EFFECT OF AQUEOUS EXTRACT *Gnetium africanum* ON SOME CARDIAC FUNCTION PARAMETERS IN ISOPRENALINE INDUCED MYOCARDIAC INFARCTION IN RATS

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BENIN CITY.

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A PROJECT WRITTEN IN THE DEPARMENT OF PHARMACOLOGY AND TOXICOLOGY AND SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DOCTOR OF PHARMACY IN THE FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

OCTOBER, 2023.

CERTIFICATION

This is to certify that this work was successfully carried out by **EDUKUGHO ORITSEGBUBEMI** in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City.

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Professor Okpo Date

(Project Supervisor)

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(Head of Department)

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Edukugho Oritsegbubemi Date

(Student)

DEDICATION

I wholeheartedly dedicate this work to God Almighty my all sufficiency, my wife Kikachukwu Edukugho, Ekene Okonkwo and my in-laws.

ACKNOWLEDGMENT

I give all the thanks to the Almighty God for seeing me through in this work.

My sincere gratitude and appreciation goes to Professor Okpo, my Project Supervisor. I am truly honored to have had the privilege of working with you throughout this project. Your guidance, encouragement and willingness to share your knowledge and insights have been invaluable to me. I am truly grateful Sir.

I would like to extend my gratitude to all members of the staff (academic and non academic) of the Department of Pharmacology and Toxicology, University of Benin, Benin City for their cooperation and support, which played an essential role in the project's success. Their contributions were crucial in achieving my project's goals and objectives.

I am also indebted to my lovely wife and the Okonkwo family for their immense support. Also to Ekene Okonkwo who has been instrumental financially and whose advice has been so helpful towards the completion of my Pharm. D programme.

In closing, I want to express my gratitude to all members of staff of Faculty of Pharmacy and fellow students for providing not only the conducive atmosphere that allowed me to do my study but also the chance to attend and interact with so many fascinating individuals.

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ABSTRACT

Cardiovascular Diseases (CVD's) remain the dominant course of mortality in developed and developing countries. Due to changing life styles, socio-economic status and decline in provision of healthcare services in developing countries such as Nigeria, myocardial infraction is making a significant contribution to national healthcare burden and mortality statistics.

Aim: This present study evaluated the effect of aqueous extract of *Gnetum africanum* on some cardiac function biomarkers in isoprenaline-induced myocardial infarction in rats.

Methodology:

Acute toxicity test and haemotological and biochemical analysis of the extract was done using standard methods. Wister rates aged 2 – 3 months weighing 150 to 200 grams were acclimatized for 2 weeks and grouped into 4 (A – D) groups.

B and C orally received graded doses of extracts (B = 50, C = 100, mg/kg body weight) daily for 28 days. Group A served as control and group D served as standard group 2ml/kg of cargradenol Blood samples (5ml) were collected into ethylene diamine tetracetic acid (EDTA) containers and analysed using haemetological automety following manufacturers guidelines. Isoprenaline was induced 85mg/kg on 26^{th} and 27^{th} day in all groups.

Result

Gnetum africanium extract displayed no accurate toxicity up to 5g/kg; at doses of 50mg/kg and 100mg/kg, it demonstrated no significant effects on cardiac biomarkers when compared with the control against isoprenaline-induced myocardial injury in rats across parameters including organ weight, body weight changes, cardiac biomarkers and oxidative-antioxidant balances.

Conclusion

Despite the traditional therapeutic use and notable tolerance in acute toxicity tests, the specific therapeutic potential of *Gnetum africanium* in cardio parameters requires further exploration.

CHAPTER ONE

1.0 INTRODUCTION

Myocardial infarction (MI) is defined as an acute coronary syndrome characterized by neo-onset or rapid worsening angina that occurs at rest with evidence of myocardial necrosis.

Incidence and risk factors occurs at virtually any age, 8 – 10% of myocardial occurs in people younger than age 65. MI frequency rises progressively with increasing age with incidence correlating significantly with genetic and behavioral predispositions increasing the relative rise of MI in their middle ages while women are relatively protected during their reproductive years. (Ziped, *et al., 2012)*.

Pathogenesis

Coronary arterial occlusion when exposed to subendotila collagen and necrotic plaque content, platelets adhere becoming activated release their agencies content and aggregate to form micro plug. Vasospasm is stimulated by mediators released from platelets.

Tissue factor activates the coagulation pathway adding to the bulk of the plug and this occluding the vessel. Lifestyle modification and diets (weight control, lipid lowering diet) are of importance. (Ashrath *et al.,* 2007; Maegrogan *et al.,* 2010).

Prevention/Treatment:

1. Regular Exercise:
2. Antiplatelet therapy (adirin and or cloxidogrel) GP IIb/IIIa inhibit B blockers) to reduce 0_2 demand, ACE inhibitors/ARB.

3. Statins

Additional therapy for control of diabetes and hypertension mineral corticoid/receptor antagonist. Anti c oagulants therapy with fractionated heparin, low molecular weight heparin direct thrombin inhibitors and/or factor X1 inhibitors to percent clothing of blood.

Nitrates to induce vasodilatation, the early biochemical consequences of myocardial ischemia is the cessation of aerobic metabolism within seconds leading to inadequate production of high energy phosphate (e.g. creatinean phosphate and adenosine triophosphate and accumulation of lactic acid because of the exquisite dependence of myocardial function on oxygen and nutrients myocardial contractility increases within a minute or so of the unset of severe ischemia. Nevertheless, these early manifestation of ischemia of ischemia injury are potentially reversible in severe ischemia lasting 20 – 40 minutes or longer irreversible damage (necrosis) of cardiac myocytes occurs. This delay is the onset of permanent myocardial injury provides the rationale for rapid diagnosis in acute MI – to permit early coronary intervention to establish reperfusion and salvage as much "at risk" myocardium as possible. (Kottwitz *et al.,* 2020).

The earliest detectable feature of myocyte necrosis is the disruption of the integrity of the sarcolemmal membrane, allowing intracellular macromolecules to leak out of necrotic cells into the cardiac interstitium and ultimately into the microvasculature and lymphatics.

This escape of intracellular myocardial proteins into the circulation forms the basis for blood tests that can sensitively detect irreversible myocyte damage, and are important for managing MI (see later). With prolonged severe ischemia, injury to the microvasculature follows injury to the cardiac myocytes. The temporal progression of these events is summarized. (Patten *et al.,* 2012).

The progression of ischemic necrosis is the myocardium is summarized in figure 1. Due to the myocardial perfusion pattern from epicardium to endocardium, ischemia is most pronounced in the subendocardium: thus, irreversible injury of ischemic myocytes occurs first in the subendocardial zone. With more extended ischemia, a wavefront of cell death moves through the myocardium to encompass progressively more of the transmural thickness and breadth of the ischemic zone. The precise location, size, and specific morphologic features of an acute MI depend on:

- The location, severity, and rate of development of coronary obstruction due to atherosclerosis and thromboses
- The size of the vascular bed perfused by the obstructed vessels
- The duration of the occlusion
- The metabolic and oxygen needs of the myocardium at risk (Mensah *et al.,* 2020)

Table 12-4 Approximate Time of Onset of Key Events in Ischemic Cardiac Myocytes

Feature	Time
Onset of ATP depletion	Seconds
Loss of contractility	<2 min
ATP reduced	
to 50% of normal	10 min
to 10% of normal	40 min
Irreversible cell injury	20-40 min
Microvascular injury	>1 hr

ATP, Adenosine triphosphate.

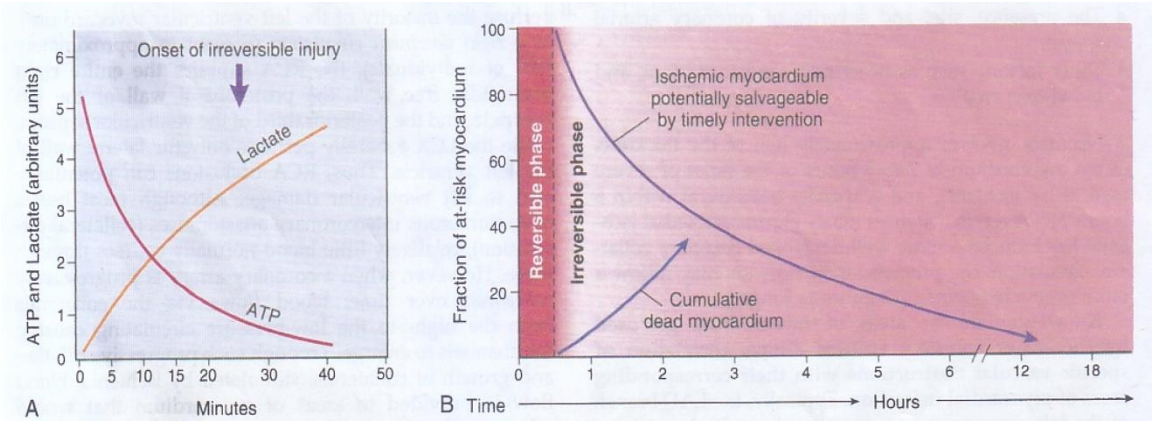


Figure 1: Temporary sequence of early biochemical findings and progression of necrosis after onset of severe myocardial ischemia. A, early changes include loss of adenosine triphosphate (ATP) and

accumulation of lactate. B, for approximately 30 minutes after the onset of even the most severe ischema, myocardial injury is potentially reversible. Thereafter, progressive loss of viability occurs that is complete by 6 to 12 hours. The benefits of reperfusion are greatest when it is achieved early and are progressively lost when reperfusion is delayed.

1.1 ISOPRENALINE

Isoprenaline (isoproterenol) is a sympathomimetic that acts almost exclusively on beta-adrenergic receptors. It is listed in the 2004 WHO Model List of Essential Medicines. It is used to increase the heart rate for the treatment of patients with severe bradycardia that is unresponsive to atropine; for the short-term emergency treatment of heart block; for ventricular arrhythmias secondary to atrioventricular nodal block during electrophysiological study, to facilitate the induction of supraventricular and ventricular tachycardias. Isoprenaline (Figure 1) is known to accelerate the sinus node and to enhance AV nodal conduction; the drug has no effect on HisPurkinje conduction time. Paradoxical bradycardia is an unusual phenomenon. Pharmacological alternatives include atropine and for Torsades de Pointes magnesium sulfate. Cardiac pacing is an option for the treatment of patients with bradyarrhythmias or Torsades de Pointes. (Zipe *et al.,* 2011).

Pharmacology

Pharmacodynamic

- **Cardiovascular system:** Isoproterenol produces powerful stimulation of the heart to increase its rate and force of contraction, causing increased cardiac output. It is as active as epinephrine in this action and, therefore, is useful in the treatment of atrioventricular block or cardiac arrest, Isoproterenol also dilates the arterioles of skeletal muscle (132 effect), resulting in decreased peripheral resistance, Because of its cardiac stimulatory action, it may increase systolic blood pressure slightly, but it greatly reduces mean arterial and diastolic blood pressure.

- **Pulmonary system:** A profound and rapid bronchodilation is produced by the drug (132 action). Isoproterenol is as active as epinephrine and rapidly alleviates an acute attack of asthma when taken by inhalation (which is the recommended route). This action lasts about 1 hour and may be repeated by subsequent doses.
- **Other effects:** Other actions on 3- receptors, such as increased blood sugar and increased lipolysis, can be demonstrated but are not clinically significant. (MacGrogan *et al.,* 2010).

Pharmacokinetics

Isoproterenol can be absorbed systemically by the sublingual mucosa but is more reliably absorbed when given parenterally or as an inhaled aerosol. It is a marginal substrate for COMT and is stable to MAO action.

Adverse effects: The adverse effects of Isoprenaline are similar to epinephrine.

- Cardiovascular: Angina, flushing, hyper/Ihypotension, pallor, palpitation, paradoxical bradycardia (with tilt table testing), premature ventricular beats, Stokes-Adams attacks, tachyarrhythmia, ventrical ventricular arrhythmia.
- Central nervous system: Dizziness, headache, nervousness, restlessness, Stokes-Adams seizure.
- Endocrine & metabolic: Hokalemia, serum glucose increased.
- Gastrointestinal: Nausa, vomiting, Neuromuscular & skeletal: Tremor, weakness.
- Ocular: blurred vision.
- Respiratory: Dyspnea, pulmonary edema.

Indications

- Mild or transient episodes of heart block that do not require electric shock or pacemaker therapy.

- Serious episodes of heart block and Adams- Stokes attacks (except when caused by ventricular tachycardia or fibrillation).
- Cardiac arrest until electric shock or pacemaker therapy is available.
- Bronchospasm during anesthesia
- Adjunct to fluid and electrolyte replacement therapy and other drugs and procedures in the treatment of hypovolemic or septic shock.
- Low cardiac output states (eg, decompensated heart failure, cardiogenic shock)

Isoprenaline forms available in market

Isoprenaline hydrochloride: Isoprenaline is available as an injection containing isoprenaline hydrochloride 20mcg/mL (I – 3ml). Isoprenaline hydrochloride contains not less than 98.0 per cent and not more than the equivalent of 101.5 per cent of (1 RS)- 1 -(3 ,4-dihydroxyphenyl)2 [(I methylethyl) amino]ethanol hydrochloride, calculated with reference to the dried substance. A white or almost white, crystalline powder, freely soluble in water, sparingly soluble in alcohol, practically insoluble in methylene chloride.

Isoprenaline sulphate lsoprenal ne sulphate contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of bis[(IRS)1 -(3,4-dihydroxyphenyl)-2-{(1-methylethyl)amino]ethanol] sulphate, calculated with reference to the anhydrous substance. A white or almost white, crystalliie powder, freely soluble in water, very slightly soluble in alcohol. It melts at about 128 °C, with decomposition. (Reynolds *et al.,* 2017).

Dosage

- The Formulary recommends isoprenaline for the treatment of adults with *bradyarrhythmias* for which it is administered by intravenous infusion at a dose of 1-4mcg/minute.

- For the treatment of adults with other cardiac disorders administered by slow intravenous injection at a dose of 20 – 60 mcg adjusted according to ventricular rate.
- For adults with heart block (acute Stokes-Adams attack) administered by intravenous infusion at a dose of 4- 8 mcg/minute.

Mechanisms Of Isoprenaline Induced Myocardial Infarction

Myocardial infarction induced by ISO has been reported to show many metabolic and morphologic aberrations in the heart tissue of the experimental animals similar to those observed in human myocardial infarction. ISO induced necrosis is maximal in the subendocardial region of the left ventricle and in the interventricular septum. Continuous infusion of ISO in rats elicits typical cardiac gene expression similar to that observed in cardiac hypertrophy caused by pressure overload. Amidst several mechanisms proposed to explain the isoproterenol-induced myocardial harm, one might say: an unbalance between oxygen supply to and demand from cardiomyocytes inwardly, which is related to myocardial hyperfunction due to increase both in chronotropism and inotropism as well as to hypotension in the coronary bed. Secondly, it is also claimed that there is an elevation of Ca^{++} overcharge inside the cell. In addition, that ion is related to the activation of the adenylate cyclase enzyme and the depletion of ATP levels on the course of the events. Eventually, there is an oxidative stress augmentation because of several metabolic products originated from isoproterenol, not to mention free radicals genesis. A schematic diagram is shown to explain the mechanism of action of Isoprenaline. (Zipe *et al.,* 2011).

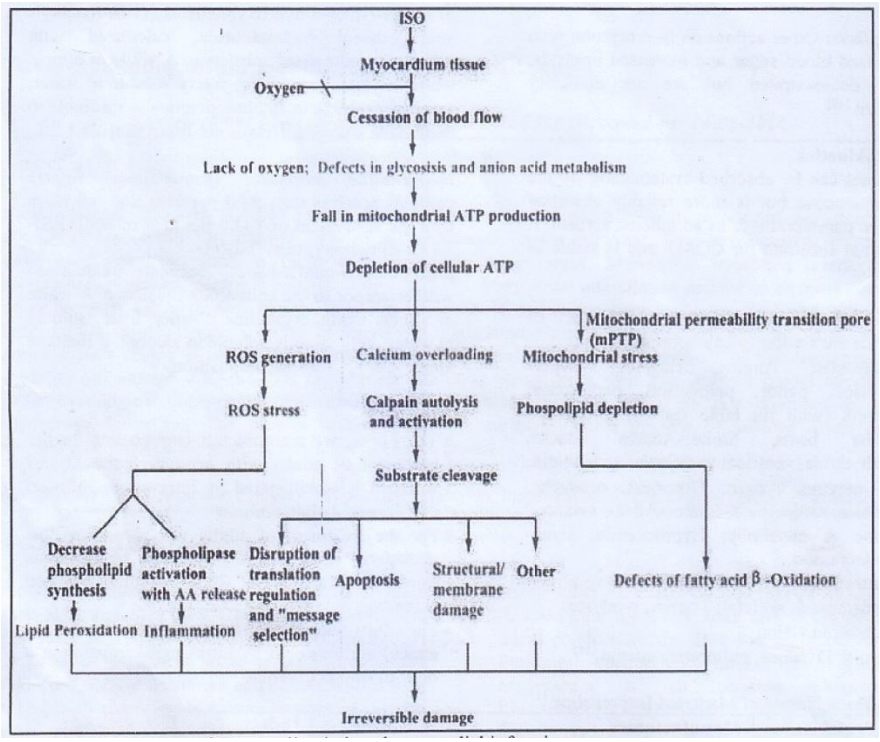


Figure 1: Mechanism of Isoprenaline Induced myocardial infarction

Mechanism Of Action/Pharmacology

Isoprenaline is a non-selective f3-adrenergic agonist. It has positive inotropic and chronotropic effects, increasing cardiac output by increasing the heart rate and cardiac contractility. Isoprenaline also decreases diastolic blood pressure by lowering peripheral vascular resistance.

Onset of action Immediate.

Duration of action (IV): 10 – 15minutes.

Half – life: 2.5 – 5 minutes.

Indications

- Heart block.
- Treatment of permanent bradycardia due to atrio-ventricular block pending with haemodynamic compromise and pending or in case of contraindication of pacemaker.
- Treatment of Adams-Stokes syndrome.

Contraindication

Hypersensitivity to isoprenaline or any of the excipients.

Concomitant use with adrenaline (if concomitant administration is required give alternatively every 4 hours).

Pre-existing ventricular arrhythmias.

Tachyarrhythmia.

Cardiac glycoside (digoxin) intoxication.

Myocardial infarction — may increase myocardial oxygen demand.

Angina — may exacerbate it.

Cautions

Hypotension due to uncorrected hypovolaemia.

Phaeochromocytoma.

Diabetic patients — isoprenaline stimulates insulin secretion thus increasing the risk of hypokalaemia.

Cases of hyperthyroidism / uncontrolled hyperthyroidism.

Cardiovascular disorders especially coronary insufficiency, arrhythmias and hypertension.

Convulsive disorders.

When using on patients who respond to sympathomimetic amines in unusual manner.

Simultaneously the muscle fibers use up oxygen in the blood, causing the hemoglobin to become totally de-oxygenated. Therefore, the infracted area takes, on a bluish-brown. In later stages, the vessel walls become highly permeable and leak fluid; the local muscle tissue becomes edematous and the cardiac.

There are two basic types of MI:

Transmural: associated with atherosclerosis involving major coronary artery. It can be sub classified into anterior, posterior, or inferior. Transmural infarcts extend through the whole

thickness of the heart muscle and are usually a result of complete occlusion of the area's blood supply.

Subendocardial: involves small area in the subendocardial wall of the left ventricle, ventricular septum, or papillary muscles. Subendocardial infarcts are thought to be a result of locally decreased blood supply, possibly from a narrowing of the coronary arteries. The Subendocardial area is farthest from the heart's blood supply and is more susceptible to this type of pathology. (Potterat, 1997).

Clinically, MI is further sub classified into ST elevation MI versus non ST elevation MI based on ECG changes.

Epidemiology

MI is a common presentation of ischemic heart disease, The WI-TO estimated that in 2002, 12.6 percent of deaths worldwide were from ischcmic heart disease. In the United States, diseases of the heart are the leading cause of death, causing a higher mortality than muscle cells begin to swell because of diminished cellular metabolism. Within a few hours of almost no blood supply, the cardiac muscle cells die. Cardiac muscle requires about 1.3 milliliters of oxygen per 100 grams of muscle tissue per minute just to remain alive. Cancer (malignant neoplasm). Coronary heart disease is responsible for I in 5 deaths in the U.S. This means that roughly every 65 seconds, an American dies of a coronary event.

In India, cardiovascular disease (CVD) is the leading cause of death. The deaths due to CVD in India were 32% of all deaths in 2007 and are expected to rise from 1.17 million in 1990 and 1.59 million in 2000 to 2003 million in 2010. Although a relatively new epidemic in Nigeria, it has quickly become a major health issue with deaths due to CVD expected to double during 1985-20 15. (Mensah GA *et al* 2020).

Legal Implication

At common law, MT is generally a disease, but may sometimes be an injury. This has implications for no-fault insurance schemes such as workers' compensation. A heart attack is generally not covered (10); however, it may be a work-related injury if it results, for example, from unusual emotional stress or unusual exertion. (11) Additionally, in some jurisdictions, heart attacks suffered by persons in particular occupations such as police officers may be classified as line- of-duty injuries by statute or policy. In some countries or states, a person who has suffered from a MI may be prevented from participating in activity that puts other people's lives at risk, for example driving a car or flying an airplane.

2. Causes

1) Oxidative Stress

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. A particularly destructive aspect of oxidative stress is the production of reactive oxygen species (ROS), which include free radicals and peroxides. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA. In humans, oxidative stress is involved in many diseases, such as atherosclerosis, MI.

ROS are metabolites of oxygen that can either strip electrons away from other molecules (oxidize), donate electrons to molecules (reduce), or react with and become part of molecules (i.e., oxidative modification). A particularly important radical for cardiovascular biology is superoxide (0_2), which is formed by the one-electron reduction of oxygen. (0_2) is important

because it can serve as both an oxidant and as a reductant in biologic systems and is a progenitor for other ROS. Endogenous production of free radicals occurs during normal aerobic metabolism. Activated oxygen intermediates arc formed by stepwise reduction of 02 to water and by secondary reactions with protons and transition metals such as Fe and Cu. The superoxide anion (0_2) is produced by many cell redox systems including isehemia-derived xanthine oxidase, aldehyde oxidase and membrane- associated NADPH oxidases. In addition, phagocytic cells, including macrophages and monocytes, increase their 0_2 uptake when stimulated and release large amounts of 0_2 – into the extracellular fluid through the action of NADPH oxidase. Although 02 is not particularly reactive, having a low second-order rate constant with biomolecules, it is capable of diffusing through relatively large distances through the cell where, in the presence of Fe and Cu, a metal-catalysed Haber-Weiss reaction is thought to occur resulting in the formation of the highly reactive hydroxyl radical (OH).

Other radicals include the lipid peroxy (L00) radical, and alkoxy- radicals (LO). Other molecules, including peroxynitrite ($ONOO^-$), hypochiorous acid ($HOCI^-$), and hydrogen peroxide (1-1202) are not radicals but have strong oxidant properties and are, therefore, included as ROS. Another relevant group of molecules are the reactive nitrogen species (RNS) including nitric oxide (NO), the nitrogen dioxide radical (NO_2), and the nitrosonium cation (NO^+). Peroxynitrite is considered both an ROS and RNS and is formed by the near diffusion-limited reaction between O_2^- and NO. RNS are important, because they often react with and modify proteins and other cellular structures and alter function of these targets.

In addition to ROS-forming enzymes, mammalian cells produce myriad molecules and enzymes that remove ROS. Some of these are small molecules, such as the thiol-containing tripeptide glutathione. Others are enzymes that catalyze removal of ROS, such as the superoxide dismutases (SODs), which catalyze dismutation of O_2 to 11202 and water;

catalase, which converts H2O2 to oxygen and water; the glutathione peroxidases, which use 1-1202 and glutathione as co-substrates to form water and glutathione disulfide; thioredoxin; and others. (Thygesenk *et al,* 2018).

Sources of reactive oxygen/nitrogen species:

NAD(P)H oxidases (Nox)

The phagocytes, including neutrophils, monocytes, and macrophages, contain a plasma membrane-bound multicomponent oxidase that utilizes NADPI-l-derived electrons and Activation of the oxidase in phagocytic cells results in large amounts of 02 - over short periods that appear to be involved in host defense. NAD(P)H oxidases in vascular cells are subject to activation by specific agonists that include angiotensin II, thrombin, platelet-derived growth factor, tumor necrosis factor-a, interleukin-, and, for endothelial cells, mechanical forces (including shear stress) and vascular endothelial growth factor.

Xanthine Oxidase

Xanthine oxidase is an iron – sulfur molybdenum flayoprotein with multiple functions that exists in two forms, xanthine dehydrogenase and xanthine oxidase, the former being predominant. The oxidation of xanthine or hypoxanthine to uric acid is associated with NADH production by the dehydrogenase, whereas the oxidase generates 0_2. The dehydrogenase is readily converted into the oxidase by proteolysis or by reversible oxidation of three groups. Xanthine oxidase contributes to impaired NO bioactivity observed with hypercholesterolemia, heavy smoking, and coronary disease.

Nitric Oxide Synthases

A relative cofactor deficiency for enzyme catalysis allows the enzyme to reduce molecular oxygen rather than transfer electrons to L arginine, thereby generating 0_2 by the oxygenase domain of the enzyme through dissociation of a ferrous – dioxygen complex that is normally

stabilized by tetrahydrobiopterin. In atherosclerosis and diabetes there is evidence that vascular tetrahydrobiopterin levels may be depressed. Nitric oxide may react with metal ions, metalloproteins, and 0_2 to form reactive nitrogen species. Perhaps the best characterized of these reactions is the combination of NO and O2 - to generate $ONOO^-$. Among the most abundant biological targets for ONOO is carbon dioxide (CO_2). The reaction of 0N00 with CO2 is complex and initially produces nitrosoperoxycarbonale which homolyzes to form a pair of caged radicals that may then diffuse apart to become free radicals, or recombine to form nitro carbonate (O_2NOCO_2), which decomposes to nitrite and CO_2. The formation of free nitrogen dioxide (NO_2) readily leads to protein tyrosine nitration and lipid peroxidation. (Monteccuco *et al.,* 2016).

Myeloperoxidase

Myeloperoxidase is the only human enzyme that generates HOC1; chlorinated biornolecules are considered specific markers of MPO-rnediated oxidation reactions. Myeloperoxidase can yield a number of products, including 3-chlorotyrosine, chlorohydrins from cholesterol and fatty acids, and tyrosyl radicals, with the latter species able to participate in single electron oxidation reactions, including the oxidation of LDL. Another activity of myeloperoxidase and HOd is to convert L-tyrosine into phydroxyphenylacetaldehyde which can react with amino phospholipids and the - amino groups of protein lysine residues. The product of these reactions may be subsequently modified by myeloperoxidase to generate a variety of reactive aldehyde residues. Furthermore, L-serine is readily converted by myeloperoxidase to Ne-(carboxyrnethyl) lysine, a well-characterized advanced glycation end-product. Thus, myeloperoxidase and 1-IOCl can generate a series of secondary oxidation products that may oxidize biornolecules, including LDL, rendering them capable of converting macrophages into foam cells.

Mitochondrial respiration

Conventional wisdom dictates that up to 1 – 2% of electron flow through the respiratory chain may be diverted to molecular oxygen. Thus; one must consider the mitochondrion as a potential major intracellular source of reactive oxygen species. Mitochondrial oxidant production is controlled, in part, by the expression of a mitochondrial Mn- containing superoxide dismutase located in the mitochondrial matrix.

2) Atherosclerosis

Atherosclerosis is the condition in which an artery wall thickens as the result of a buildup of fatty materials such as cholesterol. It is a chronic inflammatory response in the walls of arteries, in large part due to the accumulation of macrophage white blood cells and promoted by low density lipoproteins (plasma proteins that carry cholesterol and triglycerides) without adequate removal of fats and cholesterol from the macrophages by functional high density lipoproteins (FIDL). Complications of atherosclerosis are chronic, slowly progressive and cumulative. Most commonly, soft plaque suddenly ruptures causing the formation of thrombus that will rapidly slow or stop blood flow, leading to death of the tissues fed by the artery. This catastrophic event is called an infarction. One of the most common recognized scenarios is called coronary thrombosis of a coronary artery, causing MI. Even worse is the same process in an artery to the brain, commonly called stroke.

Atherosclerosis develops from low-density lipoprotein molecules (LDL) becoming oxidized by free radicals, particularly oxygen free radicals (ROS). When oxidized LDL comes in contact with an artery wall, a series of reactions occur to repair the damage to the artery wall caused by oxidized LDL. The LDL molecule is globular shaped with a hollow core to carry cholesterol throughout the body to generate brain tissues, vitamin D, and so on. Cholesterol does not dissolve in water. Cholesterol can move in the bloodstream only by being transported by LDL. (1, 20) The initial damage to the blood vessel wall results in a ‘call for

help," an inflammatory response. Monocytes enter the artery wall from the bloodstream, with platelets adhering to the area of insult. This may be promoted by redox signaling induction of factors such as VCAM-l, which recruit circulating monocytes. The monocytes differentiate macrophages which ingest oxidized LDL, slowly turning into large "foam cells" - so-described because of their changed appearance resulting from the numerous internal cytoplasmic vesicles and resulting high lipid content. Unfortunately, these white blood cells are not able to process the oxidized-LDL, and ultimately grow then rupture, depositing a greater amount of oxidized cholesterol into the artery wall. This triggers more white blood cells, continuing the cycle. Eventually, the artery becomes inflamed. The cholesterol plaque causes the muscle cells to enlarge and form a hard cover over the affected area. This hard cover is what causes a narrowing of the artery, reduces the blood flow and increases blood pressure. (Adidharma *et al.,* 2020).

3) Symptoms of MI

The onset of symptoms in Ml is usually gradual, over several minutes, and rarely instantaneous. Chest pain is the most common symptom of acute MI and is often described as a sensation of tightness, pressure, or squeezing. Other symptoms include diaphoresis (an excessive form of sweating), Shortness of breath (dyspnea), weakness, light-headedness, nausea, vomiting, and palpitations. The most common symptoms of MI in women include dyspnea, weakness, and fatigue, sleep disturbances. In women, chest pain may be less predictive of coronary ischemia than in men.

Approximately one fourth of all MI are silent, without chest pain or other symptoms. These cases can be discovered later on electrocardiograms or at autopsy without a prior history of related complaints. A silent course is more common in the elderly, in patients with diabetes mellitus and after heart transplantation, probably because the donor heart is not connected to nerves of the host.

4) Risk factors

Heart attack rates are higher in association with intense exertion, be it psychological stress or physical exertion, especially if the exertion is more intense than the individual usually performs. Quantitatively, the period of intense exercise and subsequent recovery is associated with about a 6-fold higher MI rate (compared with other more relaxed time frames) for people who are physically very fit. For those in poor physical condition, the rate differential is over 35- fold higher. One observed mechanism for this phenomenon is the increased arterial pulse pressure stretching and relaxation of arteries with each heart beat which, as has been observed with intravascular ultrasound, increases mechanical 'shear stress" on atherornas and the likelihood of plaque rupture.

Acute severe infection, such as pneumonia, can trigger Ml. A more controversial link is that between Chlamydophila pneurnoniae infection and atherosclerosis. While this intracellular organism has been demonstrated in atherosclerotic plaques, evidence is inconclusive as to whether it can be considered a causative factor. (28) Treatment with antibiotics in patients with proven atherosclerosis has not demonstrated a decreased risk of heart attacks or other coronary vascular diseases.

There is an association of an increased incidence of a heart attack in the morning hours, more specifically around 9am. (30- 32) Some investigators have noticed that the ability of platelets to ggrega1e varies according to a circadian rhythm, although they have not proven causation. (33) Some investigators theorize that this increased incidence may be related to the circadian variation in cortical production affecting the concentrations of various cytokines and other mediators of inflammation.

Risk factors for atherosclerosis are generally risk factors for MI:

Diabetes (with or without insulin resistance) - the single most important risk factor for ischaemic heart disease (IHD)

Tobacco smoking

Hypercholesterolemia (more accurately hyperlipoproteinemia, especially high LDL and low IIDL)

High blood pressure

Family history of IHD

Obesity (35) (defined by a body mass index of more than 30 kg/m2, or alternatively by waist circumference or waist-hip ratio).

Age: Men acquire an independent risk factor at age 45, Women acquire an independent risk factor at age 55; in addition individuals acquire another independent risk factor if they have a first-degree male relative (brother, father) who suffered a coronary vascular event at or before age 55. Another independent risk factor is acquired if one has a first-degree female relative (mother, sister) who suffered a coronary vascular event at age 65 or younger.

Hyperhomocysteinemia (high homocysteine, a toxic blood amino acid that is elevated when intakes of vitamins B2, B6, Bl2 and folic acid are insufficient)

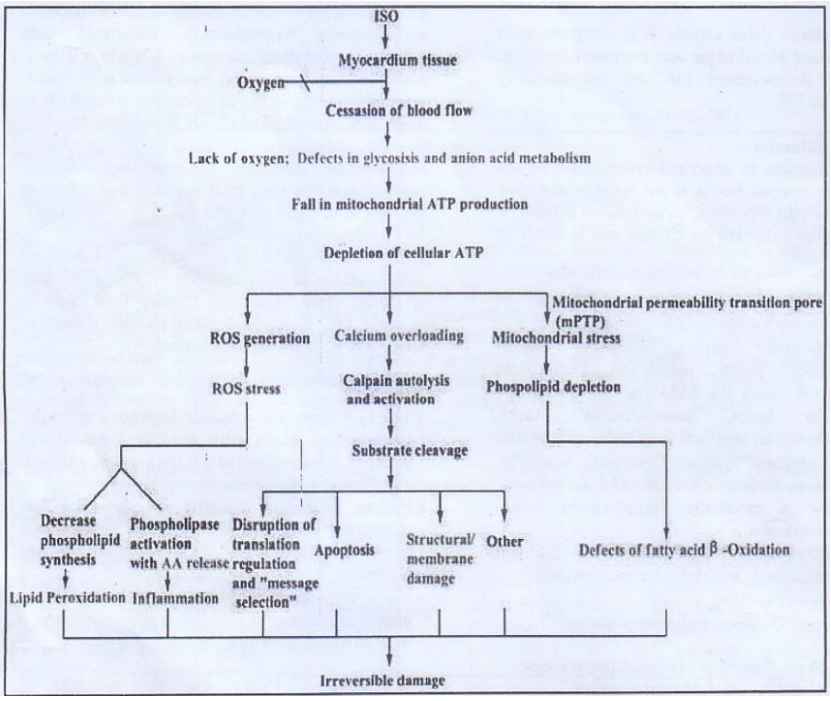
Stress (occupations with high stress index are known to have susceptibility for atherosclerosis)

Alcohol Studies show that prolonged exposure to high quantities of alcohol can increase the risk of heart attack. (Maron *et al.,* 2006).

Mechanisms Of Isoprenaline Induced Myocardial Infarction

Myocardial infarction induced by ISO has been reported to show many metabolic and morphologic aberrations in the heart tissue of the experimental animals similar to those observed in human myocardial infarction. ISO induced necrosis is maximal in the subendocardial region of the left ventrical and in the inteventricular septum. Continuous infusion of ISO in rats elicits typical cardiac gene expression similar to that observed in cardiac hypertrophy caused by pressure overload. Amidst several mechanisms proposed to explain the isoproterenol-induced myocardial harm, one might say: an unbalance between

oxygen supply to and demand from cardiomyocytes inwardly, which is related to myocardial hyperfunction due to increase both in chronotropism and inotropism as well as to hypotension in the coronary bed. Secondly, it is also claimed that there is an elevation of Ca++ overcharged inside the cell. In addition, that ion is related to the activation of the adenylate cyclase enzyme and the depletion of ATP levels on the course of the events. Eventually, there is an oxidative stress augmentation because of several metabolic products originated from isoproterenol, not to mention free radicals genesis. A schematic diagram is shown to explain the mechanism of action of Isoprenaline.



Clinical Features Myocardiac Infarction

- Diaphoresis
- Rapid seak pulse
- Nausea and vomiting

Psypnea due to impaired contractility of ischemic myocardial and resulting pulmonary congestion and edema.

Laboratory finding release of cardiac biomarker (TnT and Tn1 cardiac specific troponis T and I and creative kinase – MB from irresibly damaged myocytes. The most sensitive candlespecific biomarkers of myocardial damage are cardiac specified proteins (TnT and cTnI proteins that regulate calcium medicated contraction regulate calcium medicated contraction of cardiac and skeletal muscles.

Troponins I and T are not normally defected in the circulation following MI levels of both begin to rise at B-12 hours cTnT levels peak somewhere between 12+48 hours while cTnI levels are maximal at 24hours. Cretin kinase is an enzyme present in the brain, myocardium and skeletal muscles as a dime compound composed of two isoforms designated 'M' and 'B' while MM homodimers are found predominantly in cardiac and skeletal muscles and BB homodimers in brain, lungs and other tissues, MB heterodynes are principally located to cardiac muscles and to a lesser amount in the skeletal muscles. (Prolopio *et al,* 2017).

Thus MB form of cretine kinase (CK-MB) is sensitive but not specific to myocardial cellosine (K-MB0 can also be elevated in skeletal muscles injury. (K-MB begins to rise within 2 – 12hrs of the onset of M1 peaks at about 24hrs and returns to normal within 48 – 72 hours.

To summarize:

Time to elevation of (K-MB, cTnT and cTnI is 3 to 12hours). (K-MB and cTn1 peak at 24hrs (K-MB returns to normal in 48-72hours, cTnI in 5 – 10 days and cTnT in 5 – 14 days. (Cummins *et al.,* 1987).

1.2 CARVEDILOL

Carvedilol is nonselective β-adrenergic blocking agent with α1-blocking activity. It is (±)-1-(Carbazol-4-yloxy)-3-[[2-(omethoxyphenoxy)ethyl]amino]-2-propanol. It is a racemic mixture with the following structure:

Tablets for Oral Administration: COREG (carvedilol) is a white, oval, film-coated table containing 3.125mg, 6.25mg, 12.5mg or 25mg of carvedilol. The 6.25mg, 12.5mg and 25mg tablets are TILTAB tablets. Inactive ingredients consist of colloidal silicon dioxide, crospovidone, hypromellose, lactose, magnesium stearate, polyethylene glycol, polysorbate 80, povidone, sucrose and titanium dioxide. (Shen *et al.,* 2017).

1.3 *Gnetum Africanum*

Gnetum africanum is a dioecious forest perennial up to 10 m long but sometimes longer; branches somewhat thickened at the nodes, glabrous. Leaves decussately opposite, sometimes in whorls of 3, simple, ovate- oblong or elliptic-oblong, more rarely lanceolate, 5-13 cm long, 2- 5 cm broad, attenuate at base, abruptly acuminate, obtuse or minutely apiculate, entire, thick-papery, glabrous, pale green above, paler beneath, with 3-6 pairs of strongly curved lateral veins looped near the margin; stipules absent; petiole up to 1cm long.

Inflorescence an unbranched catkin, axillary or terminal on a short branch, solitary but male inflorescences at apex of branches often in groups of 3, up to 8cm long, jointed, peduncle 1-1.5cm long, with a pair of scale-like, triangular bracts; male inflorescence with slender internodes and whorls of flowers at nodes; female inflorescence with slightly turbinate internodes and 2-3 flowers at each node. Flowers small, c. 2 mm long, with moniliform hairs at base and an envelope; male flowers with a tubular envelope and exserted staminal column bearing 2 anthers; female flowers with cupular envelope and naked, sessile ovule.

Seed resembling a drupe, ellipsoid, 10-15 mm x 4-8 mm, apiculate, enclosed in the fleshy envelope, orange-red when ripe, with copious endosperm.

This lianoid species lacks fibre-tracheids characteristic of G. gnemon. However, tori are clearly present in tracheary elements of this species.

In Africa, there are only two species, *G. africanum* and *G. buchholzianum*. The specific epithet africanum refers to its African origin.

The plant is threatened with disappearance because of intensive gathering and cultural practices which are destroying the forests which support these plants. Possible introduction into farm systems is a step in the right direction in conserving this plant. (Okafor, 1996).

Figure 2: Leaves of *Gnetum Africanum*

Biology

This is a dioecious plant with distinct differences in male and female inflorescence structure and size. Female plants often show more vigorous growth with stronger stems than male plants.

G. africanum continues to grow during the dry season and new shoots may develop where the stem has been cut or where side shoots have been removed. New shoots are also formed from rhizomes that spread along the forest floor, The distinctly coloured drupe-like seeds are probably dispersed by birds and other animals.

Ecology

G. africanum is an endangered liane normally found in humid tropical forest. It is usually found with other climbers on middle- and under-storey trees, frequently forming thickets. It can also be found in riverine forest in areas that are otherwise too dry for the species. *Gnetum africanum* is mostly found at the periphery of primary forest and in secondary forest, it extends in distribution from SE Nigeria, to Congo and as far as Angola in the south.

Biophysical Limits

Altitude: 0-1200 m. Mean annual rainfall: 3000 mm

Documented Species Distribution

Native: Angola, Cameroon, Central African Republic, Congo, Democratic Republic of Congo, Gabon, Nigeria.

Products

Food: The leaves of this species are edible, as are those of other American and Asian Gnetum species. *G. africanum* holds an important place in the diets of many people in central Africa. In the Congo Gnetum consumption has been evaluated at 2g /capita, Women play a great role in the gathering and selling of the much relished leaves all year round. Commerce in Gnetum has increased considerably. A company, 'Paysans Centrafricains has exported leaves to Europe particularly France and Belgium.

It is a significant source of protein (16.5% dry wt.) carbohydrates (70.6% dry wt.), essential amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine), non essential amino acids (aspartic acid, serine, glutamic acid, prolineglycine, alanine, cysteine, tyrosine histidine and arginine) and mineral constituents i.e. macro and micro-elements (7.0% dry wt.). It can be eaten raw, but is generally added to meat and fish dishes after cooking the fruit and seeds are edible.

Lipids: The leaf fat content in gnetum is significant, up to 14.20% (Okafor et aI.196.).

Alcohol: The potential of African species to yield a potable sap as the Asian species is worth investigation.

Medicine: The leaves are used to treat enlarged spleen, sore throat and also used as a cathartic. The plant provides an arrow poison antidote. In West Africa chopped leaves are used as a dressing on furuncles to hasten maturation. The stem is used in making preparations to ease childbirth.

Other products: The supple stem is used in making traps for game catching and porterage straps. *Gnetum africanum* leaves contain C-glycosylflavones, including 2'-xylosylisoswertisin and 2"-glucosylisoswertisin, compounds; also presence of 2"-O-rhamnoylisoswertisin and apigenin-7-hesperidoside. (Shiembo PM, 1994).

Services

Has significant cultural value in traditional ceremonies.

Tree Management

Gnetum africanum is still mainly collected from wild stands, but farmers often retain it when clearing fields. If cultivated, farmers need to provide support, e.g. by using commercial plantations of rubber trees, oil palm and other tree crops. Fences were only found to be successful when there is enough shade, and they are generally too expensive. Fully exposed plants do not grow well: their leaves are thin and pale green, and traders reject them.

Pests And Diseases

Mealy bugs are the main pest in the nursery. When eru is grown along dead poles attacked by termites, these insects will damage adjacent leaves. Diseases have not been found to reduce productivity of eru.

1.4 AIM AND OBJECTIVES OF THE STUDY:

The aim of this study is to investigate the effect of aqueous extract *Gnetium africanum* on some cardiac function parameters in isoprenaline induced myocardial infarction in rats.

The objectives of the study were to:

a) Determine the median lethalclose (LDjo) of the ageous leaf extract of Gnetum africanum.

b) Determine the effects of the aqueous leaf extract of Gnetum africanum on some cardia biomarkers (Troponin I, CkoMB, Myoglobin, LDH, AST, ALT hsCRP) activity in rats.

c) Determine the effects of the aqueous leaf extract of Gnetum africanum on oxidant (mycloadialdehyde, MDA) and antioxidant (superoxide desmatase, catalase and reduced glutathione) activity.

d) Determine the effect of the ageous leaf extract of Gnetum africanum on the organs (heart, kidney, liver) weight to body weight ratio.

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CHAPTER TWO

MATERIALS AND METHODOLOGY

2.1 Plant Material

The leaves of *Gnetum* were purchased from the Local sellers (vendors) in New Benin Market, Benin City, Edo State. They were later identified and authenticated by a botanist at Plant Biology and Biotechnology Department, University of Benin City.

Procedures For Aqueous Extraction Of The Vegetable, *Gnetum Africanum* Procedure

The fresh leaves were picked, washed with water and ground into paste using a milling machine in the raw form, the paste was poured in a clean cloth mesh and adequately squeezed to obtain the extract.

2.2 Preparation of Extract

The wet ground leaves of *Gnetum Africanum* were extracted consecutively with 2 litres of distilled water.

Evaporation in a Rotary Vacuum Evaporator

The aqueous of *Gnetum africanum* was evaporated with the aid of a rotary vacuum evaporator to reduce the aqueous extract of the *Gnetum Africanum* filtrate into a more concentrated form for easier and faster drying in the oven.

Drying the Aqueous Extract

The aqueous extract of *Gnetum Africanum* was dried at 40 degrees centigrade. The weights of the plates before drying were 54.2g and 54.3g while the weights of the plates after drying is 70.2g and 71.6g respectively.

Experimental Animals

Female Weister rats weighing 130g to 200g were purchased from Mrs. Obi of the Department of Pharmaceutical Chemistry and the department of Pharmacology, University of Benin, Benin City. The rats were housed thereafter in the animal house, department of Pharmacology, Faculty of Pharmacy, University of Benin.

Housing and Acclimatization of Animals

The rats were acclimatized two weeks, fed with standard rat chow and had access to water adibitum Adult, male mice (n = 12) were also purchased for acute evitiy. All rats and mice handled in this study according to international Acute toxicity was carried out using the Lock's method. The test was divided into two stages.

Acute Toxicity

Stage 1

In stage 9 mice were used, divided into 3 groups consisting of 3 mice each. The 3 group were divided into different dose groups of 10mg/kg, 100mg/kg and 1000mg/kg respectively. The calculated doses of the extracts were based on the body weights of the animals using a stock solution of 100mg/ml of the *Gnetum africanum* aqueous extract. After administration of the calculated doses, the mice were observed for signs of toxicity and mortality after a 24 hours period from the last time the administration was done.

Stage 2

In the stage 2 study, 3 mice were provided each representing a dose of 1600mg/kg, 2900mg/kg and 500mg/kg respectively. The doses of the animals (mice) were calculated based on body weight. After a 24-hour period, the animals were observed for signs of toxicity and mortality.

Preparation of the Stock Solution of the Extract

3g of the dried *Gnetum Africanum* extract was weighed and dissolved in 30mls of distilled water to make a concentration of 100mg/ml of the extract. The prepared stock solution of the *Gnetum Africanum* extract was kept in the refrigerator.

To prepare a stock of 50mg/ml of the extract, 2ml of the 100mg/ml was accurately measured and 2ml of the purified distilled water was added to make a concentration of 50mg/ml of the *Gnetum Africanum* extract stock solution.

Preparation of the Stock Solution Of Carvedilol

0.7g of Isoprenaline was dissolved in 7mls of purified distilled water to make a stock concentration of 100mg/ml of Isoprenaline.

Grouping of Experimental Animals

Adult female albino rats were distributed randomly into 4 groups. Three (3) experimental groups and One (1) control group. The animals, which were from the same litter, were purchased from the animal house, Pharmacology Department, University of Benin. They were kept in the Animal House, Department of Pharmacology, faculty of Pharmacy, University of Benin to acclimatize for two weeks. They were obtained at 3 weeks old and were fed for 28 days on 3 commercial rat pellets and water ad-libitum until a range of between 120 and 160g was obtained. The animals were kept in iron cages whose dimensions are 30cm by 15cm by 25cm at room temperature. The cages and plates were cleaned thoroughly while the sawdust which served as their beddings was treated with Dettol and sun dried, and changed periodically. The rats were subsequently distributed randomly and placed in one control and three experimental groups.

Group 1 (**Distilled Water**):- This was the control group and they were fed with distilled water, without any of the extracts.

Group 2 (50mg/Kg): This group was fed with 50mg/kg body weight of *Gnetum africanum*.

Group 3 (100mg/Kg): This group was fed with an equal (100mg/kg body weight) weight of *Gnetum Africanum* extract.

Group 4 (Carvedilol 2mg/Kg): This group was treated with 2mg/kg body weight of *Gnetum Africanum* extract only. All the rats in each group were fed adlibitum with commercial rat pellet and water daily.

Treatment Protocol

Animals in the Distilled water group were fed with the calculated doses of the distilled water for 28 consecutive days, while animals in the 50mg/kg and 100mg/kg, groups were fed with the calculated doses of the *Gnetum Africanum* extract for 28 consecutive days. Animals in the Carvedilol group were also treated with the calculated doses of Carvedilol based on the individual body weight of the animal for 28 consecutive days. Administration of the extracts was through the oral with the aid of an oral gastric tube.

Induction of Myocardial Infarction Protocol

Myocardial infarction was induced in all the experimental animals by the subcutaneous administration of Isoprenaline at a dose of 85mg/kg in all animals used in this study on a day 26 and day 27 respectively. The interval of the induction between both days was a 24-hour period. Thereafter, all animals were sacrificed using chloroform as the anesthetic agent, prior to the collection of blood samples through the retrorbital plaxus and heart for analysis.

Identification Operation Strategy

Each rat across the groups were marked at notable parts of the body, using an indelible marker for each identification as follows:

1. Head
2. Neck

3. Trunk
4. Base
5. Tail

The animals were picked from the tail and the flesh of the back neck is gripped with the left hand and turned upwards with its limbs hanging up and the tail tucked between the hollow of the left hand, then a clear passage to the throat was sought before the drug was administered. Before drug administration, physical (i.e. average body weight change, feed intake and water intake) and physiological parameters (i.e. agility, fur color, nasal discharge and ocular lesion) were observed and noted. The procedure was followed for the 28-day period of the study.

Experimental Procedure

Procedure For Administering Carvedilol, Isoprenaline And The Vegetable Extracts

The animals were fed by orally using an adjustable micropipette with plastic tips and a cannula. The cannula was labeled to prevent cross contamination. The weight of the rats were taken weekly along with other physical observations before the KCN and the vegetable extracts were administered. Volume of the Carvedilol, Isoprenaline and the extract administered were based on the weight of the rats labeling was as follows:

Group 1: Distilled Water

Group 2: 50mg/kg only

Group 3: 100mg/kg

Group 4: Carvedilol Group (2mg/Kg)

Collection of Blood Samples

Tile blood samples were collected after 28 days of experimental period. The rats were fasted for 24 hours before the blood samples were collected. Capillary tubes were used to collect blood samples from the rats while they were still alive using the ocular punctures method.

The blood samples (5mg) were placed inside Lithium heparinized bottles and were centrifuged at 3000 revolutions/ minutes on table centrifuge at room temperature, after which the serum for each animal was picked and decanted into a single universal bottle, each bottle representing each animal. Thereafter the samples were taken to the Chemical Pathology Department in the University of Benin Teaching Hospital, Benin City, Edo State, for Biochemical analysis.

3.0 BIOCHEMICAL ASSAYS

The activity of serum CK-MB, cTn- 1 and serum myoglobin was determined using ARCHITECT STAT diagnolic kit (USA) according to the manufacturer procedure and protocol.

Serum CRP was measured using specific immunoassay (ELISA) technique, using a standard kit (Elabscience Rat hs-CRP (high-sensitivity C-Reactive Protein) USA) following the manufacturer's procedure.

Serum levels of Albumin, Total protein, Alkaline phosphatase, Alanine transaminase, Lactate dehydrogenase, Aspartate Transaminase, Direct Bilirubin and Total Bilirubin were quantitatively determined by ARCHITECT c Systems and the AEROSOT System (USA) following strictly, the manufacturer's procedure and protocol.

Antioxidant Assay

Standard methods of assay of antioxidant enzymes were used to assay the following enzymes, Superoxide Dismutase Activity (SOD), Catalase activity (CAT), Estimation of Glutathione Peroxidase (GPx) activity and Malondialderhyde (MDA) activity.

Catalase activity was estimated by the method described by Cohen *et al.,* (1970). Malonaldehyde was determined using the thiobarbituric acid assay (Buege and Aust, 1978). Glutathione Peroxiciase was estimated using the Nyman method (1959).

Data and Statistical Analysis

The daily record of body weight, biochemical data were subjected to statistical analysis. The results were analyzed using descriptive statistics, Microsoft excel and Kruskal-Wallis. The results were expressed as mean and standard deviation. All parameters were compared to the control group.

CHAPTER THREE

3.0 RESULTS

3.1 ACUTE TOXICITY TEST

G. africanum extract in mice showed no mortality and toxic effect up to a dose of 5g/kg after 24 hours period; this suggests that the extract when administered orally, maybe relatively non toxic.

Table 3.2: % Change in Body weight

Treatment	Week 1 (%)	Week 2(%)	Week 3(%)	Week 4(%)
Control	1.21±1.51	0.44±3.49	4.63±4.08	3.91±4.95
50mg/kg	0.71 ±1.18	4.50±1.74	10.99±3.50	10.9±4.30
100mg/kg	6.02±2 .29	10.40±2.79	12.98±2.87	10.33±3.64
Carvedilol	3.09±6.18	1.44±4.82	0.55 ± 2.91	1.54±9.05

Table 3.3: % Change in Relative organ weight

Treatment	Heart (g)	Liver(g)	Kidney	
Control	0.52 ± 0.02	4.146 ± 0.09	0.37 ± 0.02	0.37 ± 0.01
G. africanum 50mg/kg	0.52 ± 0 .02	3.94 ± 0.05	0.33 ± 0.01	0.35 ± 0.02
G. africanum 100mg/kg	0.56 ± 0.02	3.84 ± 0.09	0.36 ± 0.01	0.36 ± 0.01
Carvedilol (2mg/kg)	0.63 ± 0.10**	6.47 ± 0.26 **	0.53 ± 0.01**	0.54 ± 0.02**

Table 3.4 : Effect of aqueous extract of *Gnetum africanium* on cardiac biomarkers of isopremaline induced myocardial infraction in rats

Treatment	ACB	LDH	TROP	MYOGLO	CK-MB	CRP(HS)

Control	2.94 ± 0.08	208.2 ± 9.62	0.26 ± 0.04	0.70 ±0.07	1.21 ± 0.06	4.44 ± 0.30
G. africanum 50mg/kg	2.98 ± 0.03	201.25 ± 5.11	0.26 ± 0.04	0.63 ±0.05	1.26 ± 0.09	4.57 ± 0.54
G. africanum 100mg/kg	2.88 ± 0.07	225.17 ± 6.85	0.29 ± 0.03	0.77 ±0.08	1.31 ± 0.06	4.51 ± 0.63
Carvedilol (2 mg/kg)	2.42±0.005**	137.17±2.54**	0.64±0.01**	0.66 ±0.02	0.07±0.01**	3.83±0.16**

Table 3.5: Effect of aqueous extract of Gnetium africanum on biochemical parameters of isoprenaline induced myocardial infarction in rats.

Alkaline phosphate, TB = Total bilirubin, DB = Direct bilirubin, TP = Total protein, ALB = Albumin, LDH = Lactate dehydrogenase, CK-MB =Creatinime kinase-MB, CRP(HS) =

Treatment	AST	ALT	ALP	TB	DB	TP	ALB
Control	279.40 ± 20.55	74.20±2.27	398.80 ± 53.93	0.38 ± 0.04	0.14 ±0.02	5.84 ± 0.80	2.94 ± 0.08
G. africanum 50mg/kg	271.25 ± 26.39	64.00±3.03	415.50 ± 47.63	0.40 ± 0.04	0.15 ±0.03	6.25 ± 0.10	2.98 ± 0.03
G. africanum 100mg/kg	311.00 ± 22.76	67.60±6.12	355.40 ± 26.47	0.42 ± 0.04	0.16 ±0.02	5.70 ± 0.21	2.88 ± 0.07
Carvedilol (2 mg/kg)	61.00±24.15**	61 ±16.83*	414.2±58.01**	0.18±0.07*	0.06±0.02*	3.20±1.31*	2.42±0.005**

Creatinine protein (High sensitive)

Table 3.5 Effect of varying concentrations of *Gnetum africanum*on Oxidant- Antioxidant parameters of wister rats

Treatment	SOD (U/L)	CAT(U/L)	GPx(U/L)	MDA(U/L)

Control	0.32± 0.03	0.13± 0.01	0.63± 0.04	0.17± 0.02
50mg/kg	0.36± 0.01	0.14± 001	0.78± 0.03	0.16± 0.02
100mg/kg	0.36± 0.02	0.15 ± 0.01	0.78± 0.05	0.17± 0.02
Carvedilol	0.45 ± 0.19	0.09 ± 0.04	1.05 ± 0.43	0.12 ±0.05

Key: Values are expressed as Mean ± SEM. *p < 0.05. **p <0.01; significantly different from control.SOD-Superoxide dismutase, CAT- Catalase,GPx- Glutathione peroxidase, MDA-Malondialdehyde.

CHAPTER FOUR

4.1 DISCUSSION

The potential effects of natural remedies, particularly plant extracts has generated considerable interest due to their extensive phytochemical constituents (Shah *et al.,* 2019).

From the results, the absence of toxicity signs and death in rats treated with single dose of *Gnetum africanium* up to 5000mg/kg and monitored foe 48 hours suggests that the plant is tolerable and safe, this finding aligns with reports done on same plant by Udeh *et al.,* (2018).One of the primary indicators of the health and physiological condition of experimental animals is the alteration in body weight and muscle wasting (Faria *et al.,* 2011). Numerous studies have demonstrated that body weight loss is strongly associated with the severity of heart failure in individuals with cardiovascular diseases (Lopez *et al.,* 2008; Pocock*et al.,* 2008; Faria *et al.,* 2011). Additionally, changes in body weight may be indicative of the development of Heart Failure in animals subjected to cardiac injury (Kennedy *et al* 2006). Studies have demonstrated that substantial weight changes, particularly weight loss, may be a result of the alteration of metabolic function and physiological stress following a myocardial injury (Faria *et al.,* 2011). Consequently, rats with post-infarct chronic Heart failure display a smaller Body Weight (Bw) compared to control rats (Faria *et al.,* 2011). After experiencing an acute episode of myocardial infarction, the heart proceeds to engage in a complex sequence of alterations in order to safeguard its cardiac functionality. This progressive course of action, commonly referred to as ventricular remodeling, impacts both the infarcted and non-infarcted regions of the heart. Processes such as cell demise, inflammatory responses, collagen restructuring, neuro-hormonal activation, myocyte hypertrophy, electrical and contractile abnormalities gradually culminate in the development of heart failure (Faria *et al.,* 2011). Ultimately, decompensated heart failure can be defined as an abrupt decline in cardiac output and perfusion pressure accompanied by fluid retention and pulmonary edema, due to an increase in left and right filling pressures (Joseph *et al.,* 2009).

Results from percentage change in body weight and relative organ weight showed that neither the 50mg/kg nor the 100mg/kg doses of *Gnetum africanium* extract demonstrated significant differences in these parameters compared to the control. The Stable body and organ weights

across the groups suggest that the administration of *Gnetum africanium* did not have any adverse systemic or organ-specific toxic effects. Often, therapeutic agents can induce weight changes, either due to metabolic disturbances or systemic effects (Shah *et al.,* 2019); however, this was not observed at the doses studied. While this is reassuring from a safety perspective, the lack of significant differences might also suggest that the extract, at these doses, may not have provided the expected therapeutic benefits in modulating physiological responses associated with Myocardial Infarction at these doses.

Biochemical parameters play a crucial role in evaluating the physiological and pathological conditions of a living organism, especially in the context of myocardial infarction (Califf, 2018). Historically, cardiac biomarkers have been used as a primary tool to assess the level of damage to cardiac tissue (Aydin *et al.,* 2019). When these biomarkers are elevated, it is generally indicative of myocardial damage, which in turn indicates damage to cardiac muscle cells (Aydin *et al.,* 2019). These biomarkers are released into the bloodstream upon damage to cardiac cells, rendering them indispensable in the diagnosis and evaluation of the severity of conditions such as myocardial infarction (Aydin *et al.,* 2019). Troponins, a type of cardiac protein, are highly sensitive and precise markers of myocardial injury. They are widely regarded as the gold standard for the diagnosis of myocardial infarction. On the other hand, Myoglobin is an early indicator of muscle damage, including the heart, but is not relevant to cardiac damage (Al-Otaiby *et al.,* 2011). Additionally, elevated levels of enzymes such as Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) have been linked to liver injury or dysfunction in the past. These enzymes are mainly stored in liver cells and, if the liver is damaged, they are released into the bloodstream. An isolated increase in AST without an increase in ALT may indicate cardiac damage rather than liver damage (Giannini *et al.,* 2005) Markers such as Alkaline phosphatase, Total bilirubin, and Direct bilirubin primarily serve as indicators of liver function and the health of the biliary system. Alkaline

phosphatase or ALP, is an enzyme that can be found in various tissues, with a significant presence in the liver, bones, and kidneys. Elevated levels of ALP often suggest the presence of liver disease or bone disorders. Similarly, increased levels of bilirubin can indicate liver dysfunction, which may potentially lead to jaundice (Lowe *et al.,* 2022). Total protein measurement allows for the assessment of the overall amount of two protein classes, namely albumin and globulin, in the bloodstream. This measurement provides insights into the general health of the body. Albumin, which is produced by the liver, plays a crucial role in maintaining osmotic pressure in the blood. Decreased albumin levels may suggest the presence of liver or kidney diseases, as well as inflammatory conditions (Yoshioka *et al.,* 2023). Enzymes such as Lactate dehydrogenase and Creatinine kinase-MB hold paramount importance. Elevated concentrations of these enzymes serve as indicators of cardiac tissue damage (Farhana *et al.,* 2023). Elevated levels of high sensitive C-reactive protein serve as a significant indicator of inflammation and increased risk of cardiovascular diseases (Al-Hadi and Fox 2009). Given that there was no notable distinction in these biomarkers among the groups that received treatment and the control group, it can be inferred that the dosage of *Gnetum africanium* did not worsen or improve the myocardial injury induced by isoprenaline (Aydin *et al.,* 2019). The consistent levels of specific cardiac biomarkers such as AST, ALT, ALP, total bilirubin, creatinine, protein and albumin suggests that the myocardial injury remained the same across all groups except those treat with carvedilol which served as positive control, this findings aligns with study on same plant done by Udeh *et al.,* (2018) who reported that the extract had no significant changes to these parameters.

Another crucial factor in determining the outcome of various pathological conditions, such as myocardial infarction, is the dynamic interplay between oxidative stress and antioxidative defense mechanisms. Imbalance in the production of reactive oxygen species (ROS) and the body's natural antioxidative defenses leads to oxidative stress, which plays a pivotal role in

the pathophysiology of myocardial damage (Rahal *et al.,* 2014). At the cellular level, the production of ROS, which includes free radicals, is a constant occurrence. This production primarily takes place within the mitochondria as a natural by-product of metabolic processes. It is important to note that ROS does play crucial roles in signaling and maintaining homeostasis, however, excessive amounts of ROS can result in detrimental effects such as DNA, protein, and lipid damage. This damage can ultimately lead to cellular dysfunction and even cell death. Various factors, including inflammation, exposure to environmental toxins, elevated glucose levels, and certain medications, can significantly amplify the production of ROS, pushing cells into state of oxidative stress (Pizzino *et al.,* 2017). The human body has a variety of antioxidant mechanisms in place to neutralize the adverse effects of reactive oxygen species. These mechanisms are composed of both enzyme-based and non-enzyme-based mechanisms. Enzymic antioxidants are composed of enzymes such as superoxide dismutase (SOD), catalase, as well as glutathione peroxidase, non-enzymic antioxidants, on the other hand, are composed of molecules such as vitamins E and C (beta-carotene) and glutathione, which are capable of directly scavenging free radicals and rendering them harmless. Additionally, the body sequesters transition metals that may potentially trigger the production of reactive oxygen species through the use of metal-binding proteins such as ferritin and ceruloplasmin (Ighodaro *et al.,* 2018). Due to its high demand for energy and oxygen, the heart is particularly susceptible to oxidative stress. Reactive oxygen species (ROS) have been implicated in the development of various cardiovascular diseases, such as atherosclerosis, hypertension, and heart failure. Therefore, evaluating the oxidative-antioxidant parameters in relation to cardiac health provides valuable insights into the balance between ROS and antioxidant defenses, indicating the extent of oxidative damage and potential therapeutic interventions (Senoner and Dichtl 2019). Within the parameters evaluated, Catalase (CAT), Glutathione Peroxidase (GPx), and Superoxide Dismutase (SOD)

serve as antioxidant defense systems in the body. Catalase acts by catalyzing the decomposition of hydrogen peroxide into water and oxygen, mitigating the effects of this potentially harmful oxidant (Jena *et al.,* 2023). Glutathione Peroxidase plays its part by reducing lipid hydroperoxides to their corresponding alcohols and transforming free hydrogen peroxide into water (Jena *et al.,* 2023). Meanwhile, Superoxide Dismutase facilitates the conversion of the superoxide radicals into either ordinary molecular oxygen or hydrogen peroxide, both of which are far less reactive than the superoxide radical (Jena *et al.,* 2023). In contrast, Malondialdehyde (MDA) emerges as a marker of oxidative stress. While not an oxidant in the direct sense, its levels elevate in response to increased oxidative stress in cells. Specifically, MDA acts as an indicator of lipid peroxidation, shedding light on the extent of oxidative damage. Thus, while it doesn't induce oxidative stress directly, its presence is indicative of oxidative processes and resultant cellular damage (Jena *et al.,* 2023). The absence of significant difference in oxidative-antioxidant parameters between the treated groups and the control suggests that the administered doses of *Gnetum africanium* neither counteracted the ROS generated nor enhance the antioxidant defense mechanisms.

4.2 CONCLUSION

In conclusion, the findings from this study suggest that *Gnetum africanium* at the administered doses of 50mg/kg and 100mg/kg did not demonstrate any significant protective effect across any of the assessed parameters. Consequently, did not offer substantial benefits

against isoprenaline-induced myocardial injury also the extract may be considered non-toxic. However, for prolong use of this extract caution is advised.

4.3 RECOMMENDATIONS

Further research may be needed to explore alternative doses, extraction methods, or other potential benefits inherent to the plant. Also, its use as an adjunct/preventive medication against myocardiac infarction in therapeutic doses of isoprenaline under consultant supervision should be explored.

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