methanolic stem bark extract of *Syzygium guineense* on smooth muscle rabbit ileum

The effect of graded organ bath concentrations ($33.3x10^{-9}$ g/ml – $333.3x10^{-7}$ g/ml) of Acetylcholine, and after several washings and enough rest, those of methanolic stem bark extract of *Syzygium guineense* ($33.3 x10^{-7}$ g/ml - $333 x10^{-4}$ g/ml) on the smooth muscles of rabbit ileum was observed and recorded.

3.10.2 Effect of methanolic stem bark extract of *Syzygium guineense* and adrenaline on smooth muscles rabbit ileum

The effect of graded organ bath concentration ($33.3 x10^{-7}$ g/ml - $333 x10^{-4}$ g/ml) of methanolic stem bark extract of *Syzygium guineense*) on the smooth muscles of rabbit ileum was observed and recorded. This was followed by immediate challenge with adrenaline alone (16.67×10^{-6} g/ml). Graded bath concentrations (16.67×10^{-9} - 133.3×10^{-9} g/ml) of adrenaline interacted with phenoxybenzamine at bath concentration of 83.3×10^{-6} g/ml. These effects were observed and recorded.

3.10.3 Effect of acetylcholine and fraction SGF3 on smooth muscles rabbit ileum

The effects of graded organ bath concentrations ($33.3x10^{-9}$g/ml – $333.3x10^{-7}$g/ml) of Acetylcholine on the smooth muscles of rabbit ileum were observed and recorded. After series of washings and enough rest, the smooth muscle of the ileum was challenged with graded organ bath concentrations (33.3×10^{-7} g/ml - 333×10^{-4} g/ml) of SGF3; the effects were observed and recorded.

3.11 The Rat Uterus Preparation

The sensitivity of the rat uterus depends on the condition. It was made oestrous by injecting subcutaneously, 24hours beforehand, a dose of stilboestrol, 0.1mg/kg., i.e 0.6 -0.75 ml/ rat of a 20 µg/ml. (roughly 10^{-4}M) solution in ethanol.

The rat was sacrificed and the abdomen opened. The two horns of the uterus were dissected out and transferred to a dish containing De-Jalon's solution, which was similar to Locke's solution but contains a low concentration of calcium ions. The two horns were separated and freed from fat, and each was cut open longitudinally, and so obtains four pieces from one animal. A thread was attached at each end of each piece and the preparation was mounted in De Jalon's solution which was aerated with oxygen (95%) and carbon dioxide (5%). One thread was attached to a fixed pin and the other to a lever (Ugo basile transducer) fitted with a frontal- writing point (microdynamometer 7050) set at sensitivity of 2.0µvolt and speed of 24 mm/min. The load on the lever was about 0.5g and the magnification about 5- fold. The bath was 25 ml in volume. The temperature was usually between 30- 32 ^{0}C. The preparation took 15- 30 minutes to settle down before regular responses were obtained. Cycle used was as follows:

0 min. Microdynamometer was started.

1min. Drug was added.

1.5 min. The microdynamometer was stopped and the preparation washed.

3 min. The microdynamometer was started again.

3.11.1 Effect of oxytocin, methanolic stem bark extract of *Syzygium guineense*, salbutamol, interaction between salbutamol and oxytocin, and between methanolic stem bark extract of *Syzygium guineense* and oxytocin on rat uterus

The effects of graded concentrations (3.3×10^{-4} – 26.7×10^{-4} iu/ml) of Oxytocin and (3.3×10^{-4} – 26.7×10^{-4} g/ml) of methanolic stem bark extract of *Syzygium guineense* on rat uterus were observed and recorded. The effect of 1.3×10^{-6} g/ml bath concentration of Salbutamol, and its interaction with Oxytocin at bath concentration of 3.3×10^{-4} iu were observed and recorded. After series of washing and enough recovery, methanolic stem bark extract of *Syzygium guineense* was interacted with Oxytocin at respective bath concentrations of 26.7×10^{-4} g/ml and 3.3×10^{-4} iu/ml and the effects observed and recorded.

3.12 Phytochemical Screening

The dark brown crude methanolic stem bark extract of *Syzygium guineense* and its fraction F3 were screened phytochemically for the presence of secondary metabolites using standard conventional protocols (Sofowora, 1993; Tiwari, *et al*., 2001).

a. *Detection of carbohydrates*

0.1 g of the extract and fraction F3 were separately dissolved in 5ml of distilled water and filtered. The filtrates were used for:

i. Molisch's test: Two drops of alcoholic α-naphthol solution was added to 1.0 ml of the filtrates in a test tube and the formation of violet ring at the junction indicates the presence of carbohydrate.

ii. Fehling's test: Filtrates were hydrolysed with diluted HCl, neutralized with alkali and heated with Fehling's A and B solution; formation of brick red precipitate indicates the presence of reducing sugar.

b. *Detection of alkaloids.*

0.1 g of the extract and fraction F3 were separately dissolved in 5 ml dilute hydrochloric acid and filtered. The filtrates were used for the following tests:

i. Mayer's test: The filtrates were treated with Mayer's reagent (potassium mercuric iodide). Presence of yellow coloured precipitate indicates the presence of alkaloids.

ii. Dragendorff's test: Dragendroff's reagent (solution of Potassium Bismuth Iodine was added to 2.0 ml of the filtrates and formation of red precipitate indicated the presence of alkaloids.

iii. Wagner's test: 2.0 ml of the filtrates were treated with few drops of Wagner's reagent (iodine in potassium iodide). Formation of dense brown/reddish precipitate indicated the presence of alkaloids.

c. *Detection of flavonoids*

i. Alkaline Reagent test: 0.1 g of the extract and fraction F3 were separately dissolved in 5.0 ml distilled water and filtered. The filtrates were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour which becomes colourless on addition of dilute acid indicated the presence of flavonoids.

ii. Lead acetate test: The filtrates were also treated with few drops of lead acetate solution. Formation of yellow coloured precipitate indicated the presence of flavonoids.

d. *Detection of Saponins*

i. Froth test: The extract and the fraction F3 were dissolved with 20ml of distilled water and shaken for 15minutes in a graduated cylinder. Presence of saponins will be indicated by the formation of 1cm of foam.

e. *Detection of Tannins.*

i. Gelatin test: 1% gelatin solution containing sodium chloride was added to the filtered solutions of the extract and the fraction F3. Formation of white precipitate indicated the presence of tannin.

ii. Ferric chloride test: Filtered solutions of the extract and fraction F3 were treated with few drops of 0.1% ferric chloride solution. Formation of blue/black colour changes indicated the presence of tannins.

f. *Detection of Phytosteroids/ Terpenoids*

i. Liberman Burchard's test: 0.05 g of the extract and fraction F3 were each treated with 5.0 ml chloroform and filtered. To the filtrates were added few drops of acetic anhydride, boiled and cooled. A green colour is seen on standing. Conc. Sulphuric acid was added and the formation of brown or violet ring at the junction indicated the presence of phytosterols or Terpenoids.

g. *Detection for Anthranol Glycosides*

Borntrager's test: 0.05 g of the extract and fraction F3 were treated with 10% Ferric chloride solution and immersed in boiling water for 5 minutes. The mixture was extracted with equal volume of benzene after cooling. The benzene layers was separated and treated with ammonia solution. The formation of rose-pink colour indicated the presence of anthranol glycosides.

h. *Detection of Cardiac glycosides.*

Keller-Killani test: five milliliter of the extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. 1ml of concentrated sulphuric acid was added. A brown ring of the interface indicates deoxysugar, a characteristic of cardenolides. The appearance of a violet ring below the brown ring indicated the presence of cardiac glycosides.

3.13 High Performance Liquid Chromatography Analysis for Fingerprint

Chromatographic fingerprint of a herbal medicine is a chromatographic pattern of the extract that represents pharmaceutically active and chemically characteristic components of the herbal medicine which can be used to accurately authenticate and identify a herbal medicine (Petil and Shettigar, 2010). High performance liquid chromatograph (HPLC) fingerprinting includes recording of chromatograms, retention time of individual peaks and absorption spectra with different mobile phases (Doughari, 2010).

High performance liquid chromatography analysis was performed on the methanolic stem bark extract of *Syzygium guineenes* in the Medicinal Chemistry and Quality Control (MCQC) Department of NIPRD where the HPLC is resident using Bienvenu *et al.* (2002) method. The chromatographic system includes Shimadzu HPLC system consisting of Ultra-Fast LC-20AB equipped with SIL -20AC auto-sampler; DGU-20A3 degasser; SPD-M20A UV-diode array detector; column oven CTO -20AC, system controller CBM-20Alite and Windows LCsolution software (Shimadzu Corporation, Kyoto Japan); column, VP-ODS 5µm and dimensions (150 x 4.6 mm). The chromatographic conditions included mobile phase: solvent A: 0.2% v/v formic acid; solvent B: acetonitrile; mode: isocratic; flow rate 0.6 ml/min; injection volume 10 µl of 100 mg/ml solution of extract in methanol; detection UV 254 nm. The HPLC operating conditions were programmed to give solvent B: 20%. Column oven temperature was 40^0C. The total run time was 15 minutes.

3.14 Tyrode's Solution Preparation

The physiological solution was prepared with the different reagents mixed and stirred together the salts were weighed and dissolved in 1000ml of deionised water and poured into a 5 litre conical flask after which the volume was made to 5 litres with deionised water (Appendix I)

3.15 Preparation of De-Jalon' S Solution

Five litres of De-Jalon's solution was prepared using appropriate amounts of various salts (Appendix II).

Four (4) litres of deionised water was first obtained in a 5 litre conical flask. The solid salts were carefully weighed on the balance in the laboratory and dissolved in a beaker using small quantity of deionised water and poured into 5 litres conical flask containing deionised water. The salts in solution were measured using measuring cylinder and then poured into a conical flask. The content of the 5 litres conical flask was then made up to the 5 litres mark with deionised water.

3.16 Preparation of Ringer- Lock Solution

The solution was prepared using the following reagents with the specified amount/volume. The volume was made up to 5 litres in the conical flask with deionised water (Appendix III).

3.17 Chronic Toxicity Study

Chronic toxicity study was carried out in accordance with WHO (1992) and OECD 407 and 408 (1998) guidelines. Ninety six rats separated into 48 males and 48 females were randomized by weight into four groups of 12 rats each. The first group served as control and received 10 ml of normal saline/kg body weight orally while the rats in groups 2, 3 and 4 received 250, 500 and 1000 mg of the extract/kg body weight orally respectively daily for 90 days. The rats were observed daily before, during and after treatment for physical signs of clinical intoxication and mortality.

3.17.1 Feed intake

The amounts of feed consumed by rats in each group were measured daily as the difference between the quantity of feed supplied and the amount remaining after 24 hours respectively.

Daily feed intake (DFI) was expressed as amount of feed consumed by the rats in a group per 100 g body weight.

$$\text{DFI/100g body weight} = \frac{\text{Daily Feed Intake (g)}}{\text{Body weight per group}} \times 100\text{g body weight}$$

3.17.2 Water intake

Daily water intake (DWI) was also expressed as the amount of water taken by the rats in a group per 100g body weight.

$$\text{DWI/100g body weight} = \frac{\text{Daily Water intake (g)}}{\text{Body Weight per group}} \times 100\text{ g Body weight}$$

3.17.3 Body weight changes

Rats in all the groups were weighed twice every week during the period of treatment and on the day of sacrifice.

3.17.4 Haematology and serum biochemistry

On the 43rd and 91st day of the experiment, six rats from each treatment groups was euthanized with diethyl ether and blood samples were collected by cardiac puncture after opening up the rats surgically. One portion was collected into K^+ EDTA bottles for estimation of haematological parameters {Packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), platelets, white blood cell count (WBC) and differentials, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)} using an automated haematological machine (Cell-Dyn^{TM} Abbot, US). Another portion was dispensed into plain bottles, allowed to clot and centrifuged at 3500 rpm for 10 minutes. The sera were separated, stored at -4^{o}C and used for evaluation of biochemical parameters {alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, total and conjugated bilirubin, urea and creatinine levels} using commercial kits obtained from Randox Laboratories, UK.

3.17.5 Relative organ-body weight ratio

Different organs namely the brain, heart, spleen, liver, lungs, kidneys,brain, intestine and testis or uterus were removed from each rat, weighed (absolute organ weight) and observed macroscopically. The relative organ weight (ROW) was calculated as follows:

$$ROW = \frac{\text{Absolute Organ Weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

3.17.6 Histopathology

At the end of 42 and 90 days, six rats per group were removed randomly from each of groups 1 to 4. Each rat was then weighed, euthanized and gross pathologic examinations were conducted. On each of the posted rat selected organs (brain, heart, spleen, liver, lungs, kidneys, brain, intestine and testis or uterus) were removed, weighed and preserved in 10% formal saline for histopathologic analysis. Tissue slices of 3 to 4 cm thick were cut and kept in the automatic tissue processor where they were fixed in 10% formalin-saline solutions for 2 hours. They were then dehydrated for 2 hours. They were then dehydrated for 2 hours in each of the following ascending grades of alcohol: 85%, 90%, and 100% v/v. The dehydrated tissues were then cleared in toluene for 2 hours, after which the tissue slices were embedded in paraffin wax and left to cool. The blocks were trimmed on the microtome at 5 microns. The ribbon of sections was dehydrated in xylene and rehydrated in the following descending grades of alcohol 100%, 90%, and 70% v/v. They were then stained in haematoxylin for about 5 minutes, differentiated in 1% acid alcohol, blued in scotch's tap water and stained in eosin for 3 minutes. They were later rinsed, dehydrated in ascending grades of alcohol: 70%, 90% and 100%v/v; finally they were cleared in xylene and mounted in a pox. The slides were then examined microscopically for pathological lesions. The lesions observed were assessed for the following: mucosal atrophy, the presence of inflammatory cells in the wall, eosinophils, lymphocytes and plasma cells. These were graded according to mild

(+), moderate (++) or severe (+++). Photomicrographs of representative lesions were taken at various magnifications.

3.18 Statistical Analysis

Graph pad prism version 5.02 was used to analyze data obtained and these were expressed as mean $\pm$ standard error of mean.The differences between means were compared using One way analysis of variance (ANOVA) followed by Dunnet's post hoc test. $P \leq 0.05$ were considered significant.

CHAPTER 4.0 RESULTS

4.1 Yield of Extracts

4.1.1 The yield of crude extract

The yield of *Syzygium guineense* methanolic stem bark extract was 35.7% w/w.

4.2 Phytochemical Screening

4.2.1 Phytochemical screening of the crude extract

Glycosides, Carbohydrates, Alkaloids, Saponins, Tannins, Flavonoids, Steroids and Triterapines, Cardiac Glycosides and Polyphenols were present in crude extract of *Syzygium guineense* stem bark (Table 4.1).

4.2.2 Phytochemical screening of SGF3

Carbohydrates, flavonoids, glycosides, cardiac glycosides, phenols and terpenoids were present in SGF3 (Table 4.1).

4.2.3 HPLC analysis

The HPLC spectrum of the methanol stem bark extract of *Syzygium guineense* revealed the presence of three major peaks as shown (Fig.4.1). The first peak had retention time of 3.2 minutes. The second peak eluted at 3.67 minutes while the third eluted at 3.9 minutes respectively. The analytical chromatogram indicated the purity of the crude extract free from contaminants.

Table 4.1 Phytochemical screening of methanolic stem bark extract of *Syzygium guineense* and SGF3

Secondary metabolites	Results	
	Crude extract	**SGF3**
Carbohydrates	+	+
Alkaloids	+	-
Flavonoids	+	+
Saponins	+	-
Tannins	+	-
Phytosterols	+	-
Cardiac glycosides	+	+
Phenols	+	+
Terpenoids	+	+

Key: +: Present -: Absent; SGF3: *Syzygium guineense* fraction 3

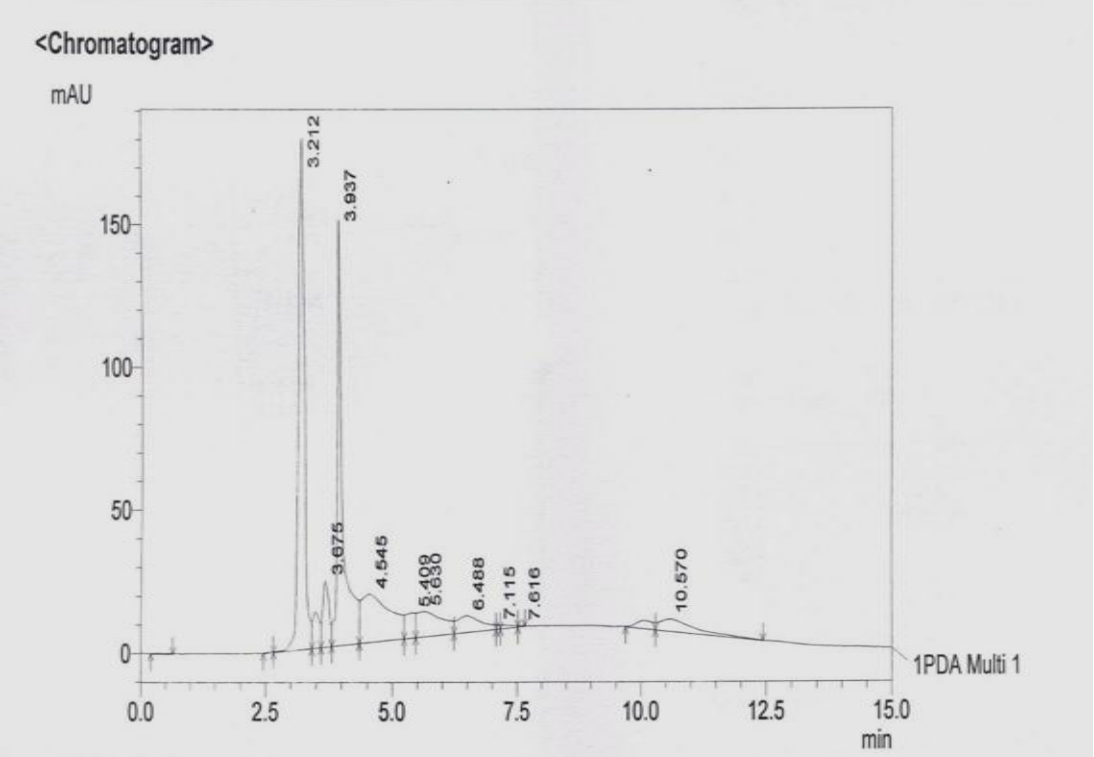


Fig. 4.1: HPLC Chromatogram for *Syzygium guineense*

4.3 Acute Toxicity Studies

4.3.1 LD_{50} determination

In the first phase of acute toxicity study using oral route, all the three doses of the extract administered 10, 100 and 1000mg/kg body weight did not produce any remarkable behavioural or physical change; there was also no casuality among the rats. In the second phase of the study, 1600, 2900 and 5000mg of the extract/kg body weight of the extract administered did not produce any mortality. There was however reduced physical activity and sedation with 5000mg/kg body weight dose. The LD50 for oral route was estimated to be greater than 5000mg/kg/body weight.

In the first phase of intraperitoneal route of administration the 10, 100 and 1000mg/kg body weight of the extract did not produce any sign of toxicity including death. The second phase of this route which involved 2900, 1600, 1000 and 600mg/kg body weight of the extract, recorded death with only 2900mg/kg body weight dose level. The physical and behavioural changes with the other lower dose levels were not remarkable. The intraperitoneal median lethal dose of *Syzygium guineense* was determined by calculating the geometric mean of 2900 and 1600mg/kg body weight; this was found to be 2154.1mg/kg in rats.

4.4 Pharmcological Studies

4.4.1 Effect of methonolic stem bark extract of *Syzygium guineense* on cat blood pressure

The extract significantly ($F4,\ 24\ =9,\ p<0.0001$) reduced diastolic pressure when compared with effect of normal saline on basal rhythm (Fig 4.2, Appendix 1V).

The extract significantly reduced systolic ($F4,\ 24=\ 8.96,\ p<0.0001$) pressure in cat when compared with effect of normal saline on basal rhythm (Fig 4. 3, Appendix 1V).

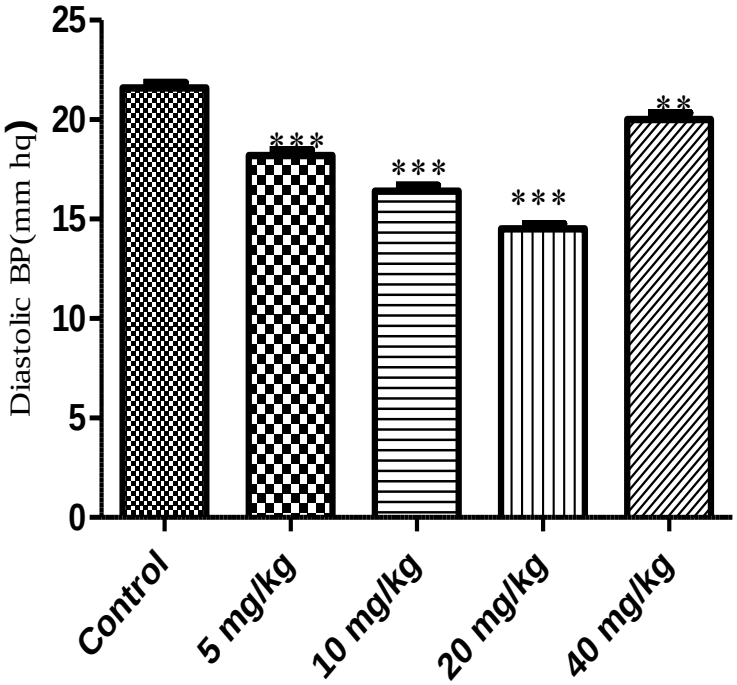


Fig 4 .2: Effect of methanolic stem bark extract of *Syzygium guineense* on diastolic blood pressure of cat.
*** significantly different from the control at , $p<0.0001$
** significantly different from control at $p< 0.05$

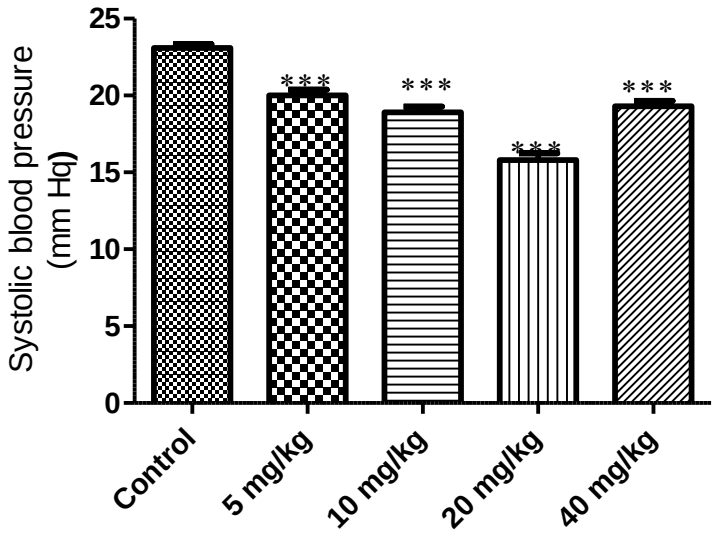


Fig.4.3: Effect of methanolic stem bark extract of *Syzygium guineense* on systolic blood pressure of cat.
*** Significantly different from the control at $p<0.0001$

4.4.2 Effect of adrenalin and methanolic stem bark extract of *Syzygium guineense* on diastolic blood pressure of cat

Adrenalin produced significant (*F6, 69=155, p<0.0001)* concentration-dependent increase in diastolic blood pressure of cat while the extract at 10 mg/kg and 20 mg/kg reduced the diastolic blood pressure (Fig 4.4, Appendix V).

4.4.3 Effect of adrenalin and methanolic stem bark extract of *Syzygium guineense* on systolic blood pressure of cat

Adrenalin produced significant (($F_{6, 69}$=108, *p=0.0013*) dependent increase in systolic blood pressure of cat while the extract at 10 mg/kg and 20 mg/kg reduced the systolic blood pressure (Fig4. 5, Appendix V).

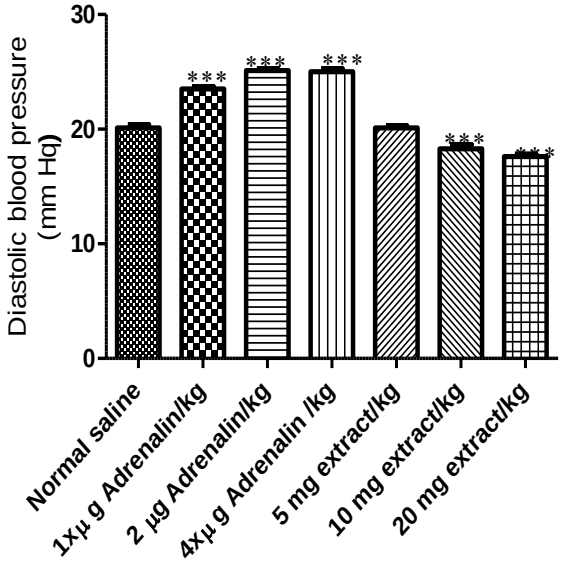


Fig 4.4: Effect of graded concentration of adrenaline alone, methanolic stem bark extract alone on diastolic blood pressure of cat

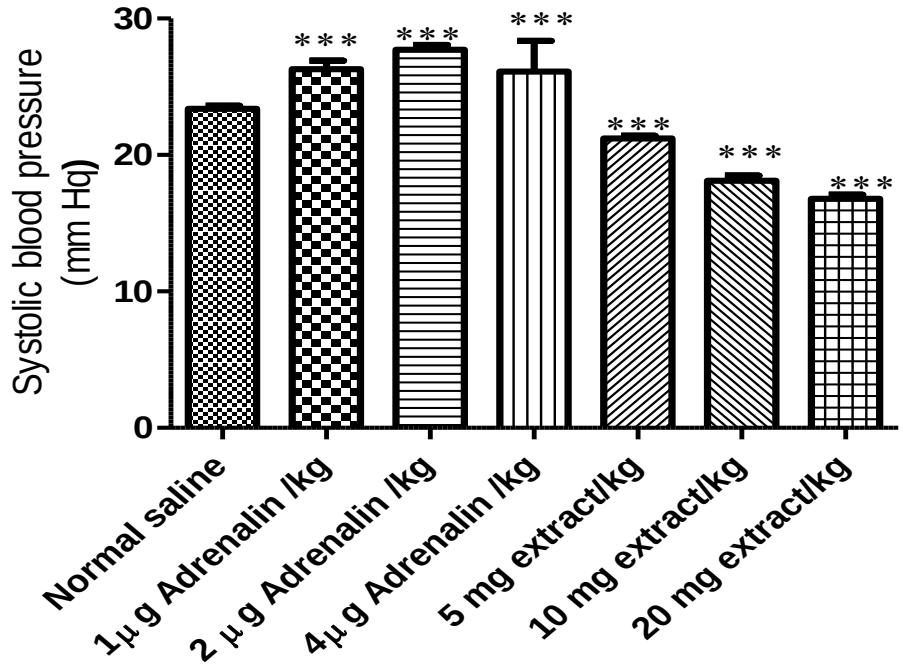


Fig 4.5: Effect of graded concentration of adrenalin and SGMSBE on systolic blood pressure of cat

4.4.4 Effect of Atropine on hypotensive effect of methanolic stem bark extract in normotensive cat

The extract (4-16 mg/kg) significantly (*p<0.05)* inhibited the antagonistic effect of 2.5 mg/kg atropine on systolic and diastolic cat blood pressure in a dose-dependent manner (Fig. 4.6 & 4.7, Appendix VI).

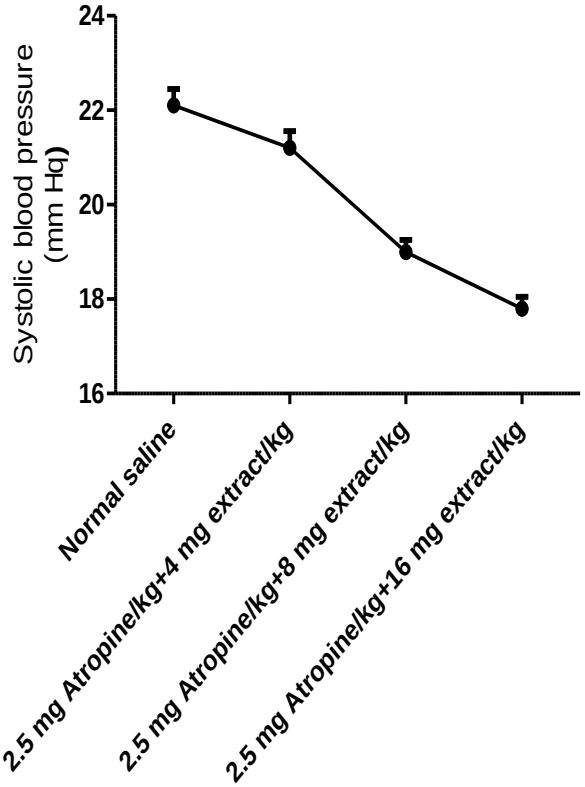


Fig 4. 6: Effect of interaction between methanolic stem bark extract of *Syzygium guineense* and atropine on systolic blood pressure of cat

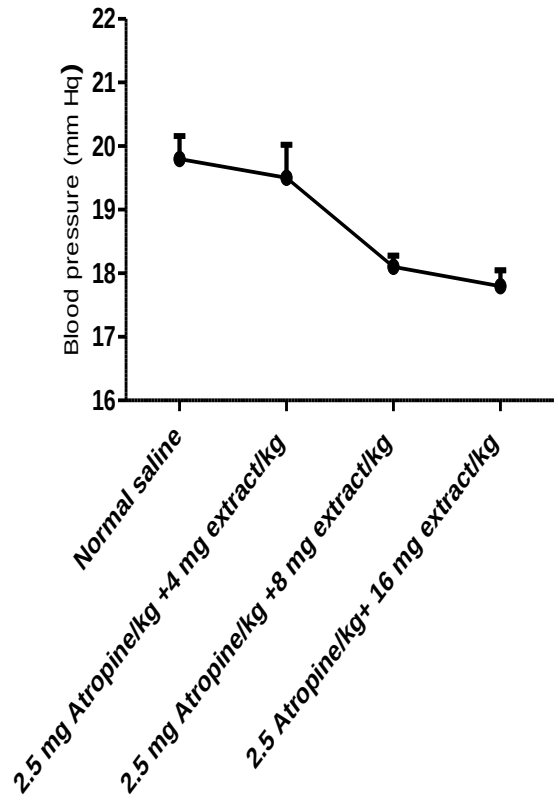


Fig 4.7:Effect of interaction of *Syzygium guineense* extract with atropine on diastolic pressure of cat

4.4.5 Effect of acetylcholine alone on diastolic and systolic BP in normotensive cat

Acetylcholine significantly reduced the diastolic (F4, 49= 31, *p<0.0001*) and systolic (F4, 49=101, *p<0.0001*) blood pressure of normotensive cat (Fig.4.8 .& Fig.4.9, Appendix VII).

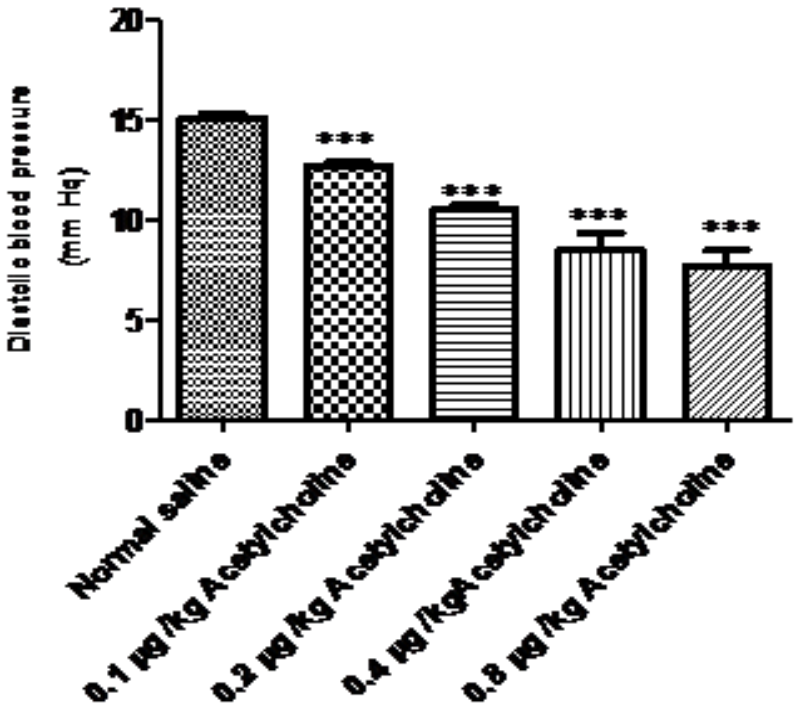


Fig. 4.8 Effect of graded doses of acetylcholine alone on the diastolic blood pressure of cat

*** Significantly different from the control at p>0.0001

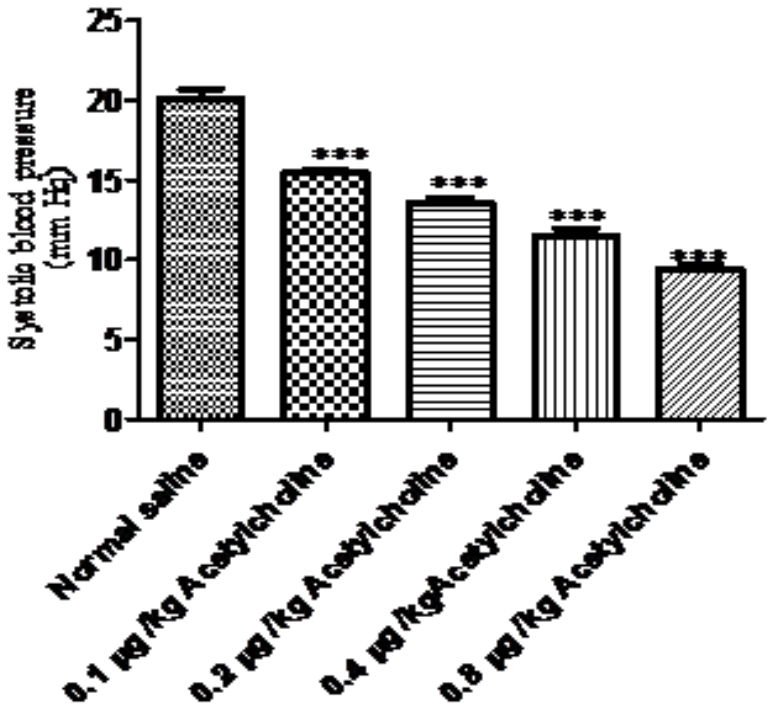


Fig. 4.9 Effect of graded doses of acetylcholine alone on the systolic blood pressure of cat

*** Significantly different from the control at p>0.0001

4.4.6 Effect of 0.9% normal saline, acetylcholine alone, *Syzygium guineense* (SG) extract, interaction between atropine and acetylcholine and interaction between atropine and SG extract on cat blood pressure

The effects of acetylcholine, Syzygium guineense extract and interractions between atropine and acetylcholine, atropine and SG extract and graded doses of the SG extract on diastolic blood pressure of cat are not significantly (*F 7, 79=0.9, p>0.05*) different from the effect of the 0.9% normal saline (Fig4.10, appendix 5). However, the effects of acetylcholine, *Syzygium guineense* extract and interactions between atropine and acetylcholine, atropine and SG extract and graded doses of the SG extract on systolic blood pressure of cat are significantly (*F 7, 79=20, p<0.0001*) different from the effect of the 0.9% normal saline (Fig. 4.11, Appendix VIII).

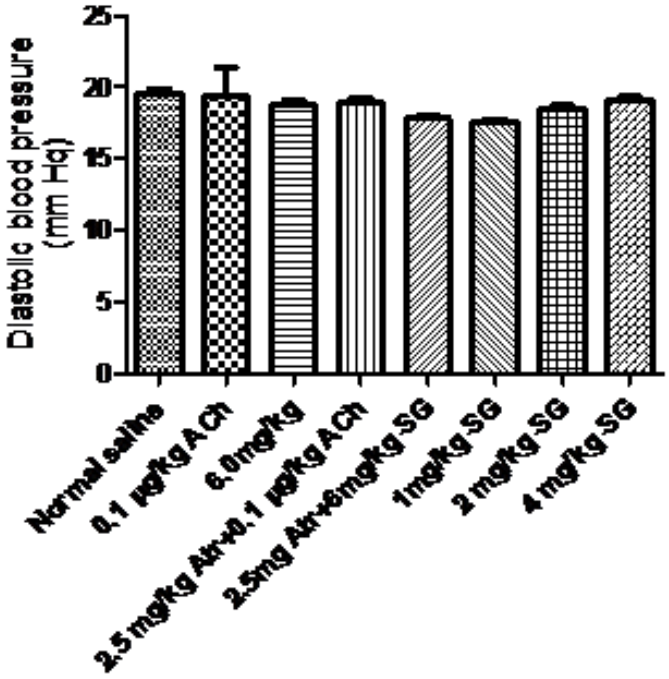


Fig.4.10 Effects of Acetylcholine (Ach), alone, *Syzigium guineense* (SG) extract, interacton between Atropine (Atr) and Acetylcholine, Atropine and SG extract on diastolic blood pressure of cat

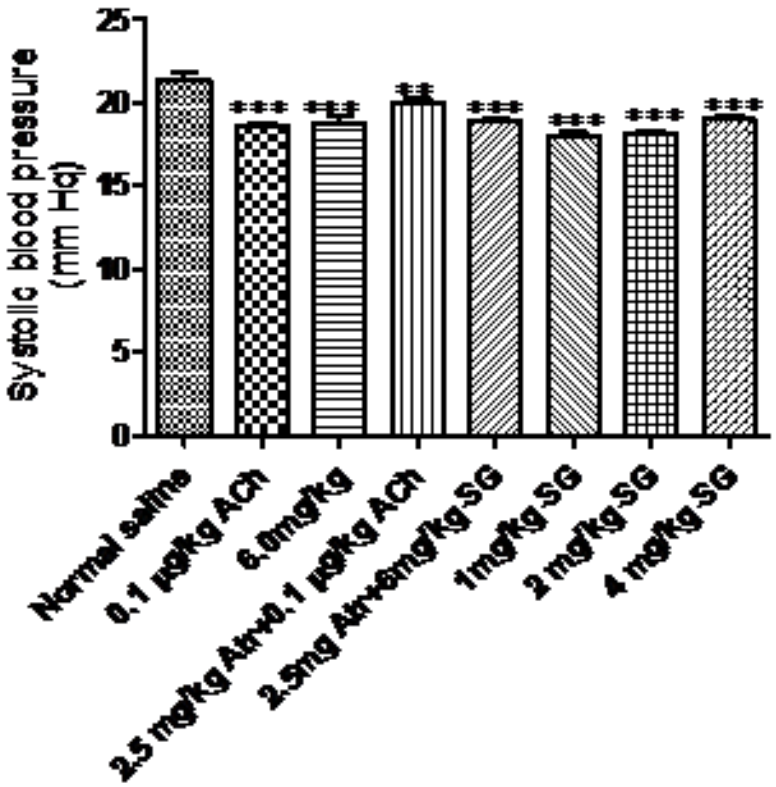


Fig.4.11 Effects of Acetylcholine (Ach), alone, *Syzigium guineense* (SG) extract, interacton between Atropine (Atr) and Acetylcholine, Atropine and SG extract on diastolic blood pressure of cat

*** Significantly different from the effect of normal saline on the cat systolic blood pressure

4.4.7 Effect of adrenaline on hypotensive effect of methanolic stem bark extract of *Syzygium guineense* in normotensive cat

Adrenalin (1µg/kg) could not reverse the hypotensive effect of the extract (4-16 mg/kg) on both systolic and diastolic blood pressure in normotensive cat (Fig 4. 12 & 4.13, appendix IX).

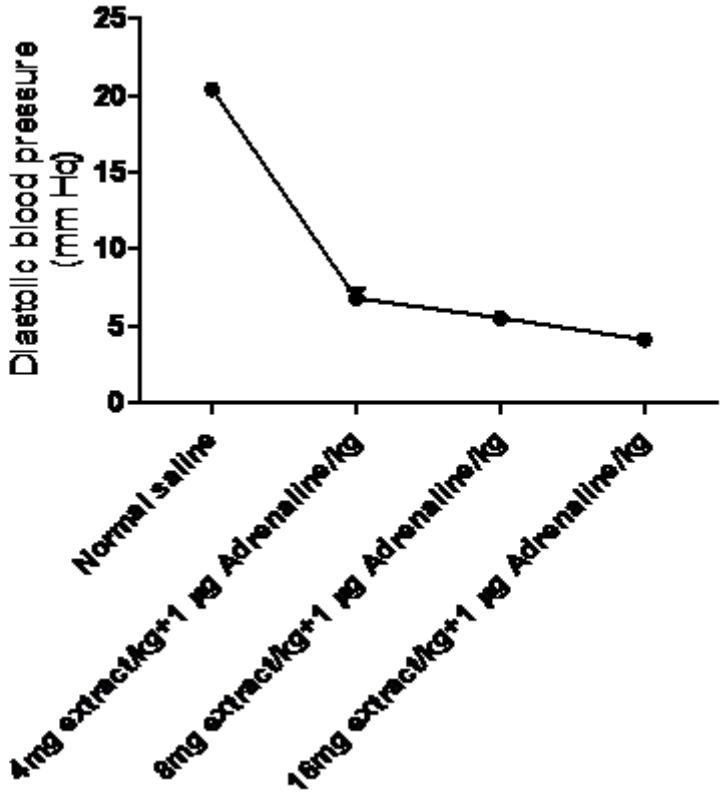


Fig. 4.12: Effect of interaction between the extract and adrenaline on diastolic blood pressure in cat

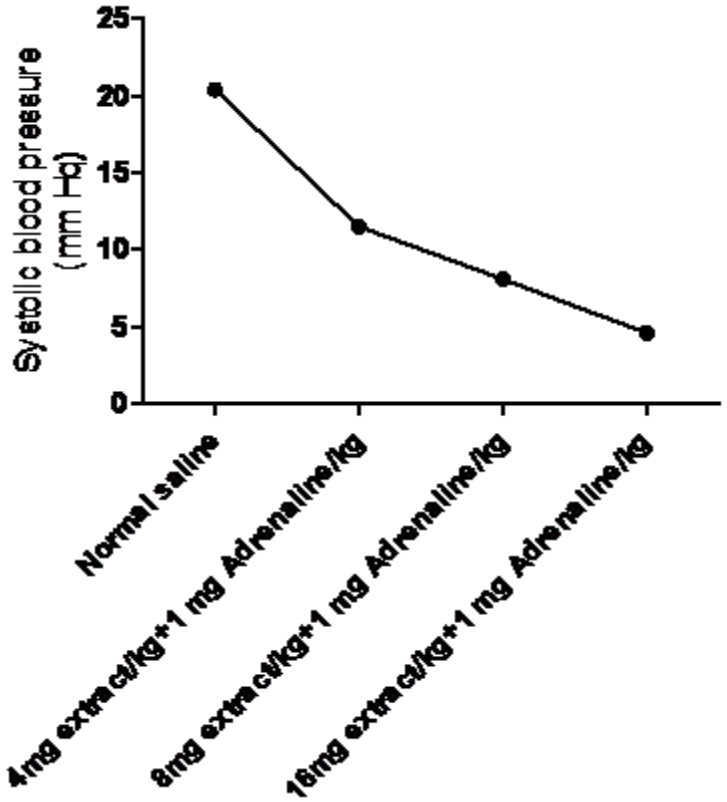


Fig. 4.13: Effect of interaction between the extract and adrenaline on systolic blood pressure in cat

4.4.8 Bioassay guided selection of active fraction (s) out of column chromatographic fractions of methanolic stems bark extract of *Syzygium guineense*

The five column fractions obtained from methanolic stem bark extract of *Syzygium guineense* were tested on the blood pressure of anaesthetized normotensive cat. Fraction SGF1 (4-32 mg/kg) increased the diastolic and systolic blood pressure while fractions SGFF2-SGF5 (4-32 mg/kg) reduced the diastolic and systolic blood pressures of anaesthetized normotensive cat to varying degree.

4.4.8.1 *Effect of Frcation SGF1 on diastolic and systolic blood pressure of normotensive cat*

Fraction SGF1 produced significant ($p<0.05$) dose –dependent increase in diastolic and systolic blood pressures of normotensive cat (Fig 4.14&4.15, Appendix X) when compared to normal saline treated control.

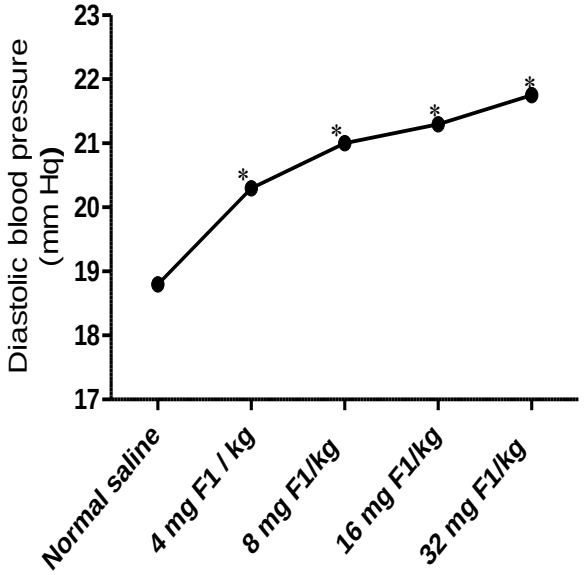


Fig. 4.14: Effecft of fraction F1 of the *Syzygium guineense* stem bark extract on diastolic pressure of anaesthetized normotensive cat

*** Significantly different from the control at p<0.05**

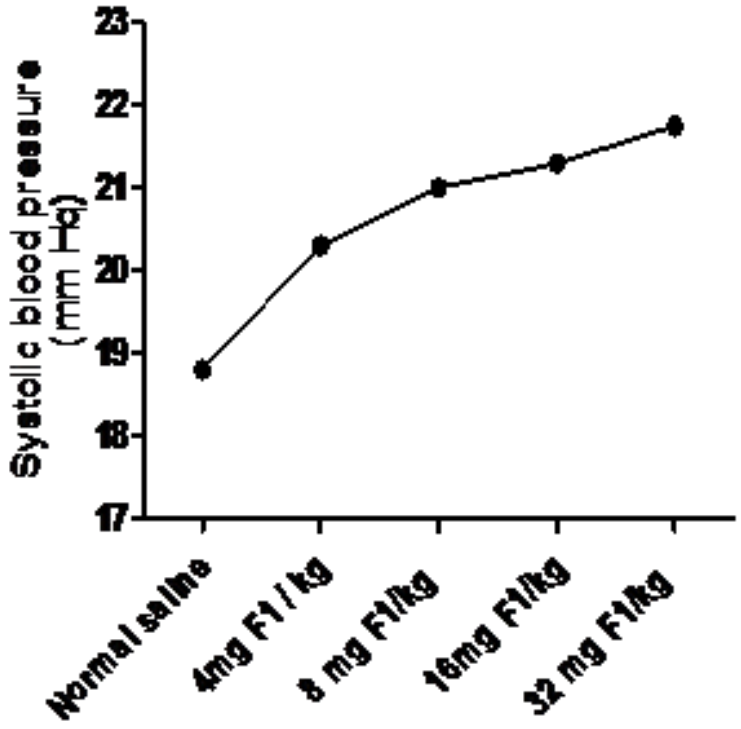


Fig. 4.15: Effect of Fraction SGF1of the *Syzygium guineense* stem bark extract on systolic pressure of anaesthetized normotensive cat

4.4.8.2 *Effect of fraction SGF2 on diastolic and systolic blood pressure of* anaesthetized normotensive *cat*

Fraction SGF2 significantly ($p<0.05$) decreased diastolic and systolic blood pressures in anaesthetized normotensive cat (Fig 4.16&4.17, Appendix XI) when compared to normal saline treated control.

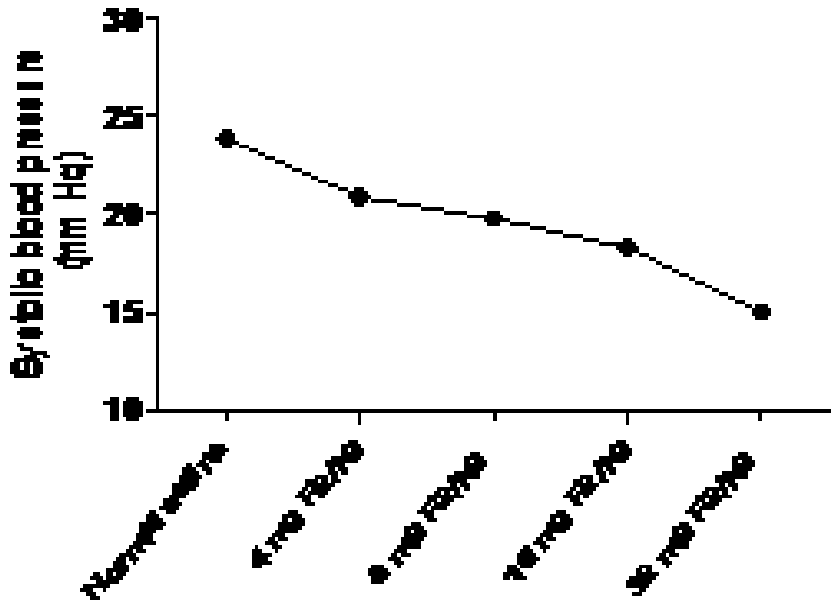


Fig. 4.16 Effect of fraction f2 of the *Syzygium guineense* stem bark extract on systolic pressure of anaesthetized normotensive cat

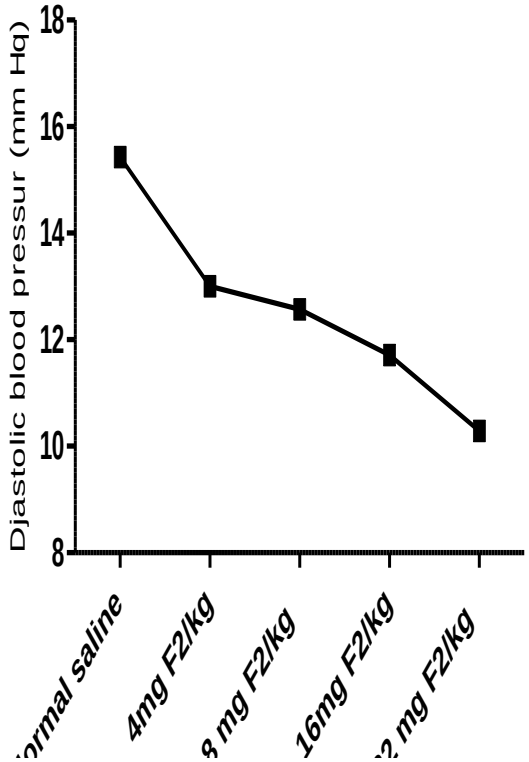


Fig. 4.17 **Effect of Fraction F2 of *Syzygium guineense* stem bark extract on diastolic pressuer of anaesthetized normotensive cat**

4.4.8.3 *Effect of fraction SGF3 on diastolic and systolic blood pressure of anaesthetized normotensive cat*

Fraction SGF3 significantly ($p<0.01$) decreased diastolic and systolic blood pressures in anaesthetized normotensive cat (Fig 4.18 & 4.19, appendix XI) when compared to normal saline treated control in a dose-dependent manner.

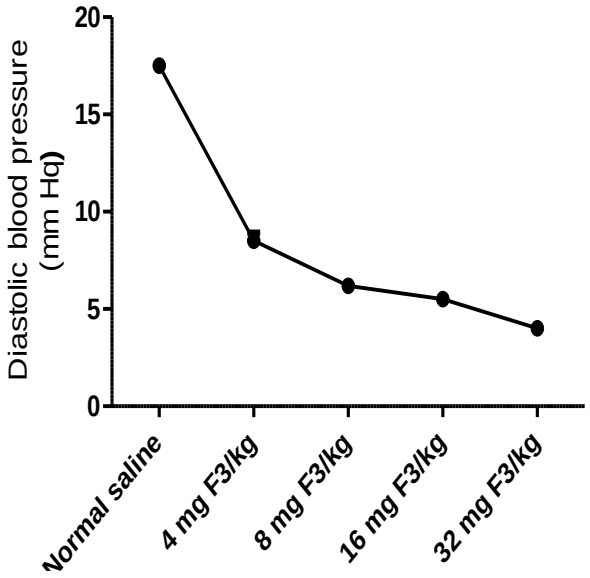


Fig. 4.18 **Effect of Fraction F3 of *Syzygium guineense* stem bark extract on diastolic pressuer of anaesthetized normotensive cat**

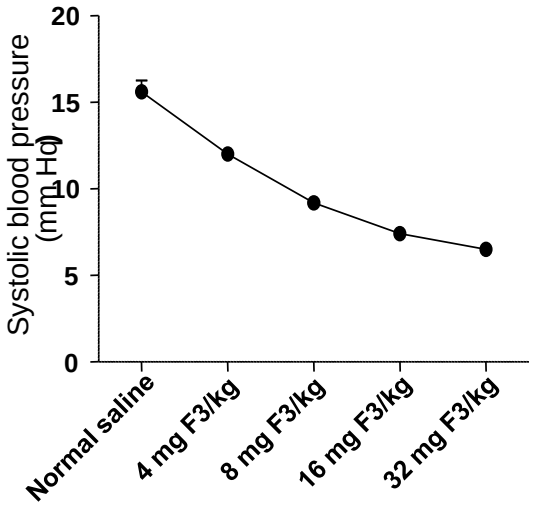


Fig 4.19: Effect of Fraction F3 of the *Syzygium guineense* stem bark extract on systolic pressure of anaesthetized normotensive cat

4.4.8.4 *Effect of Frcation SGF4 on diastolic and systolic blood pressure of* anaesthetized normotensive *cat*

Fraction SGF4 significantly ($p<0.05$) decreased diastolic and systolic blood pressures in anaesthetized normotensive cat (Fig 4.20&4.21, Appendix XII) when compared to normal saline treated control.

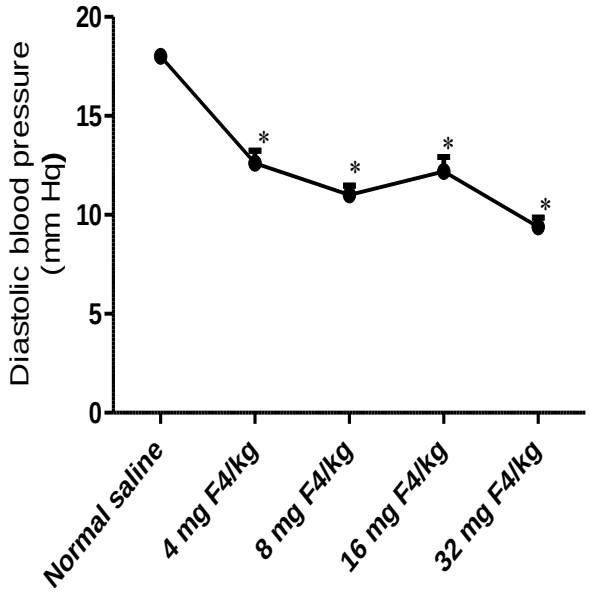


Fig. 4.20 **Effect of Fraction F4 of *Syzygium guineense* stem bark extract on diastolic pressuer of anaesthetized normotensive cat**

*** Significantly different from the control at $p<0.05$**

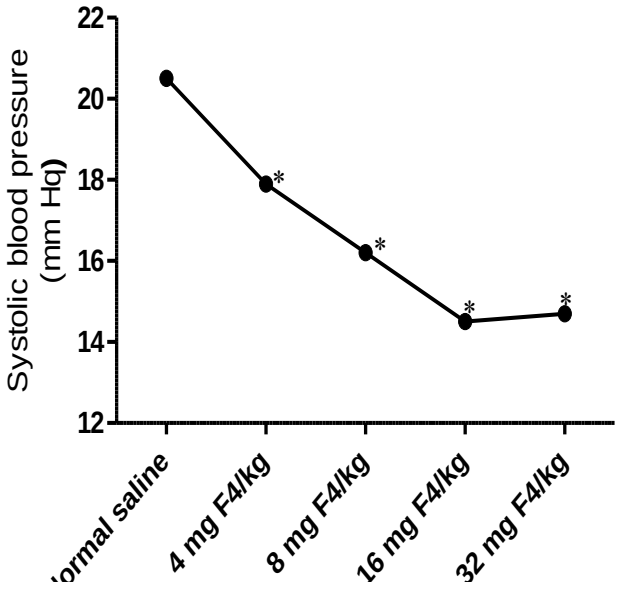


Fig. 4.21 **Effect of Fraction F4 of *Syzygium guineense* stem bark extract on diastolic pressuer of anaesthetized normotensive cat**

*** Significantly different from the control at p<0.05**

4.4.8.5 *Effect of fraction SGF5 on diastolic and systolic blood pressure of* anaesthetized normotensive *cat*

Fraction SGF 5 significantly ($p<0.05$) decreased diastolic and systolic blood pressures in anaesthetized normotensive cat (Fig 4.22&4.23, Appendix XII) when compared to normal saline treated control.

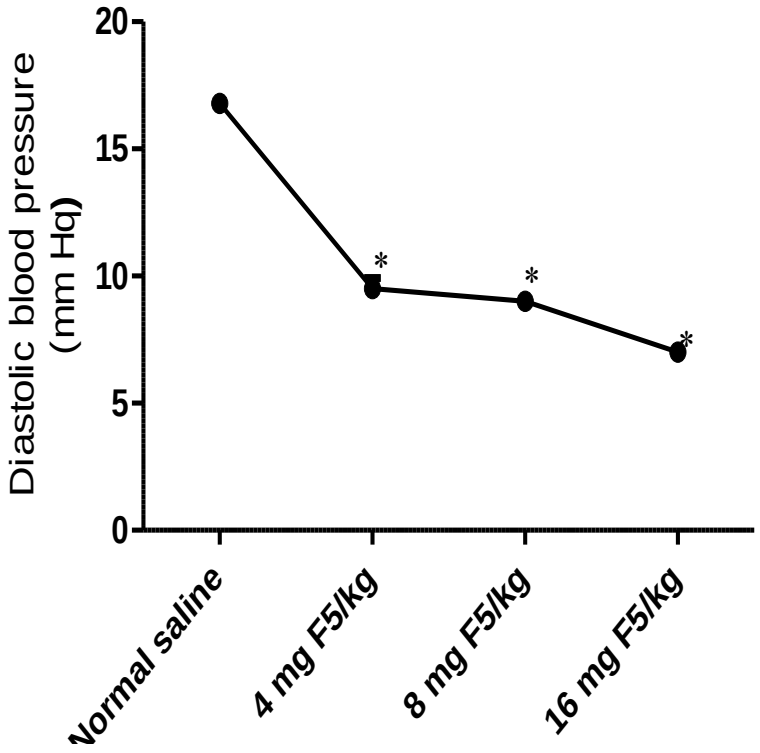


Fig. 4.22 **Effect of Fraction F5of *Syzygium guineense* stem bark extract on diastolic pressuer of anaesthetized normotensive cat**

*** Significantly different from the control at p<0.05**

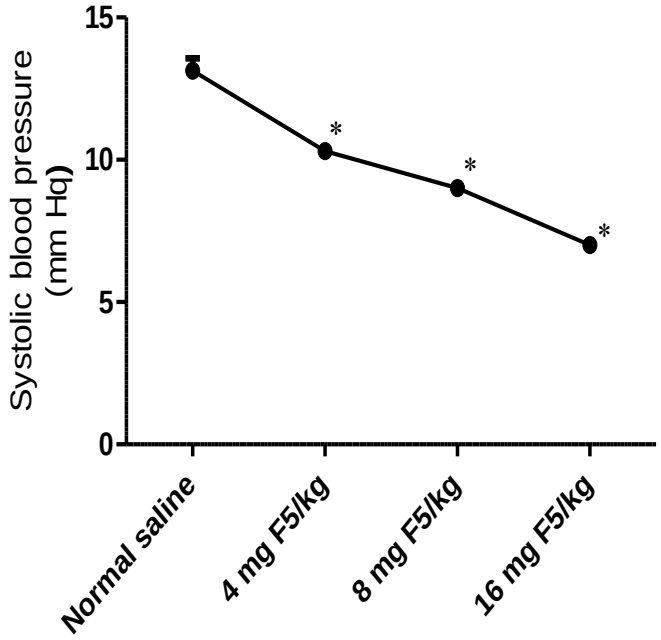


Fig. 4.23 **Effect of Fraction F4 of *Syzygium guineense* stem bark extract on diastolic pressuer of anaesthetized normotensive cat**

*** Significantly different from the control at p<0.05**

4.4.9 Effect of *Syzygium guineense* (SG) extract and its interaction with adrenalin on atrial contraction

The *Syzygium guineense* ($1x10^{-5}$g/ml - $4x10^{-3}$g /ml) significantly ($p<0.05$) and in concentration dependent - manner decreased the amplitude of contraction in guinea pig atria preparation (Fig 4.24& Appendix XIV).

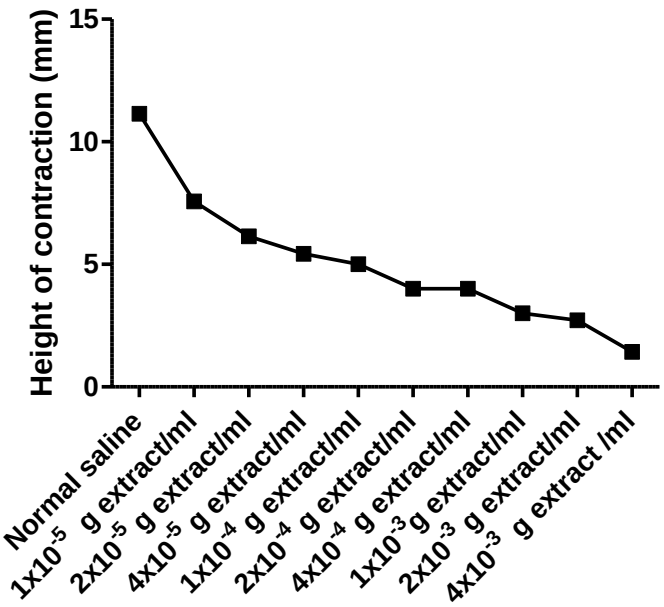


Fig. 4.24 **Effect of graded concentration of *Syzygium guineense* (SG) extract on height of contraction of Guinea pig atrium**

Adrenaline at $1.5x10^{-9}$ g/ml significantly *(p<0.05)* increased the amplitude of contraction of the guina pig atria previously treated with the extract of *Syzigium guineense* (fig 4.25 Appendix XV).

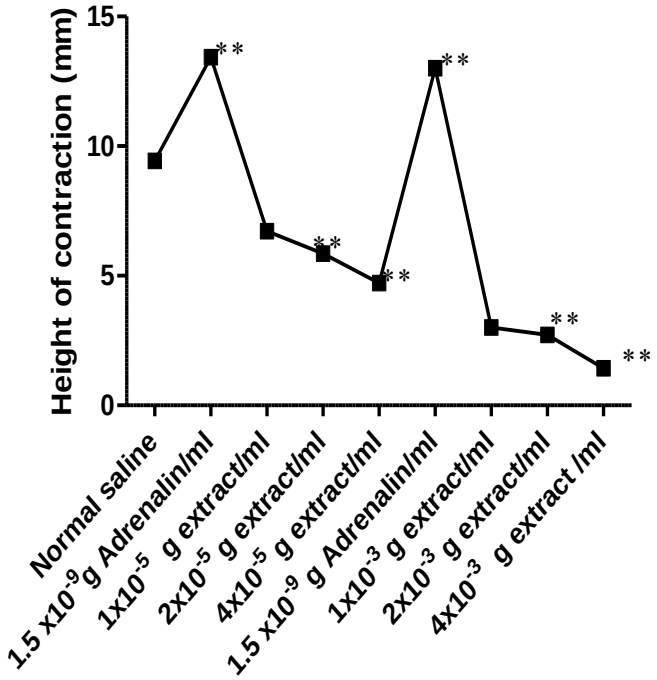


Fig. 4.25 **Effect of graded concentration of extract and *Adrenalin on height of contraction of Guinea pig atrium***

**** significatntly different from the control at p<0.01**

4.4.10 Effect of SGF3 on guinea pig atrium

Fraction SGF3 at concentration ranging between 4mg/ml to 32mg/ml significantly ($p<0.01$) reduced guinea pig atria height of contraction. (Fig.4.26 & Appendix XVI).

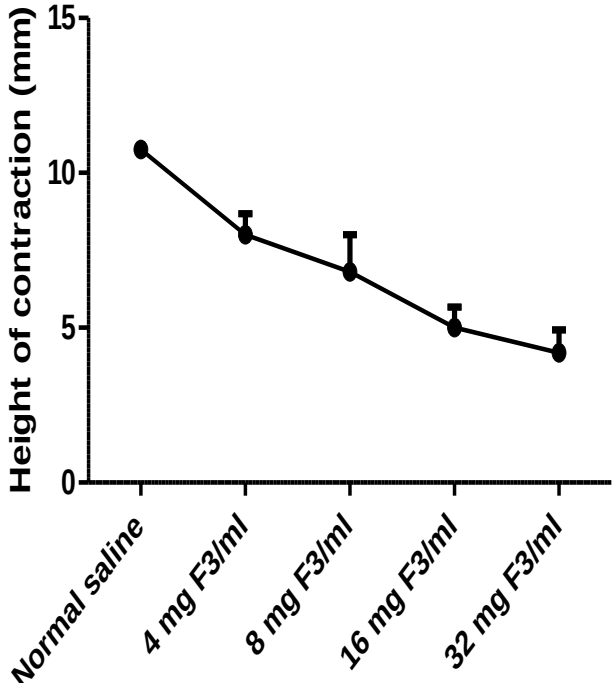


Fig. 4.26 Effect of SGF3 on height of contraction of Guinea pig atrium

4.4.11 Effect of SGF3 on atropinized guinea pig atria

Fraction SGF3 ($4x10^{-3}$ -$32x10^{-3}$ g/ml) significantly (*p<0.001*) and competitively antagonized the effect of atropine ($0.125x10^{-3}$ g/ml**)** on the height of contraction of the guinea pig atria preparation (Fig 4.27, Appendix XVII).

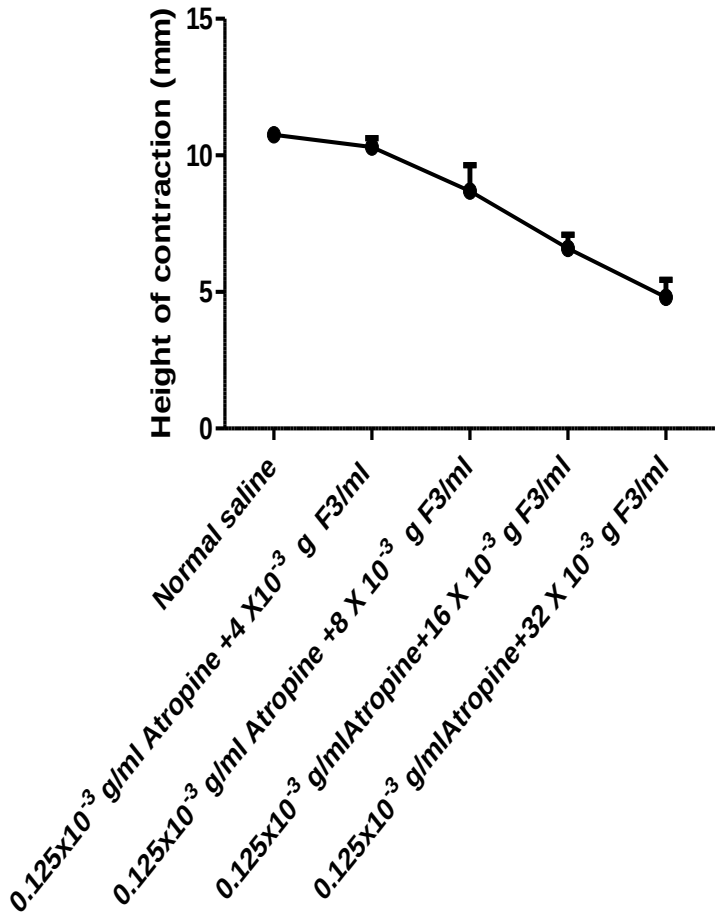


Fig. 4.27 Effect of SGF3 on height of contraction of atropinised guinea pig atrium

4.4.12 Effect of interraction between adrenalin and SGF3 on isolated guinea pig atrium

Adrenaline (1.5 $x10^{-9}$ g/ml) antagonized the effect of SGF3 at ($1x10^{-5}$g/ml, $2x10^{-5}$ g/ml and $4x10^{-5}$ g/ml). At $1x10^{-3}$g/ml, $2x10^{-3}$g/ml and $4x10^{-3}$ g/ml), SGF3 significantly (*p<0.001*) reduced the height of contraction elevated by adrenaline (1.5 $x10^{-9}$ g/ml) in a concentration dependent manner (Fig 4.28, Appendix XVIII).

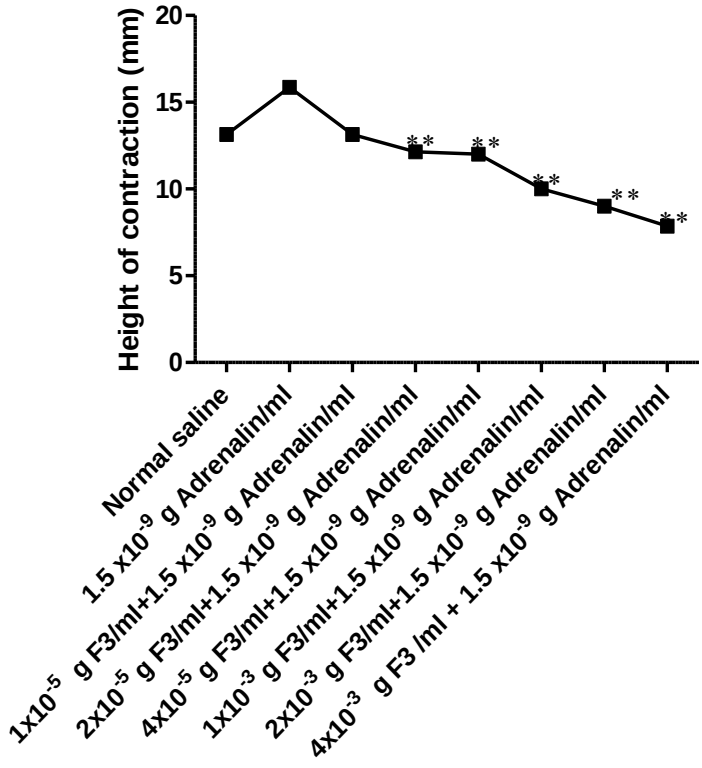


Fig. 4.28 **Effect of interaction between SGF3 and *Adrenalin on height of contraction of Guinea pig atrium***

**** significatntly different from the control at p<0.01**

4.4.13 Effect of methanolic stem bark extract of *Syzygium guineense* on isolated rabbit heart preparation

The *Syzygium guineense* (1-4 mg/ml) produced significant ($p<0.05$) decrease in height of contraction produced by the heart. The decrease was concentration dependent (Fig.4.29).

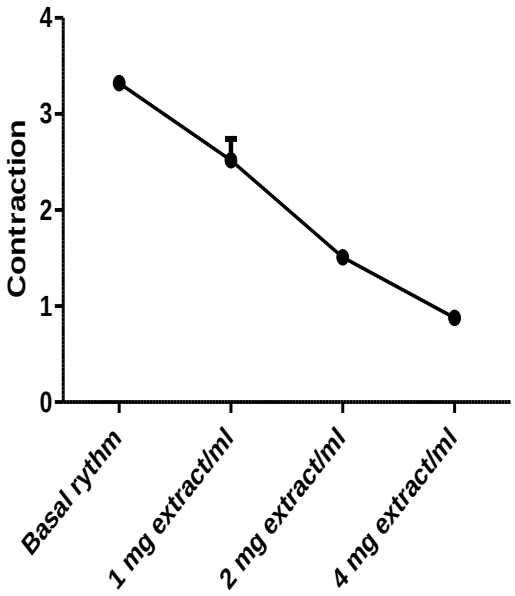


Fig. 4.29 **Effect of graded concentration of *Syzygium guineense* extract on isolated rabbit heart preparation**

4.4.14 Effect of adrenaline alone and interaction between adrenaline and SG

Adrenaline (1 µg/ml - 4 µg/ml) alone increased the height of contraction produced by the isolated rabbit heart. The interaction between Adrenaline (1 µg/ml) and (1 mg/ml – 4 mg/ml) and methanolic stem bark extract of *Syzygium guineense* produced reduction in the height of contraction produced by the isolated rabbit heart (Fig.2.30).

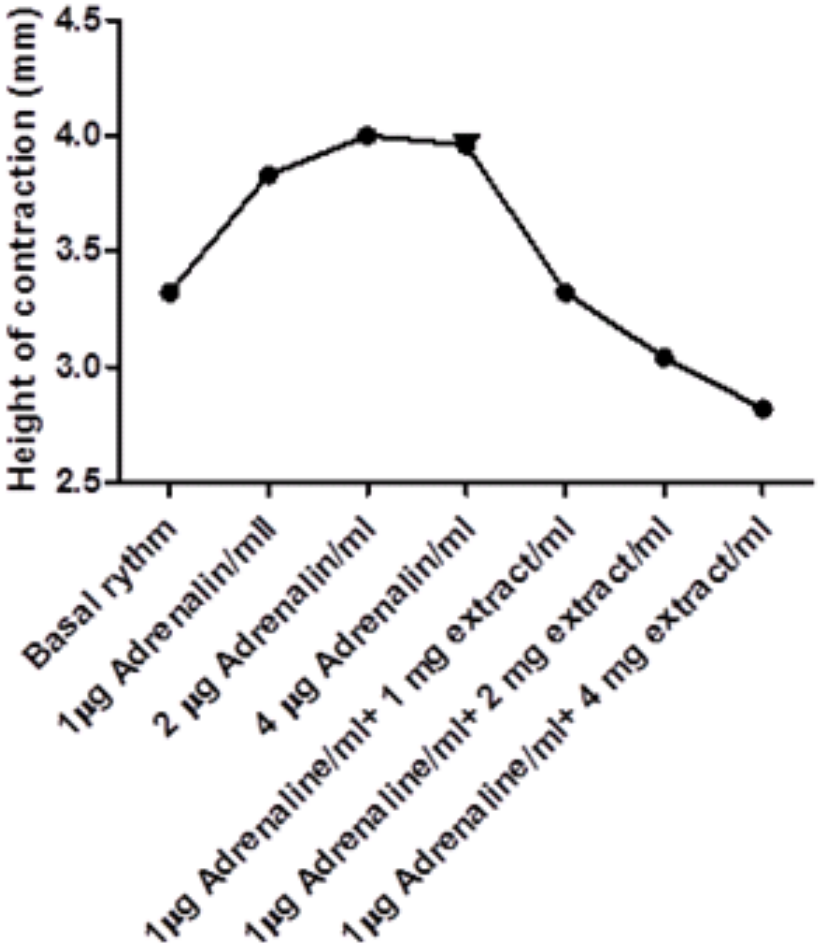


Fig. 4.30: Effect of graded concentration of *Syzygium guineese* extract alone and the presence of adrenaline on isolated heart preparation

4.4.15 Effect of SGF3 fraction on isolated rabbit heart preparation

Fraction SGF3 significantly ($p<0.001$) reduced height of contraction in isolated rabbit heart preparation in a concentration dependent manner (Fig.4.31). However, adrenaline competitively reversed the relaxant effect of SGF3 (Fig.4.32).

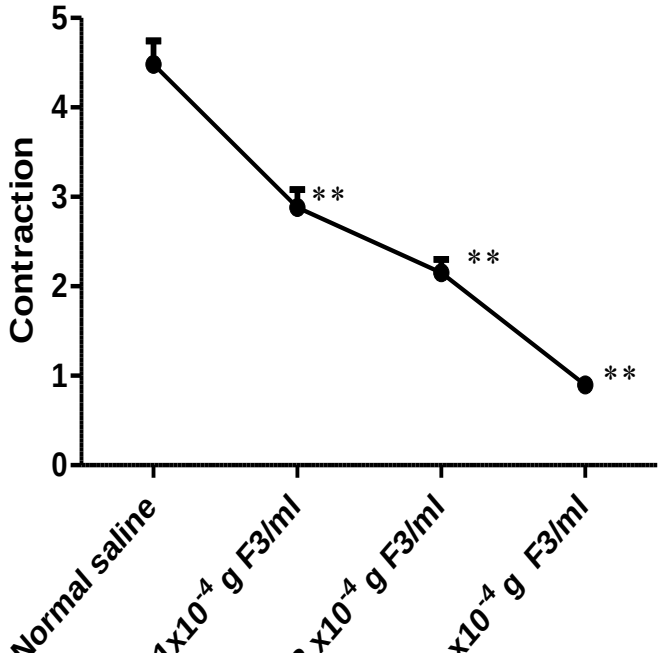


Fig. 4.31 **Effect of graded concentration of Fraction F3 on isolated rabbit heart preparation**

**** significatntly different from the control at p<0.01**

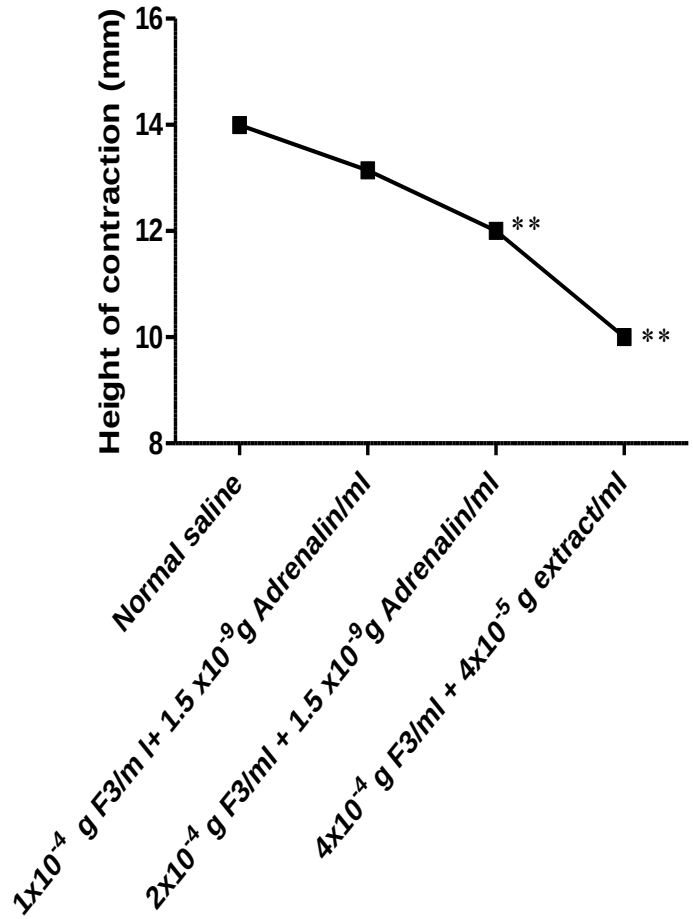


Fig. 4.32 **Effect of graded interaction between adrenaline and SG F3 on isolated rabbit heart preparation**

4.4.16 Effect of methanolic stem bark extract of *Syzygium guineense* on smooth muscle rabbit ileum

Acetylcholine ($33.3x10^{-9}$ g/ml – $333.3x10^{-7}$ g/ml) increased height of contraction in the rabbit ileum. *Syzygium guineense (*$33.3\ x10^{-7}$ g/ml - $333\ x10^{-4}$ g/ml) reduced the height of contraction produced by rabbit ileum (Fig.4. 33).

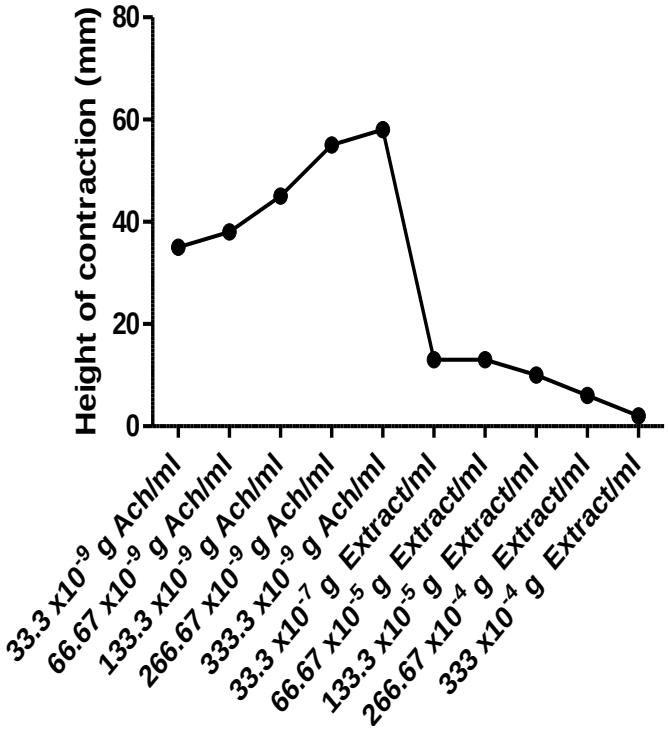


Fig. 4.33 **Effect of graded concentration of acetylcholine and *Syzygium guineense* *stem bark extract on height of contraction of rabbit ileum***

4.4.17 Effect of methanolic stem bark extract of *Syzygium guineense* adrenaline on smooth muscles of rabbit ileum

The methanolic stem bark extract of ***Syzygium guineese*** produced progressive decrease in the height of contraction indicating relaxant effect on the smooth muscle. At 2.67 mg/ml the extract completely abolished the muscle tone. Adrenaline however restored the contraction. The contraction however was pronounced in the presence of phenoxy benzamine an α-receptor blocker. The relaxant effect of the extract was however reversed in the presence of phenoxybenzamine resulting in muscle tone restoration with exaggerated contraction.

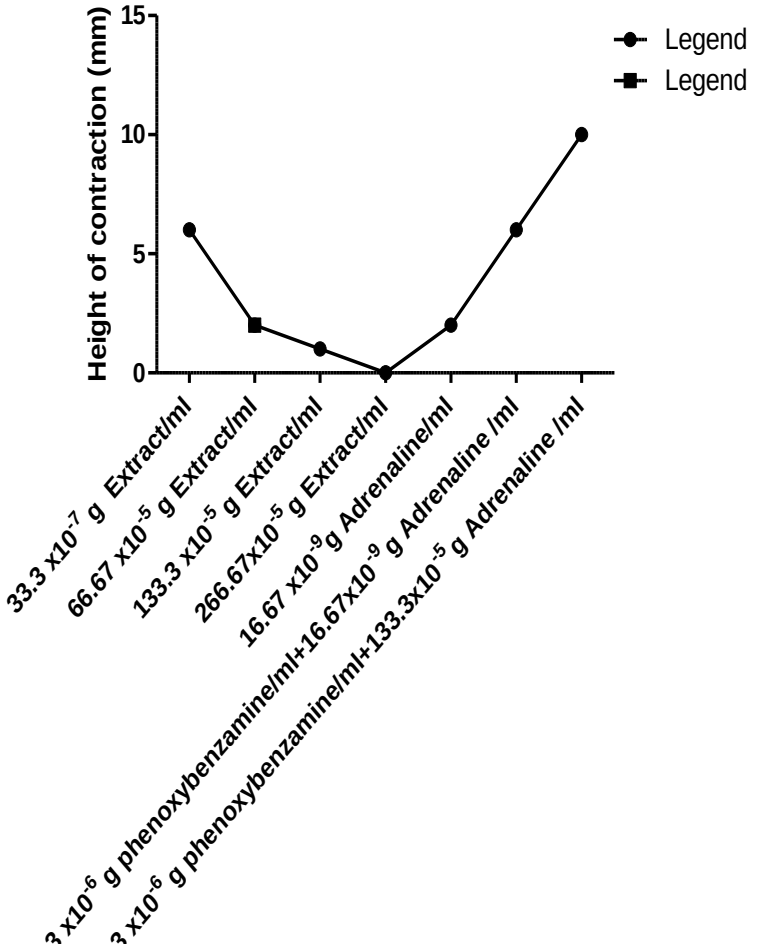


Fig. 4.34 **Effect of graded concerntration of *Szygium guineense stem bark extract, adrenaline alone, adrenaline in the presence of phenoxybenzamine and extract in the presence of phenoxybenzamine on height of contraction of rabbit ileum***

4.4.18 Effect of fraction SGF3 on rabbit ileum

Acetylcholine produced concentration dependent contraction in rabbit ileum. Fraction F3 produced significant ($p<0.05$) concentration dependent relaxation of the rabbit ileum (Fig. 4.35).

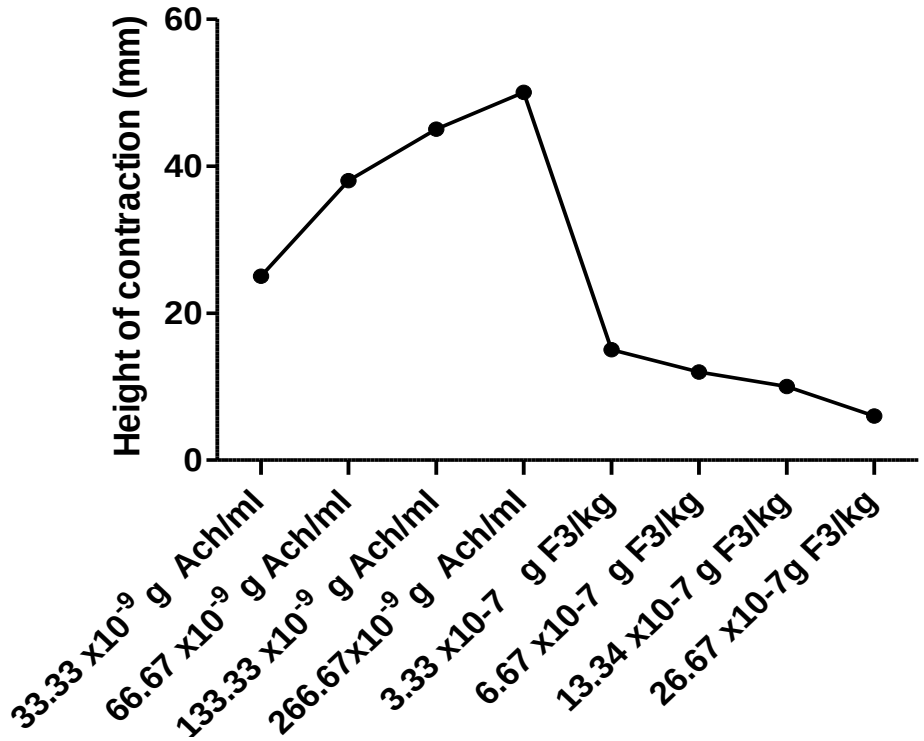


Fig. 4.35 Effect of acetylcholine, Fraction F3 on height of contraction of rabbit ileum

4.4.19 Effect of methanolic stem bark extract on uterus

Oxytocin significantly ($p<0.001$) increased the height of contraction of uterine smooth muscle preparation. *Syzygium guineese* as well as salbutamol did not elicit contractile response in the uterine tissue. However, oxytocin produced significant contractile response in the presence of salbutamol.

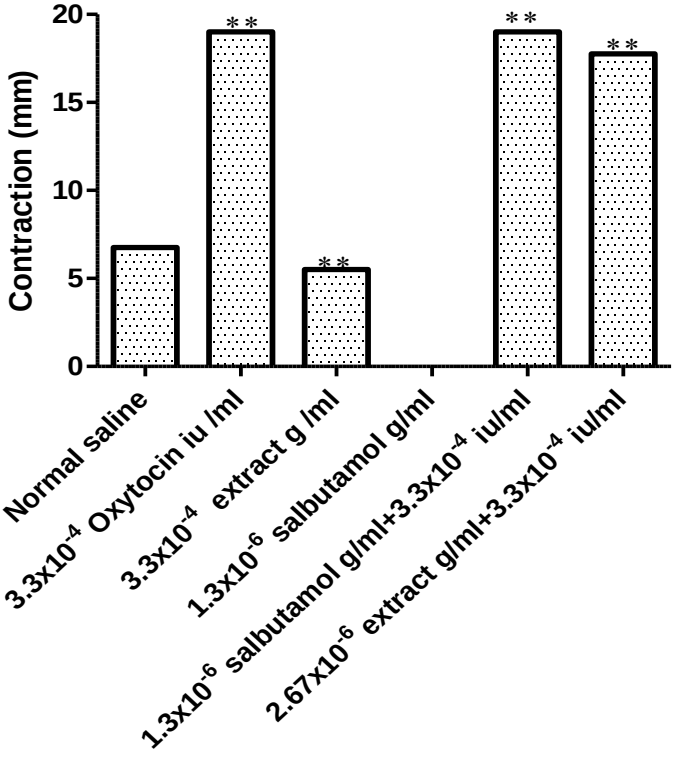


Fig. 4.36 **Effect of oxytocin, methanolic stem bark extract of *Syzygium guineense and salbutamol on Rat uterus***

**** significantly different from the control at p<0.001**

4.5 Chronic Toxicity Studies

4.5.1 Effect of methanolic stem bark extract of *Syzygium guineense* on clinical signs

The animals appeared normal before, during and after treatment with extract. All the rats survived until scheduled euthanasia.

4.5.2 Effect of 90 days oral adminsaration of methanolic stem bark extract of *Syzygium guineense* on feed intake of male and female rats

There was significant (p<0.05) increase in feed intake of treated male and female rats when compared to the corresponding controls throughout the 90-days study (Fig 4.37 and 4.38). Significant increase (p<0.05) in water intake was observed in male and female rats treated with methanolic stem bark extract of *Syzygium guineense* in a dose-dependent manner (Fig 4.39 and 4.40).

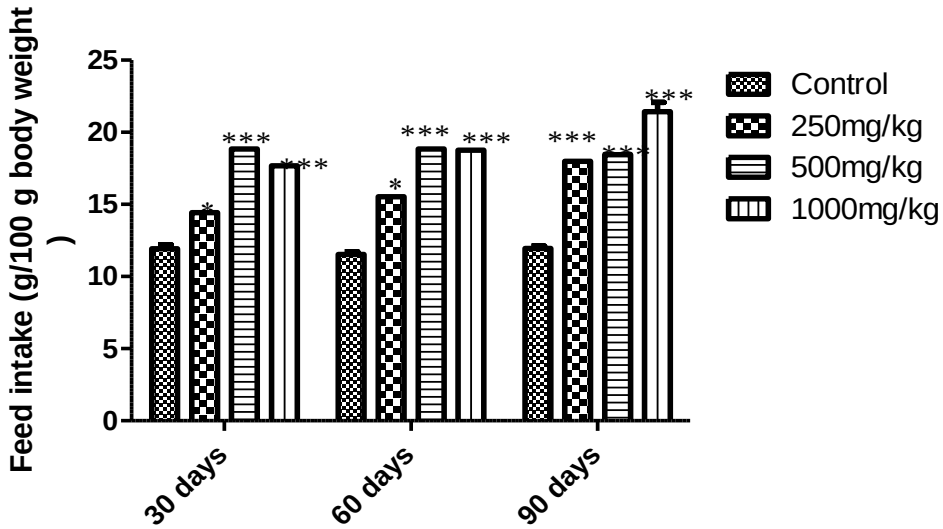


Fig. 4.37 **Effect of extract of *syzygium guineense* on feed intake of male rats**
*** significantly different from thecontrol at p<0.0001
* significantly different from the control at p<0.05

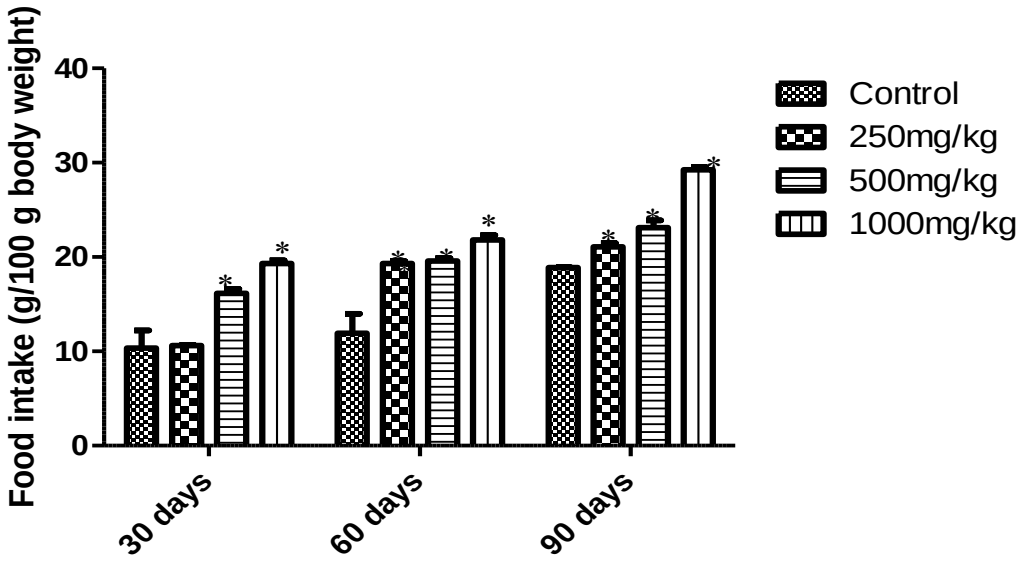


Fig. 4.38 Effect of extract of *syzygium guineense* on feed intake of female rats

***** Significantly different from the control at $p<0.05$.

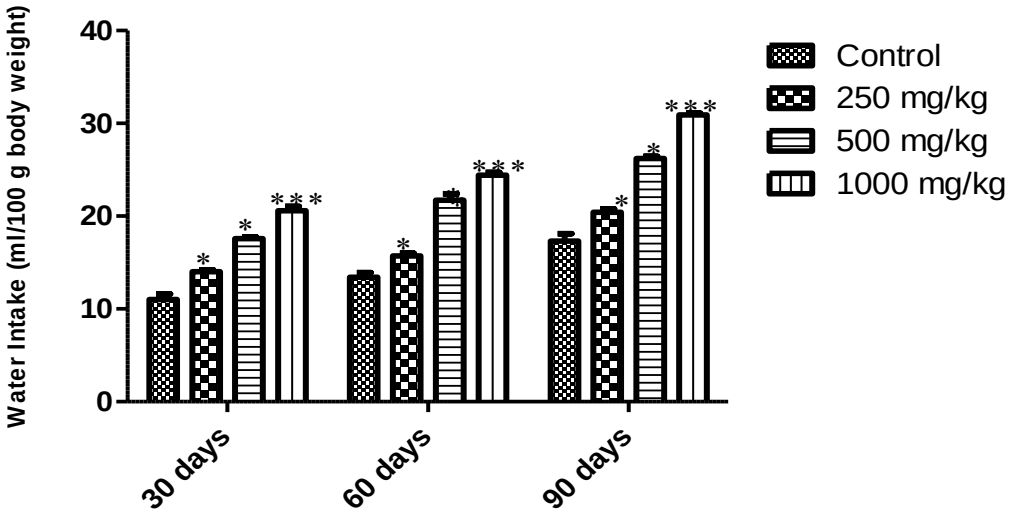


Fig. 4.39 Effect of extract of *syzygium guineense* on water intake in male rats.
*** Significantly different from the control at p<0.05**
***** Significantly different from the control at p<0.0001**

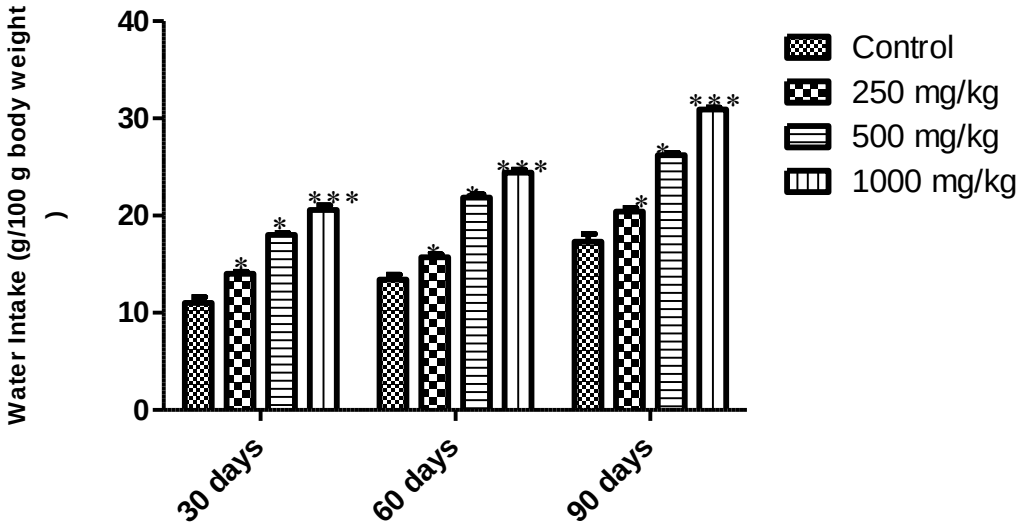


Fig. 4.40 Effect of extract of *syzygium guineense* on water intake in female rats.
***** Significantly different from the control at p<0.0001**
*** Significantly different from the control at p<0.05**

4.5.3 Effect of 90 days oral adminsaration of methanolic stem bark extract of *Syzygium guineense* on body weight

The methanolic stem bark extract of *Syzygium guineense* produced significant (p<0.05) increase in body weight of male and female rats (Fig.41 and 42).

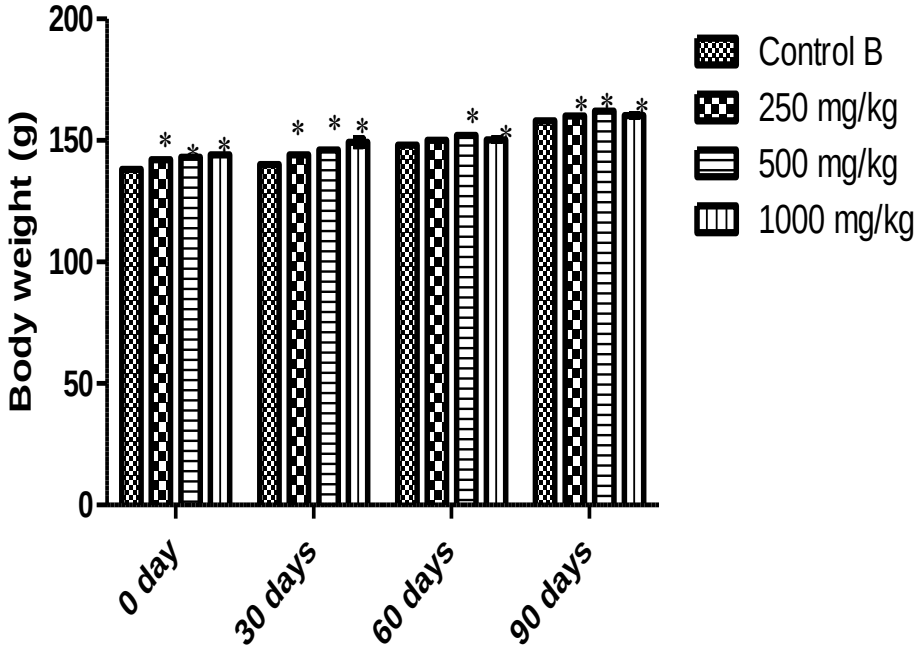


Fig. 4.41 Effect of extract of syzygium guineense on body weight gain of male rats

* Significantly different from the control at p<0.05

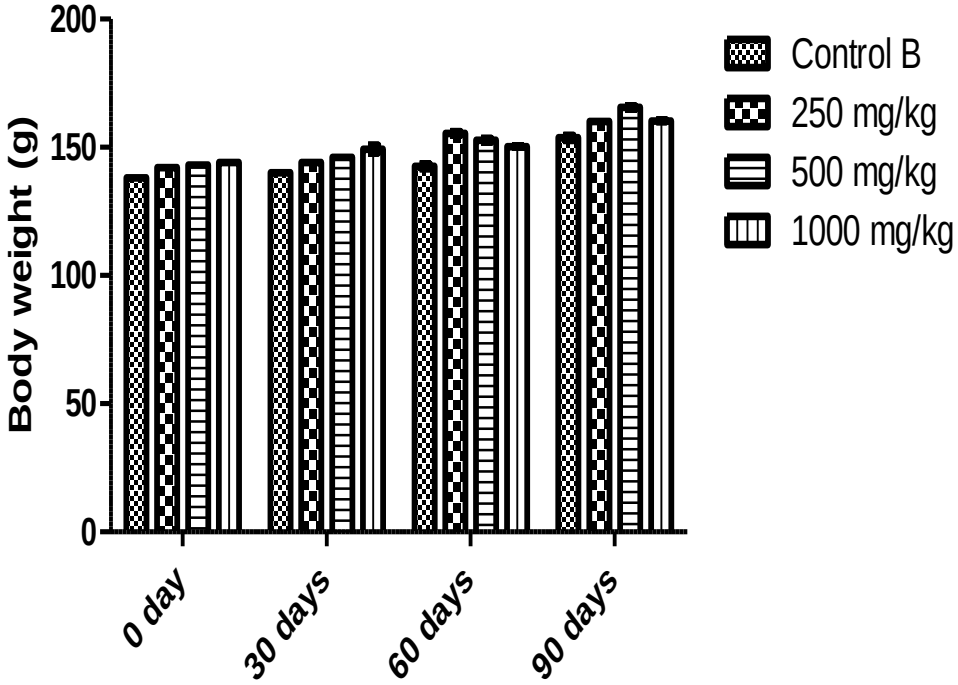


Fig. 4.42 **Effect of extract on body weight gain of female rats**

4.5.4 Effect of 42 days oral administration of methanolic stem bark extract of *Syzygium guineense* on haematological parameters in male and female rats

The heamatological parameters were normal in male and female rats (Table 4.2).

4.5.5 Effect of 90 days oral administration of methanolic stem bark extract of *Syzygium guineense* on haematological parameters in male and female rats

The heamatological parameters were normal in male and female rats (Table 4.3).

Table 4.2: Effect of 42 days oral administration of mtethanolic stem bark extract of *Syzygium guineense* on haematological parameters in male and female rats

Male rats	**control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
WBC(10^9/L)	11.00 ± 0.32	11.50 ± 0.74	11.40 ± 0.25	11.00 ± 0.63
RBC(10^{12}/L)	9.40 ± 0.50	9.20 ± 0.28	9.40 ± 0.45	9.70 ± 0.15
HB (g/dL)	10.00 ± 0.40	10.00 ± 0.50	10.00 ± 0.09	10.80 ± 0.97
HCT %	36.30 ± 0.81	36.67 ± 0.60	36.54 ± 0.29	37.00 ± 0.98
MCV(μm^3)	44.86 ± 0.16	45.14 ± 0.63	46.47 ± 1.03	46.86 ± 1.35
MCH (Pg)	21.35 ±0.41	21.61 ± 0.30	21.40 ± 0.36	21.69 ± 0.83
MCHC(g/dL)	39.96 ± 0.64	39.05 ± 0.24	39.73 ± 0.26	39.20 ± 0.54
PLT (10^3/µL)	751.00±7.37	750.00±8.34	752.20±2.10	753.60±4.83
LYM %	72.00±4.60	73.00±1.80	71.00±1.50	70.00±0.80
MNC %	7.60±0.90	7.80±0.50	7.50±0.60	7.40±0.54
NEUT %	42.80±3.50	43. 45±4.40	42.78±3.10	43.10±2.67

Female rats	**control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
WBC(10^9/L)	17.00 ± 0.41	17.70 ± 0.14	18.40 ± 0.45	18.00 ± 0.03
RBC(10^{12}/L)	7.30 ± 1.10	7.20 ± 0.28	7.40 ± 0.45	7.70 ± 0.15
HB (g/dL)	15.00 ± 0.77	15.00 ± 0.50	15.00 ± 0.69	14.00 ± 0.17
HCT %	43.00 ± 2.8	42.00 ± 1.6	43.00 ± 2.9	44.00 ± 1.20
MCV (μm^3)	45.86 ± 0.16	45.14 ± 0.63	46.47 ± 1.03	46.86 ± 1.35
MCH (Pg)	23.29 ±0.11	23.71 ± 0.30	23.00 ± 0.16	23.29 ± 0.63
MCHC(g/dL)	30.96 ± 0.64	30.05 ± 0.24	30.73 ± 0.26	31.20 ± 0.54
PLT (10^3/µL)	501.00±7.37	502.00±8.34	502.20±2.10	503.60±4.83
LYM %	72.00±4.60	72.00±9.80	72.00±3.50	73.00±2.80
MNC %	7.60±1.30	7.80±1.40	7.50±1.60	7.70±1.50
NEUT %	20.00±0.50	21.00±0.40	22.00±0.10	22.00±0.67

Table 4.3: Effect of 90 days oral administration of methanolic stem bark extract of *Syzygium guineense* on haematological parameters in male and female rats

Male rats	control	250 mg/kg	500 mg/kg	1000 mg/kg
WBC(10^9/L)	12.74 ± 0.41	12.08 ± 0.14	12.20 ± 0.17	12.96 ± 0.03
RBC(10^{12}/L)	7.50 ± 0.35	7.58 ± 0.22	7.60 ± 0.25	7.63± 0.17
HB (g/dL)	12.78 ± 0.55	12.88 ± 0.41	12.86 ± 0.05	12.88 ± 0.37
HCT %	45.80 ± 0.08	44.80 ± 0.39	44.60 ± 0.68	44.90 ± 0.30
MCV (μm^3)	63.20 ± 0.65	62.30 ± 0.52	62.83 ± 0.65	63.03 ± 0.54
MCH (Pg)	18.60 ± 0.16	18.87 ± 0.17	18.68 ± 0.60	18.93 ± 0.42
MCHC(g/dL)	26.80 ± 0.41	26.73 ± 0.13	26.76± 0.41	26.83 ± 0.27
PLT (10^3/µL)	651.00±7.37	652.00±8.34	655.20±2.10	653.60±4.83
LYM %	84.80±0.11	85.60±1.44	85.60±2.54	88.60±1.08
MNC %	3.20±0.86	3.20±0.57	3.20±0.37	3.20±0.58
NEUT %	11.00±1.63	11.20±1.20*	11.20±2.44	11.40±1.36

Female rat	**control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
WBC(10^9/L)	10.60 ± 0.77	10.30 ± 1.36	10.46 ± 2.35	10.40 ± 2.46
RBC(10^{12}/L)	5.32 ± 0.27	5.32 ± 0.43	5.60 ± 0.13	5.88 ± 0.14
HB (g/dL)	10.22 ± 0.32	10.90 ± 0.79	10.80 ± 0.08	10.98 ± 0.40
HCT %	39.60 ± 1.32	40.80 ± 2.04	41.00 ± 0.71	42.40 ± 1.44
MCV (μm^3)	50.20 ± 0.65	50.00 ± 0.82	53.83 ± 1.45	52.83 ± 1.54
MCH (Pg)	15.20 ± 0.16	15.17 ± 0.17	15.83 ± 0.60	15.33 ± 0.42
MCHC(g/dL)	28.80 ± 0.31	28.83 ± 0.31	31.50 ± 1.41	28.83 ± 0.17
PLT (10^3/µL)	719.20± 1.52	720.40±1.60	724.00±1.40	722.20±1.76
LYM %	72.40±1.82	72.50±0.63	72.32±2.36	73.60±2.14
MNC %	5.60±0.25	5.40±0.68	5.42±0.66	5.50±0.49
NEUT %	14.60±1.94	14.80±3.37	14.00±1.67	14.20±0.07

4.5.6 Effect of 42 days oral administration of methanolic stem bark extract of *Syzygium guineense* on liver function parameters in male and female rats

The methanolic stem bark extract did not produce adverse effect on the liver function parameters in both male and female rats after 42 days treatment (Table 4.4).

4.5.7 Effect of 90 days oral administration of methanolic stem bark extract of *Syzygium guineense* on liver function parameters in male and female rats

There was no significant difference in liver function parameters in male and female rats following 90 days of oral administration of Syzygium guineense stem bark extract (Table 4.5).

Table 4.4: Effect of 42 days oral administration of methanolic stem bark extract of *Syzygium guineense* on liver function parameters in male and female rats

Male rats	**control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
ALP (IU/L)	45.00±0.60	44.00±0.39	45.60±0.77	46.00±0.51
AST (IU/L)	35.00±0.37	35.00±0.25	35.00±0.19	35.00±0.33
ALT (IU/L)	37.00±0.13	38.00±0.11	36.50±0.60	36.00±0.11
T/Bil(mg/dL)	4.10±0.28	4.30 ± 0.37	4.20 ± 0.37	4.30±0.70
T/chol(mg/dL)	33.00±0.51	34.00±0.44	32.40±0.63	33.10±0.45
Trig (mg/dL)	133.40±0.50	134.00±0.54	133.00±0.56	133.21±0.51
HDL-chol (mg/dL)	38.00±0.25	40.00±0.26	40.00±0.39	40.00±0.40
LDL-chol (mg/dL)	15.85±0.60	16.00±0.58	16.44±0.04	15.70±0.50
Female rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
ALP (IU/L)	56.00±0.22	54.00±0.49	56.00±0.55	56.00±0.51
AST (IU/L)	34.00±0.37	35.00±0.25	35.00±0.19	35.00±0.33
ALT (IU/L)	56.00±0.13	57.00±0.11	56.50±0.60	56.00±0.11
T/Bil(mg/dL)	5.10±0.28	5.30 ± 0.37	5.20 ± 0.37	5.30±0.70
T/chol(mg/dL)	83.00±0.51	84.00±0.44	82.40±0.63	85.10±0.45

Trig (mg/dL)	124.00±0.24	125.00±0.24	123.00±0.76	123.21±0.21
HDL-chol (mg/dL)	37.00±1.25	37.00±1.26	38.00±2.39	38.00±1.40
LDL-chol (mg/dL)	19.00±0.60	20.00±0.68	20.44±0.24	20.00±0.59

Table 4.5: Effect of 90 days oral administration of methanolic stem bark extract of *Syzygium guineense* on liver function parameters in male and female rats

Male rats	**control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
ALP (IU/L)	156.00±0.28	156.60±0.58	157.00±.1.66	178.40±0.60
AST (IU/L)	141.00±0.69	142.20±0.20	141.20±1.77	142.60± 0.40
ALT (IU/L)	100.20±0.44	102.00±0.36	101.80±0.16	103.40± 1.50
T/Bil(mg/dL)	5.40±0.81	5.45±0.50	5.32 ±0.58	5.56±0.82
T/chol(mg/dL)	60.60±0.23	59.60±0.71	60.20±0.40	60.40±0.40
Trig (mg/dL)	34.80±0.18	35.80±0.74	34.60±0.99**	33.20±0.42
HDL-chol (mg/dL)	46.80±0.16	46.60±0.21	47.00±1.27	46.40±0.043
LDL-chol (mg/dL)	24.00±0.75	25.20±0.89	24.40±0.75	25.40±0.65
Female rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
ALP (IU/L)	146.00±1.28	145. 60±1.38	146.00±.1.66	146.40±2.60

AST (IU/L)	125.7.2 ± 0.80	127.20±0.80	125.20±1.77	126.60± 1.40
ALT (IU/L)	130.20±0.44	131.00±0.36	132.80±0.16	131.40±0.50
T/Bil(mg/dL)	4.26± 0.31	4.25±0.50	4.30 ±0.38	4.27±0.82
T/chol(mg/dL)	56.40±0.23	55.80±0.71	56.20±0.40	55.40±0.40
Trig (mg/dL)	33.80±0.18	34.80±0.74	34.60±0.99	35.20±1.42
HDL-chol (mg/dL)	34.80±0.16	34.60±0.21	35.00± 1.7	35.40±0.04
LDL-chol (mg/dL)	20.00±0.12	21.20±0.36	21.40±1.74	22.90±0.47

4.5.8 Effect of 42 days oral administration of methanolic stem bark extract of *Syzygium guineense* on electrolyte, urea and creatinine in male and female rats

There was no significant change in serum levels of electrolytes, urea and creatinine in male and female rats, after 42 days of oral administration of methanolic stem bark extract, values were within the normal range (Table 4.6).

4.5.9 Effect of 90 days oral administration of methanolic stem bark extract of *Syzygium guineense* on electrolyte, urea and creatinine in male and female rats

There was no significant change in serum levels of electrolytes, urea and creatinine in male and female rats, after 90 days of oral administration of methanolic stem bark extract, values were within the normal range (Table 4.7).

Table 4.6: Effect of 42 day oral administration of methanolic stem bark extract of *Syzygium guineense* on Electrolyte, Urea and Creatinine in male and female rats

Male rats	**control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Na+ (mmol/L)	134.00±0.76	133.00±0.55	135.00±1.97	135.00±2.73
K+ (mmol/L)	14.00±0.27	13.98±0.12	14.00±0.13	13.60±0.60
CL- (mmol/L)	97.40±0.20	99.20±1.58	97.85±0.11	96.40±0.86
HCO^-_3 (mmol/L)	24.60±0.51	24.20±0.97	24.20±0.58	23.60±0.13
Urea (mg/dL)	10.50±0.25	8.60±0.56	8.65±0.73	8.70±0.10
Creatinine(mg/dL)	54.00±0.31	52.90±0.34	53.00±0.31	54.00±0.31
Female rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Na+ (mmol/L)	122.00±0.76	122.50±0.55	123.60±1.97	123.80±2.73
K+ (mmol/L)	13.00±0.67	12.50±0.72	12.80±0.63	13.60±0.60
CL- (mmol/L)	85.00±1.20	84.00±1.58	86.00±1.11	87.00±1.86
HCO^-_3 (mmol/L)	22.60±0.51	22.20±0.97	23.20±0.58	23.60±0.13
Urea (mg/dL)	6.15±0.85	6.60±0.56	6.30±0.73	6.70±0.10
Creatinine(mg/dL)	70.54±0.31	70.90±0.34	70.60±0.31	70.50±0.31

Table 4.7: Effect of 90 day oral administration of methanolic stem bark extract of *Syzygium guineense* on electrolyte, urea and creatinine in male and female rats

Male rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Na+ (mmol/L)	134.00±0.76	133.00±0.55	135.00±1.97	135.00±2.73
K+ (mmol/L)	14.00±0.27	13.98±0.12	14.00±0.13	13.60±0.60
CL- (mmol/L)	85.40±0120	85.20±1.58	86.85±0.11	86.40±1.86
HCO^-_3 (mmol/L)	24.60±0.51	24.20±0.97	24.20±0.58	23.60±0.13
Urea (mg/dL)	12.80±0.25	12.70±0.56	12.71±0.73	12.70±0.10
Creatinine(mg/dL)	34.00±0.31	35.90±0.34	35.00±0.31	34.00±0.31
Female rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Na+ (mmol/L)	122.00±0.76	122.50±0.55	123.60±1.97	123.80±2.73
K+ (mmol/L)	13.00±0.67	12.50±0.72	12.80±0.63	13.60±0.60
CL- (mmol/L)	85.00±1.20	84.00±1.58	86.00±1.11	87.00±1.86
HCO^-_3 (mmol/L)	22.60±0.51	22.20±0.97	23.20±0.58	23.60±0.13
Urea (mg/dL)	6.15±0.85	6.60±0.56	6.30±0.73	6.70±0.10
Creatinine(mg/dL)	70.54±0.31	70.90±0.34	70.60±0.31	70.50±0.31

4.5.10 Effect of 42 days administration of methanolic stem bark extract on relative organ weight ratio in male and female rats

The relative organ weight ratio values were not significantly changed after 42 days of oral administration of methanolic stem bark extract (Table 4.8).

4.5.11 Effect of 90 days administration of methanolic stem bark extract on relative organ weight ratio in male and female rats

The relative organ weight ratio values were not significantly changed after 90 days of oral administration of methanolic stem bark extract (Table 4.9).

Table 4.8: Effect of 42 days administration of methanolic stem bark Extract on Relative Organ Weight ratio in Male and Female rats

Male rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Liver	3.20±0.29	3.20±0.54	3.32 ± 0.20	3.28±0.23
Stomach	1.20±0.11	1.23±0.39	1.28±0.71	1.30±0.65
Lungs	1.76±0.15	1.74±0.34	1.75±0.22	1.77±0.58
Kidneys	0.74±0.52	0.73±0.00	0.73±0.42	0.72±0.11
Spleen	0.98±0.01	0.91±.06	0.98±0.05	0.93±0.05
Brain	0.80±0.35	0.87±0.44	0.67±0.57	0.69±0.36
Testes	0.75±0.27	0.60±0.13	0.61±0.16	0.90±0.21
Intestine	0.51±0.11	0.65±0.06	0.39±0.07	0.48±0.04
Heart	0.45±0.04	0.42±0.07	0.48±0.03	0.48±0.08
Female rats	**control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Liver	3.40±0.09	3.30±0.64	3.35±0.29	3.38±0.23
Stomach	1.20±0.01	1.22±0.09	1.24±0.01	1.25±0.05
Lungs	0.76±0.25	0.74±0.24	0.75±0.22	0.76±0.18
Kidneys	0.63±0.02	0.62±0.02	0.67±0.02	0.65±0.01
Spleen	0.38±0.71	0.37±0.60	0.38±0.70	0.38±0.55

Brain	0.64±0.05	0.65±0.04	0.65±0.07	0.63±0.06
Ovaries	0.62±0.27	0.60±0.13	0.61±0.16	0.60±0.21
Intestine	0.51±0.11	0.65±0.06	0.39±0.07	0.48±0.04
Heart	0.45±0.04	0.41±0.04	0.41±0.63	0.43±0.42

Table 4.9: Effect of 90 days administration of methanolic stem bark Extract on Relative Organ Weight ratio in Male and Female rats

Male rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Liver	3.20±0.29	3.20±0.54	3.32 ± 0.20	3.28±0.23
Stomach	1.20±0.11	1.23±0.39	1.28±0.71	1.30±0.65
Lungs	1.76±0.15	1.74±0.34	1.75±0.22	1.77±0.58
Kidneys	0.74±0.52	0.73±0.00	0.73±0.42	0.72±0.11
Spleen	0.98±0.01	0.91±.06	0.98±0.05	0.93±0.05
Brain	0.80±0.35	0.87±0.44	0.67±0.57	0.69±0.36
Testes	0.75±0.27	0.60±0.13	0.61±0.16	0.90±0.21
Intestine	0.51±0.11	0.65±0.06	0.39±0.07	0.48±0.04
Heart	0.45±0.04	0.42±0.07	0.48±0.03	0.48±0.08
Female rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Liver	3.40±0.09	3.30±0.64	3.35±0.29	3.38±0.23
Stomach	1.20±0.01	1.22±0.09	1.24±0.01	1.25±0.05
Lungs	0.76±0.25	0.74±0.24	0.75±0.22	0.76±0.18
Kidneys	0.63±0.02	0.62±0.02	0.67±0.02	0.65±0.01
Spleen	0.38±0.71	0.37±0.60	0.38±0.70	0.38±0.55

Brain	0.64±0.05	0.65±0.04	0.65±0.07	0.63±0.06
Ovaries	0.62±0.27	0.60±0.13	0.61±0.16	0.60±0.21
Intestine	0.51±0.11	0.65±0.06	0.39±0.07	0.48±0.04
Heart	0.45±0.04	0.41±0.04	0.41±0.63	0.43±0.42

4.5.12 Effect of 42 and 90 days oral administration of methanolic stem bark extract of *Syzyggium guineense* on histology of organs

There were no abnormalities seen in organs studied in both male and female rats after the two treatment periods of 42 and 90 days.

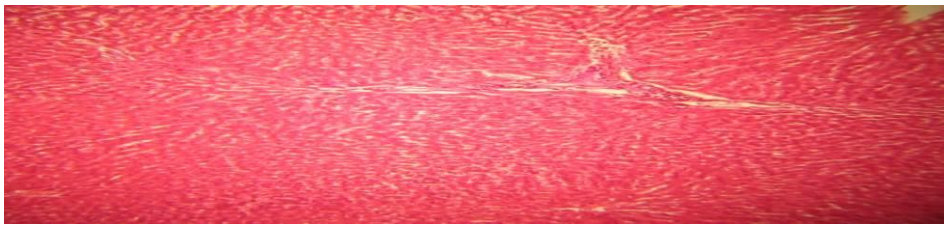


Plate III: Histotological findings in liver from control groups treated with 10 ml normal saline/kg body weight orally for 42 and 90 days of treatment showing **normal lobular architecture of the liver (HE x40)**

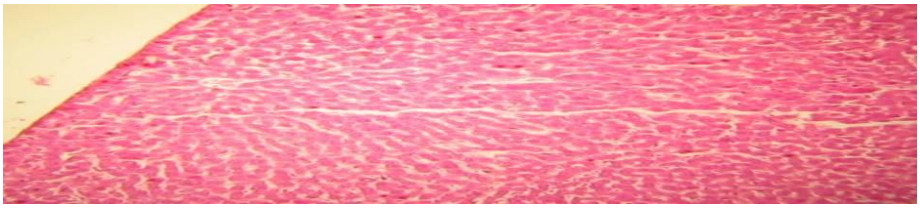


Plate IV: Histotologic findings in liver of rats treated with 250,500 and 1000 mg/kg of methanolic stem bark extract of *Syzygium guineense* orally for 42 and 90 days showing **normal lobular architecture of the liver** (HE x40)

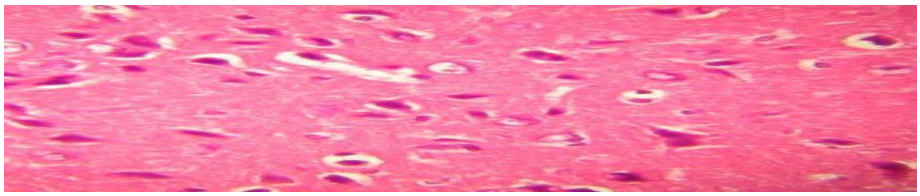


Plate V: Histotologic findings in brain of control group rats treated with 10 ml normal saline/kg body weight orally for 42 and 90 days showing **normal brain architecture (HE x40)**

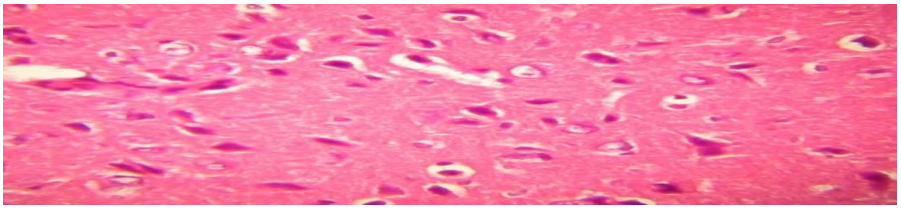


PLATE VI: Histotologic findings in brain from groups treated with 250, 500 and 1000 mg/kg body weight of methanolic stem bark extract of *Syzygium guineense* orally for 42 and 90 days showing **normal brain architecture (HE x40)**

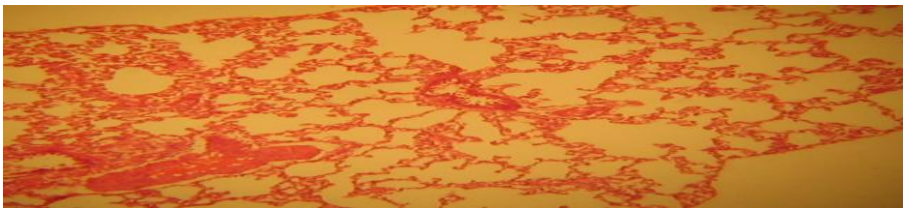


Plate VII: Histopathologic findings in lungs of control rats treated with 10 ml normal saline/kg body weight orally for 42 and 90 days showing **normal normal lung architecture (HE x40)**

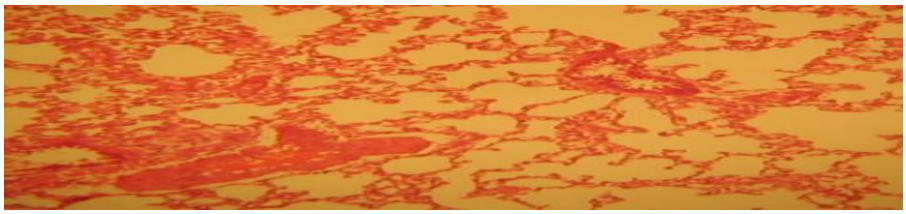


Plate VIII: Histopathologic findings in lungs of rats treated with 250, 500 and 1000 mg /kg body weight of methanolic stem bark extract of *Syzygium guineense* orally for 42 and 90 days showing **normal normal lung architecture (HE x40)**

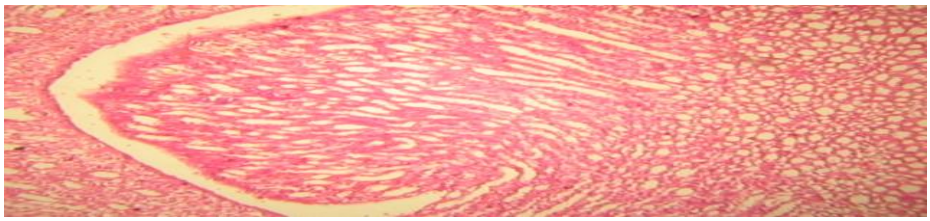


Plate IX: Histopathologic findings in kidney of control rats treated with 10 ml normal saline/kg body weight orally for 42 and 90 days showing **no abnormality (HE x40)**

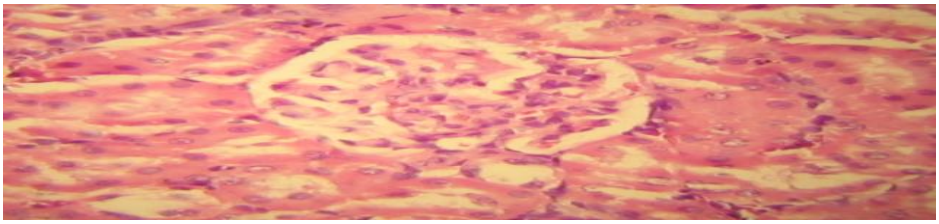


Plate X: Histotologic findings in kidney from groups treated with 250, 500 and 1000 mg/kg body weight of methanolic stem bark extract of *Syzygium guineense* orally for 42 and 90 days **showing no abnormality (HE x40)**



Plate XI: Histologic findings in testes of control group treated with 10 ml normal saline/kg body weight orally for 42 and 90 days showing **fairly uniform seminiferous tubules and spermatogenesis (HE x40)**

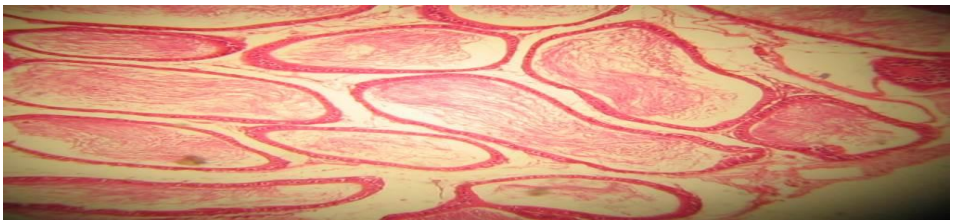


Plate XII: Histologic findings in testes of male rats treated with 250, 500 and 1000 mg/kg body weight of methanolic stem bark extract of *Syzygium guineense* orally for 42 and 90-days **showing normal distribution of seminiferous tubules and spermatogenesis (HE x40)**

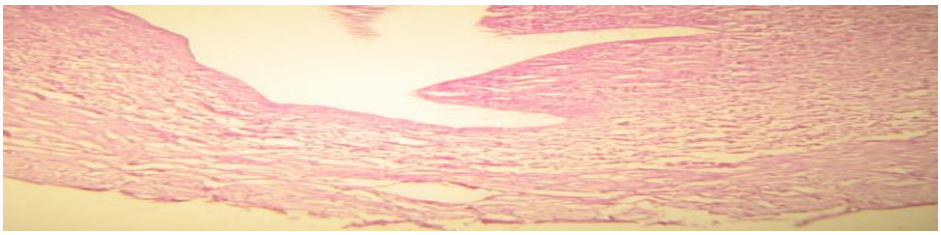


Plate XIII: Histologic findings in ovary from control group (female rats) treated with 10 ml normal saline/kg body weight orally for 42 and 90-days showing **no abnormality (HE x40)**

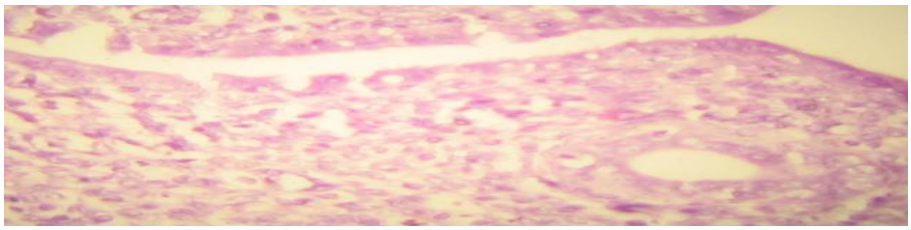


Plate XIV: Histologic findings in ovary from groups (female rats) treated with 250, 500 and 1000 mg/kg body weight of methanolic stem bark extract of *Syzygium guineense* orally for 42 and 90-days **showing no abnormality**

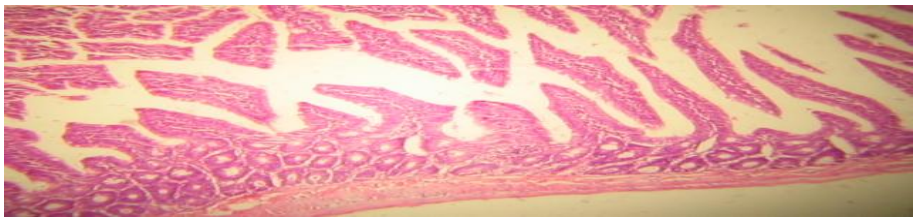


Plate XV: Histotological findings in small intestine of control rats at treated with methanolic stem bark extract of *Syzygium guineense* orally for 42 and 90 days showing **normal mucosa of the intestine (HE X40)**

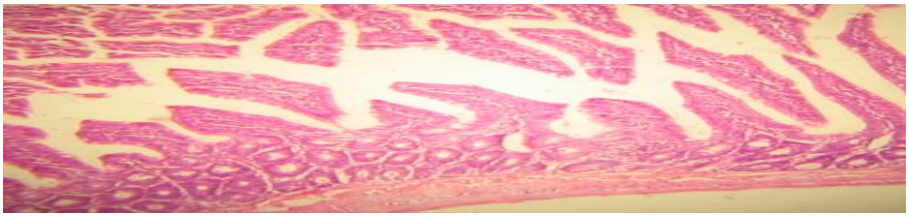


Plate XVI: Histological findings in small intestine of rats treated with 250, 500 and 1000 mg /kg body weight of methanolic stem bark extract of *Syzygium guineense* orally for 42 and 90 days showing **unremarkable changes in the intestine (HE X40)**

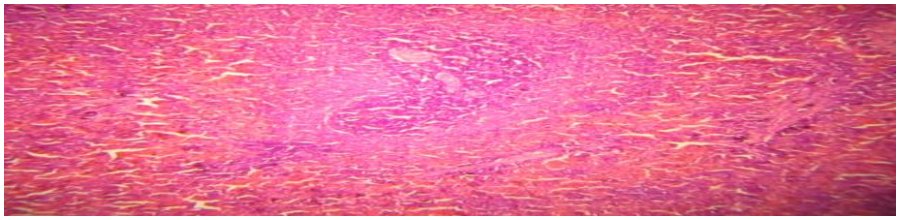


Plate XVII: Histological findings in spleen of controlrats treated with 10 ml normal saline/kg body weight orally for 42 and 90 days showing **no abnormality (HEX40)**

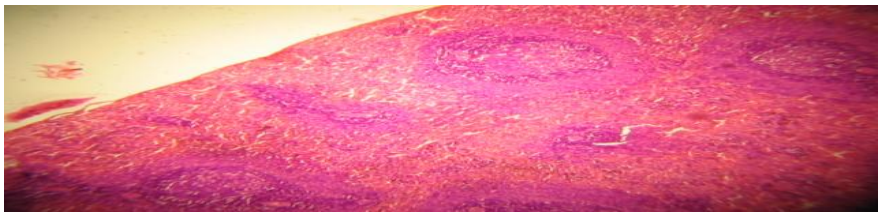


Plate XVIII: Histological findings in spleen of rats treated with 250, 500 and 1000 mg /kg body weight of methanolic stem bark extract of *Syzygium guineense* orally for 42 and 90 days showing **no abnormality (HEX40)**

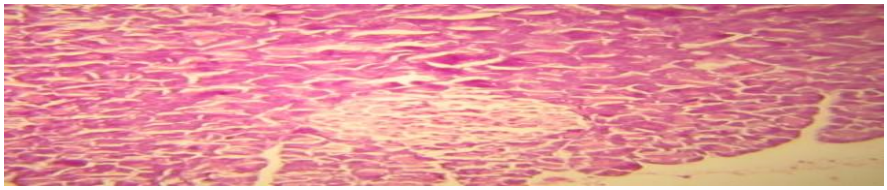


Plate XIX: Histological findings in pancreas of control groups treated with 10 ml normal saline/kg body weight orally for 42 and 90 days post treatment showing **no abnormality (HEX40)**

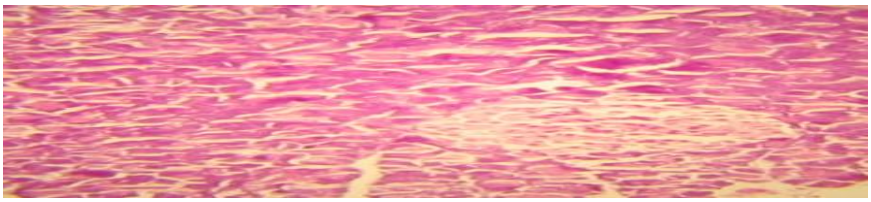


Plate XX: Histological findings in pancreas of groups treated with 250, 500 and 1000 mg /kg body weight of methanolic stem bark extract of *Syzygium guineense* orally for 42 and 90 days **showing normal architecture (HEX40)**

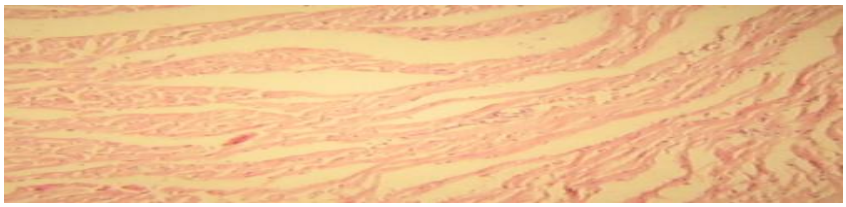


Plate XXI: Histological findings in heart from control group treated with 10 ml normal saline/kg body weight of methanolic stem bark extract of *Syzygium guineense* orally for 42 and 90- days showing **normal architecture (HE x40)**

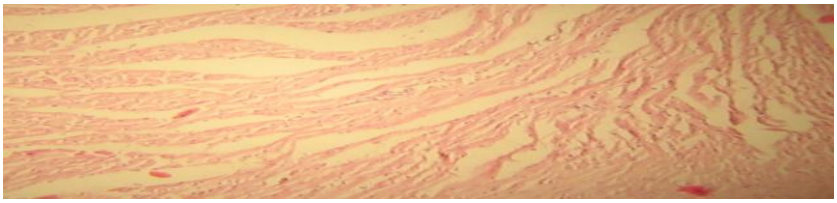


Plate XXII: Histologic findings in heart of groups treated with 250, 500 and 1000 mg /kg body weight of methanolic stem bark extract of *Syzygium guineense* orally for 42 and 90 day showing **normal architecture of the heart (HEX40)**

CHAPTER 5.0 DISCUSSION

Medicinal plants are the richest bio-source of drugs of traditional systems of medicine, folk medicines, modern medicines, pharmaceutical intermediates, chemical entities for synthetic drugs, nutraceuticals and food supplements (Hammer *et al*., 1999; Ncube *et al*., 2008). The standard extraction methods (e.g. maceration, percolation etc) used pharmaceutically involves the separation of biologically active potion of plant tissues from the inactive/inert components by using selective solvents. The extract may contain complex mixture of many medicinal plant metabolites such as alkaloids, terpenoids or flavonoids. Properties of a good solvent in plant extractions include, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The choice of the solvent is influenced by what is intended with the extract. It should not interfere with the process of bioassay (Ncube *et al*., 2008; Das *et al.,* 2010). Water is a universal solvent, used to extract plant products. Traditional healers use water primarily for plant extraction, however, extracts from organic solvents have been found to give more consistent activity compared to water extracts (Das *et al.*, 2010).

Methanol is one of the most versatile solvents. It has both polar and non-polar characteristics. It has been used to extract bioactive compounds from various parts of plants and plants based food-fruits & vegetables (Peschel *et al.*, 2006). It is capable of extracting polar and non-polar bioactive components from plant material such that the extract would be rich in chemicals responsible for its biological activities (Chan *et al.*, 2000). The therapeutic indications of extracts from medicinal plants used in folk medicine are attributed to combinations of secondary metabolites (Amos *et al.*, 2001).

The acute toxicity studies recorded no adverse effects or death in the animals treated with oral administration of methanolic stem bark extract of *Syzygium guineense* at over 5000mg extract/kg body weight. The results indicate that aqueous methanolic stem bark extract of *Syzygium guineense* is acutely safe and practically non-toxic orally (Salawu *et al*., 2008). The safety margin of the extract with oral median lethal dose of greater than 5000mg extract/kg may have been responsible for its long term use in ethnomedicine for managing ailments.

Phytochemicals that were found to be present in methanolic stem bark extract of *Syzygium guineense* have been reported to have cardiac inhibitory activities in laboratory animals and clinically. Orallo (2003), reported inhibitory effect of (+) nanetine, an alkaloid isolated from *Platycapnos spicata* in several rat isolated tissues. He attributed the observed effect to α_1- adrenergic receptor blocking and 5- HT 2A receptor blocking properties whereas at high concentrations it may also be due to decreased Ca^{2+} influx through trans-membrane Ca^{2+} channels (Ca antagonist activity).

Hippachine, an alkaloid from *Crinum macowanni* was shown to exhibit a negative chronotropic and ionotropic effects in the isolated rat heart and was responsible partly or fully for the cardiovascular effects of *C. Macowann* (Mugabo *et al., 2012).*

Antioxidant properties of flavonoids have a major effect in the vascular system. A few clinical studies have suggested that flavonoids intake protects against Chronic Heart Disease (Cook and Samman, 1996; Aggrawal, 2011). Roza *et al.* (2007), reported an inverse correlation between flavonoids intake and total plasma cholesterol.

Physiological evidence by Lahlou *et al.,* (2002) showed that 1, 8-cineole, a terpenoid oxide present in many plant essential oils elicits hypotensive in either anaesthesised or

conscious rats due to active vascular relaxation rather than withdrawal of sympathetic tone. Cardioprotective effects appear to involve an inhibition of free radical formation rather than direct free radical scavenging.

Pharmacologically, cardiac glycosides have two prominent effects. Firstly, they increase contractility of the heart by allowing Ca to accumulate in the cell with resultant increase in force and duration of the contraction and decrease the ability of the heart to pump Ca out of the cardiac cell leading to increased contractility of the heart (increased ionotropic effect).

Secondly, they decrease AV node conduction. The AV node thus stays closed for longer period and the electrochemical activity going from the atrium to the ventricles is decreased (decreased chronotropic effects). This helps prevent atrial fibrillation from becoming ventricular fibrillation.

An additional effect of cardiac glycosides is negative dromotropic activity i.e. decreased conduction of the heart cells.

The results showed that the crude methanolic stem bark extract of *Syzygium guineense* (5– 40 mg/kg) has dose-dependent significant lowering of both systolic and diastolic effects on the blood pressure (BP) of anaesthetized normotensive cat. Extracts, fractions and phytochemicals from many medicinal plants including *Mareya micrantha* (Euphorbiaceae) (Abo *et al.,* 2000), *Bumbusa vulgaris* (Poaceae) (N'guessan *et al*., 2009), *Anarcadium occidentale* (Anacardiaceae) (Tchikaya *et al*., 2003), *Gossypium barbadense* (Malvaceae) (Hasrat *et al*., 2004) *and Hibiscus sabdariffa* (Malvaceae) (McKay *et al*., 2010) have been reported to possess blood pressure lowering effects in many animal models. The decrease in BP induced by the

methanolic stem bark extract of *Syzygium guineense* is observed to be similar to those produced by acetylcholine (Ach).

The comparative study of the interaction of atropine and methanolic stem bark extract of *Syzygium guineense* and that of atropine and Ach, support the possible involvement of cholinergic mechanism in the hypotensive effect of methanolic stem bark extract of *Syzygium guineense*. Ach is one of the many neurotransmitters in the autonomic nervous system (ANS) and the principal neurotransmitter in all autonomic ganglia and parasympathetic postganglionic nerve ending. At the latter site, Ach interacts with both muscarinic and nicotinic receptors to produce its pharmacological actions. The muscarinic receptors are subdivided into M_1, M_2, M_3, M_4 & M_5 receptors. The M_2 are non-neuronal receptors of smooth muscles, cardiac muscles and glandular epithelium (Brown and Laiken, 2010).

In cardiac tissue, Ach has an inhibitory effect. When it is released from vagal parasympathetic neurons, it has a direct vasodilator action on arterioles. It has three primary effects upon the heart: vasodilatation in all beds including pulmonary and coronary, decreased heart rate (negative chronotrophic effect) and decrease in force of contraction (negative inotropic effect). The dilatation of vascular beds is due to the presence of muscarinic receptors in the endothelium, which when stimulated release some factor (endothelium derived relaxing factor) which diffuses through the smooth muscle in the media and causes relaxation. It decreases spontaneous depolarisation by reducing the rate of phase 4 depolarisation and hyperpolarising the resting cell membrane in the SA node. It also slows conduction in atrial muscles, AV node bundle of His and Purkinje System.

Atropine is antimuscarinic agent. It has little effect on nicotinic receptors, autonomic ganglia and neuromuscular junction (NMJ). It is a non-selective antagonists of M_1 and M_2 receptors. Small doses of atropine causes a transient fall in heart rate due to central effect by acting on the sinoatrial node or by its effects on central muscarinic receptors increasing vagal activity. Atropine is capable of blocking peripheral vasodilatation caused by Ach or other choline esters; when administered alone at usual doses it has little effect (Julu, 1992.; Montano *et al*., 1998; Brown and Laiken, 2010).

The result indicated that adrenaline (1-4µg/kg) produced significant concentration dependent increase in both the diastolic and systolic blood pressure of anesthetized normotensive cat. Comparative study of interaction of methanolic stem bark extract of *syzygium guineense* and adrenaline however, indicated the failure of adrenaline to reverse the hypotensive effect of the extract on both the diastolic and systolic blood pressure of anaesthetized normotensive cat; and this could suggest the involvement of non adrenergic neurones in the hypotensive activity of extract.

Noradrenaline, adrenaline and dopamine are collectively referred to as catecholamines. These endogenous compounds and the drugs that resemble them functionally and structurally are also called sympathomimetics. Adrenoceptor subtypes can be subtly distinguished by their affinity for different agonists and antagonists and, in even more cases by their structures. The following classification scheme had been adopted: α_1, α_2, β_1, β_2 and β_3 for adrenoceptors and D_1, D_2, D_3, D_4 D_5 for dopamine receptors. β_1 receptors predominate in cardiac tissues while B_2 receptors predominate in smooth muscles and gland tissue; individual tissues may however contain both receptors in varying concentrations (Westfall *et al*., 2010). Adrenaline is a very potent vasopressor that evokes a characteristic rise in blood pressure, rapidly peaking in direct proportion to the administered dose. The

mechanism of the rise in blood pressure with adrenaline is three fold: direct myocardial stimulation (positive *inotropic* effect), increased heart rate (positive *chronotropic* effect) and peripheral vasoconstriction. Vasoconstriction is most noticeable in the skin, mucosa and kidney along with marked constriction of veins. The action is on smaller arterioles and precapillary sphincters. The direct stimulation of the heart is via β_2 receptors of the myocardium and conducting tissues. The effect of adrenaline on smooth muscles depends largely upon the receptor type predominant in the muscle concerned. The predominant receptors on the stomach are α_2 and β_2, while those on intestine are α_1 and $\beta_{1,2}$. The effect of adrenaline on them is relaxation with constriction of their sphincters (Westfall *et al.,* 2010).

Phenoxybenzamine an α adrenergic blocker. It binds covalently to α receptor, producing irreversible and insummountable antagonism. It is a haloalkylamine, selectively and relatively block α_1 receptor directly. The blockade of α_1 receptors produces peripheral vasodilatation and consequent fall in blood pressure (Westfall, *et al*. 2010).

The hypotensive effect of the methanolic stem bark extract of *Syzygium guineense* observed in normotensive cat suggests that the stem bark extract possesses therapeutic potential in managing hypertension. The observed hypotensive effect was dose dependent. Cholinergic antagonist atropine, blocked the hypotensive effect of the extract. This could suggest that the extract might have acted as cholinergic agonist on non neuronal M_2 cholinergic receptors on myocardium to exert negative ionotropic and chronotropic effect. It might also act on muscarinic receptors on the vascular beds to cause vasodilatation. Another suggestion is also hyperpolarisation of the resting membrane potential in the sino atrial node there by slowing conduction in arterial muscles, Atrioventricular node and purkinjee system

The methanolic stem bark extract of *Syzygium guineense* decreased contraction of isolated guinea pig atria in a concentration dependent manner. The effect of the contraction was however reversed by adrenaline. This could suggests competitive antagonism of β_2 adrenergic receptor at lower doses on this isolated tissue.

The result obtained from the isolated perfused heart preparation (Longendorf Experiment) showed decrease in the contraction of the heart. This might result from blockade of myocardial muscarinic receptors, non-specific β blockade of adrenergic receptors or hyperpolarisation of the resting membrane potential of the sino atrial node. This further confirmed the hypotensive effect observed in the blood pressure study on anesthesized normotensive cat, and the lowering of contraction in isolated atrial preparation.

The relaxant effect of the methanolic stem bark extract of *Syzygium guineense* on smooth muscles of the rabbit ileum was blocked by phenoxybenzamine and this would suggest that the extract might have effect on adrenergic receptors; this effect also supports its antispasmodic and antidiarrhoeal action (Nigatu, 2004).

The five fractions, SGF_1 – SGF_5 obtained from partial purification of the methanolic stem bark extract of *Syzygium guineense* through column chromatography produced different effects on the cat blood pressure. They were tested for blood pressure lowering effect and all produced a decrease in the blood pressure except fraction SGF_1 which increased it. Fraction F_3 was found to be the most active in the blood lowering effect. It also decreased the contractions of Guinea pig atria and isolated rabbit heart. Phytochemical screening of SGF_3 revealed the presence of flavonoids, glycosides, phenols, cardiac glycosides and terpenoids. All of which have been reported to have antihypertensive activity. Blood pressure is a product of cardiac

output and the total peripheral resistance. The cardiac output is determined by the force of contraction of the myometrium (ionotropic effect) and the heart rate (chronotropic effect). The relaxant effect may result in reduced heart rate, force of contraction and ultimately decrease in cardiac output. The decrease in cardiac output and possible relaxation of peripheral smooth muscles in the peripheral arterioles and venules will decrease the blood pressure. The observed hypotensive effect of the methanolic stem bark extract of *Syzygium guineense* and its fractions on whole animal study and intact preparations may have been due to the biological effects of the phytochemical constituents on the cardiovascular system. The actions might involve multiple mechanisms: cholinergic receptor mechanisms, competitive antagonism at the adrenergic receptor sites, and direct vasodilatation of the blood vessels through the release of endothelium derived relaxing factor (EDRF).

A subchronic toxicity study in animals is aimed at predicting of toxicity and provision of guide line for selecting a safe dose of a drug for human use (Rhiouani *et al*., 2008). The World Health Organisation (WHO, 1992) recommends 90 days subchronic toxicity testing through the intended route of administration for any drug meant for use for up to 1-6 months.

A decrease in body weight is a useful indicator of adverse effects of drugs and chemicals (Raza *et al*., 2002; Teo, *et al*, 2002). The administration of the methanolic stem bark extract of *Syzygium guineense* for 42 and 90 days respectively was not accompanied by decrease in body weight.

Data on haematological parameters from preclinical animals have a higher predictive value for human toxicity when translated into clinical use (Olson *et al*., 2000). The 42 and 90 days oral administration of the methanolic stem bark extract of *Syzygium*

guineense had no adverse effect on haematological parameters of male and female rats. This could suggest that the extract does not affect the pluripotent stem cells or any of its products in heamopoeitic system in the bone marrow. There was no significant difference in the haematological parameters from those of the control. The RBC, WBC, Hb, PLT, LYM, MNC, NeuT were all normal.

The kidney function parameters were also not affected. The values of the electrolytes, BUN and creatinine obtained were not different from those of the control. This could suggest that the integrity of the nephrons had not been affected by the extract.

Elevated serum levels of Aminotransferases in general (AST, ALT) are associated with hepatocellular damage (lifeinthefastlane.com; liverfoundation.org).They are not generally associated with cholestasis. AST is released into serum in propotion to cellular damage and is most elevated in acute cellular necrosis. It is found in decreasing level in: liver heart, skeletal muscles, brain, pancreas, red blood cells. It is useful in detection and diagnosis of hepatic diseases. ALT is mainly in the liver cells and is relatively a specific indicator of hepatocellular damage. ALT, AST and ALP levels are good indicators of liver function (Martin *et al*., 1981). The 42 and 90 days oral administration of the methanolic stem bark extract of *Syzygium guineense* did not produce adverse effects on the liver enzymes and lipid profile studied in both male and female rats. The biomarkers of liver function ALP, AST, ALT, T/BIL, T/Chol, Trig, HDL and LDL were not different from those of the control. This may suggest that the extract is not hepatotoxic and there was no heamolysis or cholestasis as the level of total bilirubin was normal compaired to control.

In multi-dose general toxicology studies of 7 days to one year in duration, it is recommended that liver, heart, kidneys brain, adrenal glands and testes/ovary should

be weighed in all specie (Sellers *et al*., 2007). There was no significant change observed in the relative organ to body weight of the target organs: liver, kidney brain, stomach, heart, testes, intestine after 42 and 90 days administration of the methanolic stem bark extract to male and female rats. The increase or decrease of organ weight may suggest treatment related abnormality. The abnormalities could be hepatocellular hypertrophy in liver, cardiomegaly in the heart, renomegaly in kidneys encephaledema in the brain. None of these was recorded and the extract could be considered safe and non toxic to target organs. This is confirmed by the results of histopathological studies of all the organs studied which did not reveal lesions in all the tissues. The methanolic stem bark extract of *Syzygium guineense* may thus be considered safe and effective for therapeutic management of hypertension.

CHAPTER 6.0 SUMMARY CONCLUSION AND RECOMMENDATION

Hypertension, sustained rise in blood pressure (BP), is a global public health issue. It is one of the risk factors for cardiovascular diseases. Access to good quality, and therapeutically cost effective medicines particularly at primary care level are vital to the treatment and control of blood pressure. Many medicinal plants are used in traditional system of medicine for the treatment of hypertension. These claims have to be scientifically evaluated to justify their continued use in folkloric medicine. The search for effective and affordable alternative therapeutic agent in treating hypertension from natural resource with minimal adverse effect using scientific phyto-pharmacological models necessitated this study.

The results of the study revealed that the methanolic stem bark extract of *Syzygium guineense* possesses potent hypotensive activity. Extraction of the dry powdered plant material was performed by maceration using methanol. Phytochemical screening and column chromatography of the extract were performed. Fingerprinting, an important quality control tool for for qualification,quantification and standardisation of the components of herbal samples was done using shimadzu HPLC system and employed Bienvenu *et al* (2000) method. The hypotensive effect of the methanolic stem bark extract of *Syzygium guineense* and fractions were studied in vivo and *in vitro* after the acute toxicity studies. Blood pressure of anaesthesized normotensive cat was determined by method of cannulation of carotid artery. Isolated tissue preparations of guinea pig atria, rabbit ileum, perfused rabbit heart and rat uterus were performed to identify the possible mechanism of action.

The oral median lethal dose (LD_{50}) of methanolic stem bark extract of *Syzygium guineense* was found to be greater than 5000mg/kg body weight which can be considered practically profit. Results of acute and chronic toxicity studies thus indicated that the crude extract is non-toxic and safe.

Phytochemical analysis suggested the presence of alkaloids, flavonoids, cardiac glycosides terpenoids, saponins, sterols, glycosides and phenols. The partial purification of the crude extract yielded five fractions and fraction SG F_3 was found to be most active.

The methanolic stem bark extract of *Syzygium guineense* and fraction SGF_3 revealed hypotensive effects in whole animal and intact preparation studies. The hypotensive activity may have been mediated by a combination of cholinergic receptor mechanism (muscarinic receptors), irreversible antagonism of adrenoceptors and/or direct relaxant effect via release of endothelial derived relaxing factors.

No treatment- related adverse effects were observed after 42 and 90 days repeat dose toxicity studies in the physiological parameters examined or in the evaluation of blood tissue samples taken from the rats at the end of the studies. This could be considered an indication of its safety. The ability of the methanolic stem bark extract of *Syzygium guinense* to lower systolic and diastolic blood pressures of anaesthetised normotensive cat, reduce height of contraction in atrial preparation; and reduce force of contraction, height of contraction and rate of heart beat in isolated heart preparation have produced useful data in identifying it as an alternative potent and safe therapeutic agent for managing hypertension. It has also established the scientific basis for its folkloric use in the treatment of hypertension. Further research on its antihypertensive and safety pharmacology and the establishment of the chemical structure and class of the active

principle(s) are strongly recommended. The studies that can be carried out on the cardiovascular activities of the methanolic stem bark extract of *Syzygium guineense* include: antihypertensive activities in established animal models, *in vivo* and *in vitro* cardio protective, anti-oxidative and anti-inflammatory activities of the plant and safety pharmacological studies.

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APPENDICES

Appendix I: Tyrode Solution

Reagents	Quantity/ 5 Litre
NaCl	45g
10%KCl	10ml
10%$NaH_2PO_4 2H_2O$	2.5ml
D. glucose	5g
$NaHCO_3$	5g
10%$CaCl_2$	10ml
$MgCl_2.6\ H_2O$	0.5ml
Aerating gas	O_2 or air

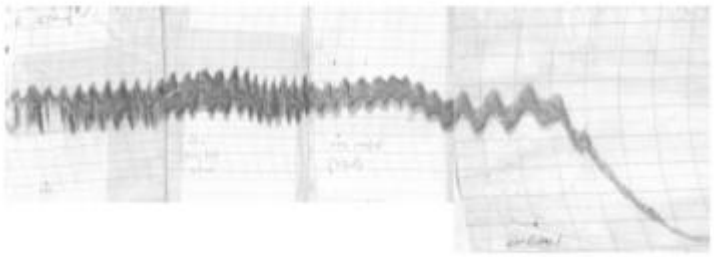
Appendix II: De- Jalon's Solution

Reagents	**Quantity/ 5 Litre**
NaCl	45g
10%KCl	21ml
D. glucose	2.5g
$NaHCO_3$	2.5g
$CaCl_2$(Molar)	10ml
$MgCl_2.6\ H_2O$	1.35ml
Aerating gas	$95\%O_2 + 5\%CO_2$

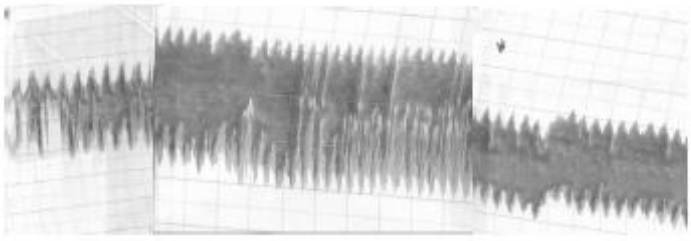
Appendix III: Ringer-Lock Solution Preparation

Reagents	Quantities for 5 litres
NaCl	45g
10% KCl	41ml
D. glucose	5g
$NaHCO_3$	2.5g
$CaCl_2$ (molar)	5.4ml
Aerating gas	Pure O_2

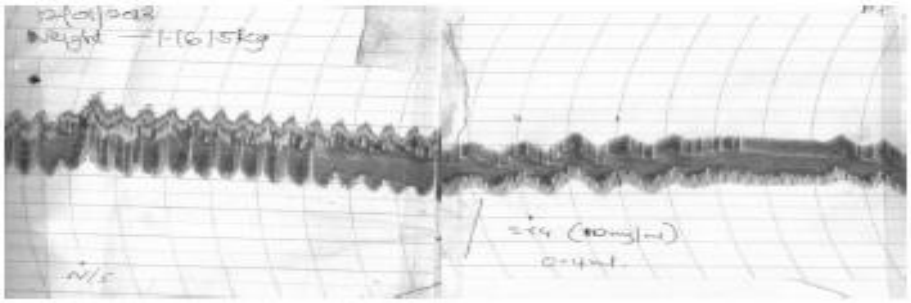
Appendix IV: Tracing showing the effect of methanolic stem bark extract of *Syzygium guineense* in normotensive cat



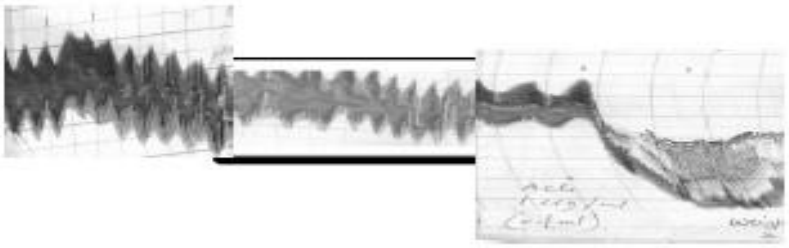
Appendix V: Tracing showing the effect of adrenalin and methanolic stem bark extract of *Syzygium guineense* on blood pressure of cat



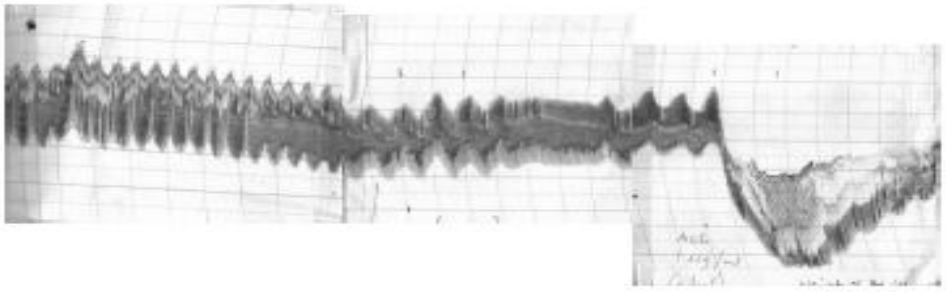
Appendix VI: Tracing showing the interaction of Atropine and methanolic stem bark extract of *Syzygium guineense* on Blood Pressure of normotensive cat



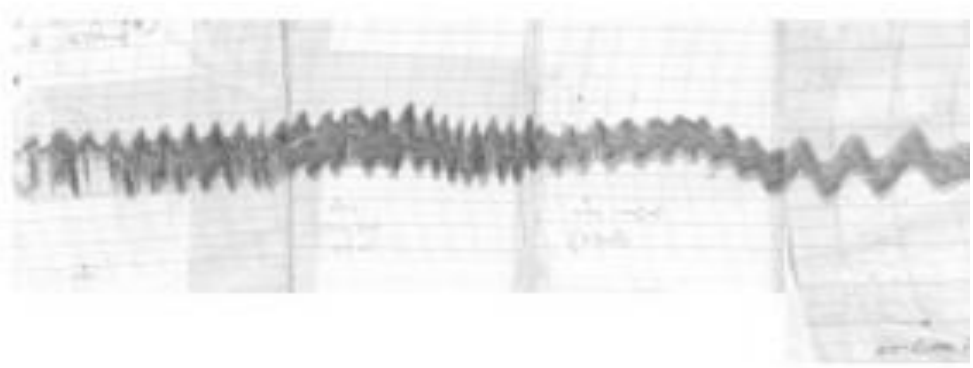
Appendix VII: Tracing showing the effect of acetylcholine alone on Blood Pressure of normotensive cat



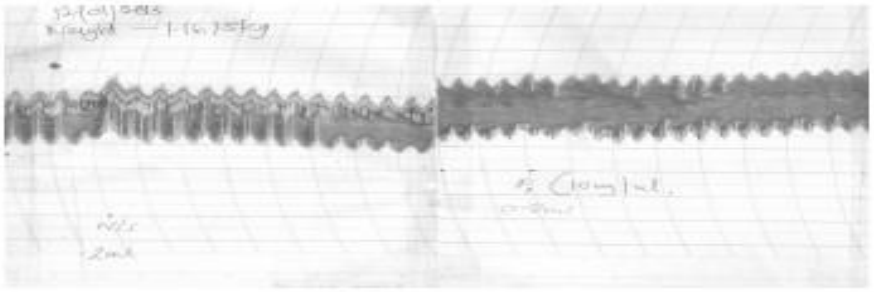
Appendix VIII: Effect of normal saline, Acetylcholine alone, *Syzygium guineense* extract, interaction between atropine and acetylcholine and interaction between atropine and SG extract on Cat blood pressure



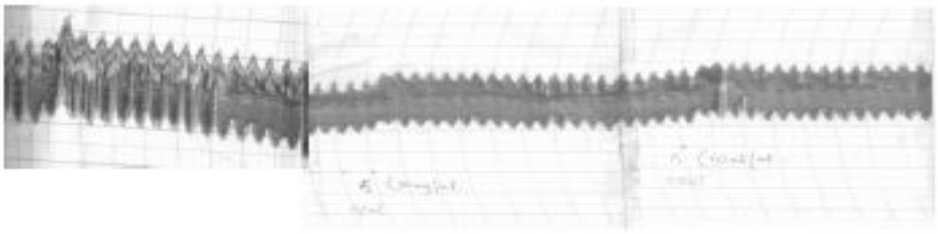
Appendix IX: Effect of adrenaline on hypotensive effect of methanolic stem bark extract of *Syzygium guineense* in normotensive cat



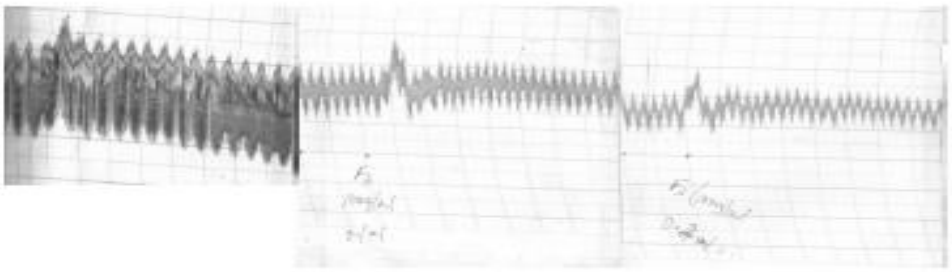
Appendix X: Effect of Fraction SGF1 on diastolic and systolic blood pressure of normotensive cat



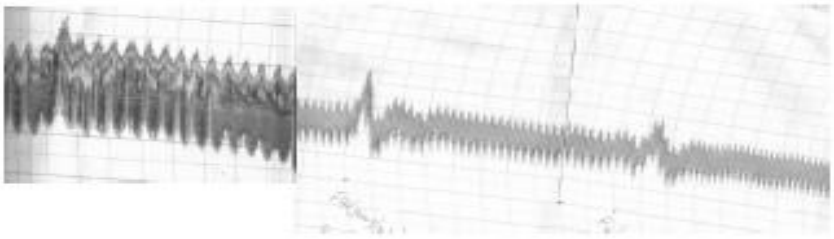
Appendix XI: Effect of Fraction SGF2 on diastolic and systolic blood pressure of normotensive cat



Appendix XII: Effect of Fraction SGF3 on diastolic and systolic blood pressure of normotensive cat



Appendix XIII: Effect of Fraction SGF4 on diastolic and systolic blood pressure of normotensive cat



Appendix XIV: Effect of Fraction SGF5 on diastolic and systolic blood pressure of normotensive cat

