USE OF GARLIC (*Allium sativum)* EXTRACT TO AMELIORATE POSSIBLE EFFECTS OF ORAL CONTRACEPTIVES IN FEMALE WISTAR RATS

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BEING A CITATIONS IN THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE SUBMITTED TO THE COLLEGE OF POST-GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M. Sc) IN MEDICAL LABORATORY SCIENCE (CLINICAL CHEMISITRY) IN UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

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DEDICATION

I dedicate this project to my father Igbikibere Roland Amadi Rtd DSP (late)

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ABSTRACT

Ischemic heart disease (IHD) is a major cardiovascular disorder characterized by dyslipidemia and myocardial injury, and its risk may be increased in females using oral contraceptives (OCs) due to alterations in lipid metabolism and endothelial function. Garlic (*Allium sativum*) has been reported to possess cardioprotective properties, including lipid modulation and antioxidant effects. This study evaluated the therapeutic potential of garlic (*Allium sativum*) extract in ammeliorating biochemical markers of ischemic heart disease in female Wistar rats exposed to varying doses of combined oral contraceptives (COCs). A total of fifty-six adult female rats were randomly assigned into seven groups comprising a control group, COC-only group, COC plus graded doses of garlic extract groups, and a garlic-only group, and treatments were administered orally for four weeks. Serum lipid profile parameters; total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined using enzymatic colorimetric methods, while cardiac troponin I levels were quantified using sandwich ELISA. Data were expressed as mean ± standard error of mean (SEM) and analyzed by one-way ANOVA ($p < 0.05$). Total cholesterol levels were not significantly different across groups (Control: 3.75 ± 0.12 mmol/L; Group B: 3.68 ± 0.10 mmol/L; Group C: 3.72 ± 0.11 mmol/L; Group F: 3.80 ± 0.13 mmol/L). Triglyceride levels varied significantly (Control: 1.45 ± 0.05 mmol/L; Group B: 1.20 ± 0.04 mmol/L; Group C: 1.38 ± 0.06 mmol/L; Group F: 1.78 ± 0.07 mmol/L). HDL levels were significantly increased in lower-dose groups (Control: 0.98 ± 0.03 mmol/L; Group B: 1.25 ± 0.05 mmol/L; Group C: 1.22 ± 0.04 mmol/L), while LDL levels were significantly reduced in these groups (Control: 1.90 ± 0.06 mmol/L; Group B: 1.50 ± 0.05 mmol/L; Group C: 1.55 ± 0.07 mmol/L). Cardiac troponin I levels showed no statistically significant differences among groups (Control: 0.330 ± 0.012 ng/mL; Group B: 0.315 ± 0.010 ng/mL; Group C: 0.305 ± 0.009 ng/mL; Group F: 0.345 ± 0.013 ng/mL). Body weight remained stable across all groups, indicating good tolerability of treatments. Histological examination of the heart, liver, and kidney revealed normal tissue architecture in all examined groups, with no observable pathological lesions. In conclusion, garlic extract demonstrated a modest protective effect on lipid metabolism, particularly at lower COC doses, without evidence of organ toxicity, may have a potential supportive role in mitigating cardiovascular risk associated with oral contraceptive use.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Ischemic heart disease (IHD), also known as coronary artery disease, remains a significant global health burden and is the leading cause of death among women worldwide. According to the World Health Organization (2023), cardiovascular diseases account for nearly 35% of all female deaths annually. IHD arises primarily from a reduction in coronary blood flow, often due to atherosclerosis, thrombosis, or endothelial dysfunction. While historically considered more common in men, recent trends reveal an alarming rise in cardiovascular events among women, driven by urbanization, sedentary lifestyles, dietary habits, and widespread use of hormonal contraceptives (Garde *et al.*, 2020; James *et al.*, 2023).

Combined oral contraceptives (COCs), comprising synthetic estrogen and progestin, are widely used by women for birth control and hormonal regulation. However, substantial evidence indicates that long-term use of COCs can increase the risk of cardiovascular complications. These include elevated blood pressure, unfavorable alterations in lipid profiles such as increased low-density lipoprotein (LDL) and triglyceride levels and decreased high-density lipoprotein (HDL) and enhanced oxidative stress and systemic inflammation, all of which contribute to IHD development (Kchaou *et al.,* 2017; Ghosh *et al.,* 2022; Sun *et al.*, 2023). Moreover, studies have shown that COCs may induce a hypercoagulable state, increasing the risk of thromboembolic events and endothelial damage (Booker *et al.,* 2020; Mogadam *et al.*, 2021).

These risks have prompted the search for safe, natural agents that can counteract or reverse the adverse cardiovascular effects of COCs. Garlic (*Allium sativum*), a traditional medicinal plant, has received considerable attention due to its cardioprotective potential. It contains bioactive sulfur compounds like allicin, ajoene, and diallyl disulfide, which exhibit lipid-lowering, vasodilatory, anti-inflammatory, and antioxidant properties (He *et al.,* 2022; Kim *et al.,* 2022). These compounds have been shown to modulate lipid metabolism, enhance nitric oxide bioavailability, and reduce oxidative stress, making garlic a strong candidate for cardiovascular protection.

Experimental studies have demonstrated garlic extract's ability to reduce blood pressure, improve lipid profiles, suppress inflammatory mediators, and protect myocardial tissue in models of hypertension, hyperlipidemia, and myocardial infarction (Zhou *et al.,* 2022; Adegbite *et al.,* 2023). In particular, garlic has been shown to reduce serum troponin levels—biomarkers of cardiac injury—suggesting its potential role in cardiac repair and recovery.

Despite these promising findings, there is limited research on garlic's ability to reverse established cardiovascular dysfunction induced by hormonal contraceptives. Most studies have focused on prevention or acute intervention, not on therapeutic reversal after prolonged contraceptive exposure. Bridging this gap is essential, especially in low-resource settings where access to conventional cardiovascular therapies is limited and where COC use continues to rise.

This study aims to investigate the therapeutic efficacy of garlic extract in reversing markers of ischemic heart disease such as elevated blood pressure, dyslipidemia, tachycardia, and troponin elevation in female Wistar rats subjected to different oral contraceptive regimens.

1.2 Statement of the Problem

Cardiovascular disease remains the leading cause of mortality in women globally, with ischemic heart disease (IHD) accounting for over 30% of these deaths (World Health Organization, 2023). The widespread and often long-term use of combined oral contraceptives (COCs) has raised significant concerns about their contribution to cardiovascular risk, particularly in women predisposed to metabolic or vascular dysfunction. Contemporary formulations of COCs, though lower in estrogen than earlier versions, have still been shown to induce pro-thrombotic states, elevate blood pressure, and negatively alter lipid metabolism—key contributors to atherosclerosis and myocardial ischemia (Ghosh *et al.*, 2022; Sun *et al.*, 2023).

Recent animal and human studies have reported that long-term use of hormonal contraceptives leads to increased serum LDL levels, triglycerides, and a decrease in HDL cholesterol—factors that accelerate endothelial dysfunction and plaque formation (Mogadam *et al.*, 2021; Bouchemal *et al.*, 2023). In parallel, there is also growing concern about contraceptive-induced oxidative stress and systemic inflammation, both of which impair cardiac function and elevate biomarkers such as cardiac troponins, even in young, otherwise healthy females (Olaniyi *et al.*, 2022).

Despite these known risks, there is currently no standard non-pharmacological intervention aimed specifically at reversing the cardiovascular side effects of COC use. Furthermore,

the use of synthetic medications to manage IHD often involves side effects, high cost, and reduced accessibility in low-resource settings.

This raises a critical need to explore affordable, natural alternatives that may offer cardioprotective benefits. Garlic (*Allium sativum*), known for its potent antioxidant, lipid-lowering, and anti-inflammatory properties, has shown promise in reducing cardiovascular risk factors in various preclinical and clinical settings (Zhou *et al.,* 2021; Kim *et al.,* 2022). However, there remains a significant gap in research evaluating the *therapeutic* potential of garlic extract to *reverse* established cardiac dysfunction caused by contraceptive use, especially using female models that reflect the hormonal milieu affected by such drugs.

Thus, there is an urgent need to investigate whether garlic extract can serve as an effective intervention for preventing or reversing contraceptive-induced IHD—an increasingly relevant issue in reproductive and cardiovascular health research.

1.3 Justification of the Study

Given the rising prevalence of contraceptive use and the corresponding increase in cardiovascular complications among women, it is essential to develop accessible and safe therapeutic strategies. Pharmacological treatments for cardiovascular conditions often come with high costs and side effects, which limits their applicability in low-resource settings. Natural remedies such as garlic, with documented antioxidant, anti-inflammatory, and lipid-lowering properties, provide a valuable alternative (Kim *et al.,* 2022).

Garlic has demonstrated efficacy in improving cardiovascular parameters in various animal models and human studies. Its active components are known to enhance endothelial

function, reduce blood pressure, modulate lipid metabolism, and protect myocardial tissues against ischemic injury (Zhou *et al.*, 2022; Li *et al.*, 2023). Despite this, the specific effect of garlic extract in the context of contraceptive-induced ischemic heart disease remains largely unexplored.

This study is particularly justified by the increasing rates of hormonal contraceptive use among women of reproductive age, a demographic that is often overlooked in cardiovascular research. Investigating garlic's therapeutic potential in reversing such damage could provide a low-cost, natural, and accessible intervention to reduce cardiovascular risk. Moreover, it may offer additional data to support broader public health recommendations for the use of integrative medicine in managing chronic diseases.

Furthermore, utilizing an animal model such as the Wistar rat allows for controlled experimentation and closer observation of physiological changes, enabling more robust conclusions on causality and effect.

1.4 Aim

This study aimed to investigate the therapeutic efficacy of garlic extract in reversing markers of ischemic heart disease such as dyslipidemia troponin I elevation and histological assessment in female Wistar rats subjected to combined oral contraceptive doses.

1.5 Objectives of the Study

The specific objectives of the study were as follows

1. To evaluate effect of garlic extract on the lipid profile (total cholesterol, LDL, HDL, triglycerides) in female Wistar rats.
2. To determine the level of cardiac troponin I, a biomarker of myocardial injury, in treated vs. untreated groups.
3. To evaluate the histology of heart, kidney and liver in treated vs. untreated groups.

1.6 Research Question

1. To what extent does garlic extract modulate lipid alterations induced by oral contraceptives?
2. Does garlic administration reduce cardiac troponin I levels, indicating reduced myocardial injury in OC-treated rats?
3. Does treatment affect the histological structure of the heart, kidney, and liver compared to untreated groups?

1.7 Hypotheses

Null Hypothesis

1. **Null Hypothesis (H_{01}):** Garlic extract does not significantly reverse alterations in lipid profile in female rats exposed to oral contraceptives.
2. **Null Hypothesis (H_{02}):** Garlic extract does not significantly reverse alterations in troponin levels in female rats exposed to oral contraceptives.
3. **Null Hypothesis (H_{03}):** Garlic extract does not significantly reverse lipid-related histopathological changes in female rats exposed to oral contraceptives.

Alternative Hypothesis (H1):

1. **Alternative Hypothesis (H_{11}):** Garlic extract significantly reverses alterations in lipid profile in female rats exposed to oral contraceptives.
2. **Alternative Hypothesis (H_{12}):** Garlic extract significantly reverses alterations in troponin levels in female rats exposed to oral contraceptives.
3. **Alternative Hypothesis (H_{13}):** Garlic extract significantly reverses lipid-related histopathological changes in female rats exposed to oral contraceptives.

CHAPTER TWO

LITERATURE REVIEW

2.1 Ischemic Heart Disease (IHD)

Ischemic heart disease (IHD), also known as coronary artery disease (CAD), is a condition characterized by inadequate blood supply to the myocardium, primarily due to narrowing or obstruction of the coronary arteries, which impairs oxygen delivery to cardiac tissue (World Health Organization [WHO], 2023). This reduction in coronary perfusion creates an imbalance between oxygen supply and myocardial oxygen demand, potentially leading to ischemia, metabolic disturbances, reduced contractility, and, if prolonged, myocardial necrosis (Zhang *et al.,* 2025). Clinically, IHD manifests as angina pectoris, shortness of breath, fatigue, or in some cases, silent ischemia, where myocardial injury occurs without overt symptoms, making early detection challenging (Ogunmola *et al.,* 2021). This impaired blood supply typically results from the development of atherosclerosis, a chronic and progressive process where lipids, inflammatory cells, and connective tissue accumulate within the arterial walls and gradually restrict blood flow (Gao *et al.,* 2024). As atherosclerotic plaques enlarge and become unstable, they significantly increase the risk of acute rupture, platelet aggregation, and the formation of obstructive thrombi that can suddenly block coronary circulation (Amoah and Kengne, 2023). When coronary perfusion falls below the metabolic demands of the myocardium, the heart muscle experiences an oxygen deficit that triggers metabolic disturbances, loss of contractile efficiency, and eventual necrosis of cardiac cells if the ischemia persists (Zhang *et al.,* 2025). Individuals suffering from IHD may present with angina pectoris, shortness of breath, fatigue, or even silent ischemia, all of which reflect the heart's inability to cope with restricted blood flow

(Ogunmola *et al.,* 2021). Because of its complex pathophysiology and profound clinical consequences, IHD remains one of the most significant cardiovascular disorders worldwide, affecting millions of people and placing substantial demands on healthcare systems (American Heart Association [AHA], 2025).

The primary pathological mechanism of IHD is atherosclerosis, a chronic process involving the accumulation of lipids, fibrous tissue, and inflammatory cells within the arterial walls, resulting in plaque formation and progressive narrowing of the coronary arteries (Gao *et al.,* 2024). Plaque growth and instability increase the likelihood of acute rupture, triggering platelet aggregation and thrombus formation, which can acutely block blood flow and precipitate myocardial infarction (Amoah and Kengne, 2023). This cascade demonstrates the complex interplay between structural vascular changes and hemostatic mechanisms in the pathogenesis of IHD.

Endothelial dysfunction is another central mechanism in IHD development. The endothelium normally regulates vascular tone, inhibits platelet adhesion, and maintains anti-inflammatory and anticoagulant properties. However, factors such as chronic oxidative stress, hypertension, hyperglycemia, and dyslipidemia impair endothelial nitric oxide production, leading to vasoconstriction, inflammation, and a pro-thrombotic state (Vaziri *et al.,* 2020; Ogunmola *et al.,* 2021). Reduced nitric oxide bioavailability also contributes to impaired coronary vasodilation, limiting oxygen delivery to the myocardium during increased demand.

Inflammation significantly accelerates plaque progression and destabilization. Chronic exposure to pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis

factor-alpha (TNF-α), promotes monocyte recruitment, macrophage activation, and foam cell formation within the arterial wall. This inflammatory milieu not only facilitates plaque growth but also increases the risk of rupture, which is a primary trigger of acute ischemic events (Zhang *et al.,* 2025). Studies in Nigerian populations have documented elevated inflammatory markers in patients with IHD, emphasizing the role of systemic inflammation in disease pathophysiology (Ogunmola *et al.,* 2021; Adebayo *et al.,* 2022).

Metabolic derangements such as insulin resistance, obesity, and dyslipidemia further exacerbate coronary artery narrowing. Elevated low-density lipoprotein cholesterol (LDL-C) undergoes oxidative modification, becoming highly atherogenic and accelerating endothelial injury and plaque formation (Gao *et al.,* 2024). Simultaneously, impaired glucose metabolism and chronic hyperglycemia increase oxidative stress and inflammatory signaling, creating a vicious cycle that magnifies myocardial vulnerability to ischemia.

Thrombosis represents the final common pathway in acute IHD events. The rupture of vulnerable plaques exposes subendothelial collagen and tissue factor, activating platelets and the coagulation cascade. This rapid thrombus formation can entirely obstruct coronary blood flow, resulting in myocardial infarction (Amoah and Kengne, 2023). In low-resource settings, including parts of sub-Saharan Africa, delayed access to emergency cardiac care often exacerbates outcomes, leading to higher rates of morbidity and mortality (African Union Health Report, 2023).

Genetic and environmental factors also modulate IHD susceptibility. Family history of early-onset cardiovascular disease, polymorphisms in lipid metabolism genes, and epigenetic modifications influence the development and severity of atherosclerosis (Gao *et*

al., 2024). Environmental influences such as urbanization, sedentary lifestyles, smoking, poor diet, and exposure to air pollution further heighten the risk, particularly in developing countries where rapid lifestyle transitions are occurring (Adebayo *et al.,* 2022).

IHD is a multifactorial disease arising from the convergence of structural vascular changes, endothelial dysfunction, inflammatory processes, metabolic derangements, and thrombotic events. Its complex pathophysiology underscores the need for targeted preventive strategies, early detection, and therapeutic interventions, including lifestyle modification, pharmacological treatment, and complementary approaches such as antioxidant and cardioprotective plant extracts (Ried *et al.*, 2016; Banerjee and Maulik, 2021). Understanding these mechanisms in the context of Nigerian and broader African populations is essential for designing effective interventions that address both clinical and socio-economic burdens of IHD.

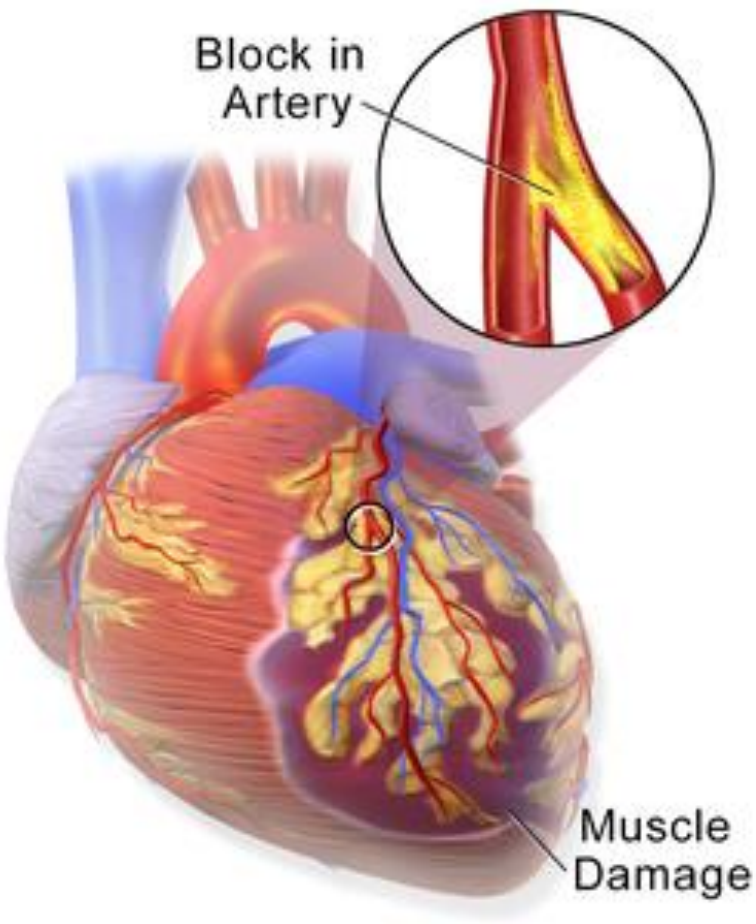


Figure 2.1 Coronary Artery Blockage and Resulting Heart Muscle Damage (Mendis *et al.*, 2011)

The image illustrates the pathophysiology of a heart attack (myocardial infarction). A blockage in a coronary artery, usually due to atherosclerotic plaque or a blood clot, restricts blood flow to part of the heart muscle. The zoomed inset shows the arterial blockage in detail. Reduced blood flow deprives the affected cardiac tissue of oxygen, causing muscle damage and potential necrosis if blood supply is not restored promptly. The diagram highlights the relationship between coronary artery obstruction and localized myocardial injury.

2.1.1 Ischemic Heart Disease (IHD) and Its Global Significance

Globally, IHD continues to rank as a leading cause of death and disability, contributing heavily to the burden of cardiovascular diseases, which accounted for approximately 19.8 million deaths in 2022 alone (WHO, 2023). This staggering figure underscores the critical role IHD plays in shaping global health trends and influencing mortality patterns across both developed and developing nations (Gao *et al.,* 2024). Despite improvements in medical technology and greater awareness of cardiovascular risk factors, the absolute number of people developing or living with IHD continues to rise due to population aging, urbanization, and persistent exposure to harmful lifestyle factors such as smoking, poor diet, and physical inactivity (Zhang *et al.,* 2025). Projections based on global modeling studies suggest that by the year 2050, annual IHD deaths may reach as high as 16 million, and more than 510 million individuals may be living with the disease worldwide, highlighting the urgent need for enhanced preventive and therapeutic strategies (Amoah and Kengne, 2023). The global economic impact of this growing burden is immense, with cardiovascular diseases—including IHD—costing an estimated US$417.9 billion in direct medical expenses and lost productivity in the United States alone between 2020 and 2021 (AHA, 2025). These statistics emphasize the profound social, economic, and public-health significance of IHD at a time when chronic diseases increasingly dominate global health challenges (WHO, 2023).

Importantly, the burden of IHD is not evenly distributed across the world, as low- and middle-income countries are experiencing rapid increases in morbidity and mortality from this condition (Amoah and Kengne, 2023). While high-income countries have seen

declines in age-standardized IHD mortality due to improved screening, early diagnosis, and better emergency cardiac care, low-resource regions continue to face rising rates driven by epidemiological transition and limited access to quality healthcare (Zhang *et al.,* 2025). Countries in sub-Saharan Africa, including Nigeria, are witnessing a sharp increase in cardiovascular risk factors such as hypertension, obesity, and diabetes, which are directly associated with rising IHD incidence (Adebayo *et al.,* 2022). Data from African health surveys reveal that deaths attributable to IHD increased by more than 50% across the continent between 1990 and 2019, indicating a dramatic shift in disease patterns previously dominated by infectious illnesses (African Union Health Report, 2023). In Nigeria, tertiary hospital records show a steady rise in admissions for acute coronary syndromes, as well as increasing mortality rates, reflecting both the increased burden of disease and the inadequate availability of specialized cardiac care services (Ogunmola *et al.,* 2021). These regional trends demonstrate that IHD is no longer a disease confined to affluent societies but has now become a major contributor to premature death and disability in developing countries (Amoah and Kengne, 2023).

Beyond its clinical implications, IHD has a profound effect on the social and economic well-being of affected individuals and their families (AHA, 2025). People living with IHD often grapple with chronic chest discomfort, reduced physical endurance, emotional stress, and the fear of recurrent cardiac events, all of which significantly reduce their quality of life (WHO, 2023). Families frequently bear the burden of caregiving, financial strain from medical costs, and reduced household productivity when breadwinners become ill (Adebayo *et al.,* 2022). At the community level, IHD contributes to workforce shortages, increased dependency ratios, and constrains national economic growth, especially in

countries with already limited resources (African Union Health Report, 2023). Public-health experts continue to emphasize that prevention—through risk-factor modification, early screening, and health education—remains the most effective strategy for reducing the global burden of IHD (Gao *et al.*, 2024). However, achieving widespread prevention requires not only individual behavior change but also systemic interventions such as improved access to healthcare, strengthened emergency response systems, and policies that promote healthier environments (WHO, 2023).

Ischemic heart disease stands as one of the most pressing global health challenges of the 21st century, with increasing significance in both developed and developing nations (AHA, 2025). The rising prevalence of risk factors, combined with socioeconomic changes, urbanization, and inadequate healthcare systems in many regions, continues to fuel the global expansion of IHD (Zhang *et al.*, 2025). Understanding its global significance not only highlights the human suffering associated with this disease but also underscores the need for innovative research, enhanced public-health strategies, and the exploration of new therapeutic approaches, including the potential use of natural plant extracts such as garlic in reversing myocardial injury (Amoah and Kengne, 2023). As the global community works toward reducing the burden of heart disease, comprehensive strategies that integrate medical, social, and preventive approaches will be essential in addressing the widespread impact of IHD on health and development worldwide (WHO, 2023).

2.1.2 Epidemiology and Burden of Disease

Ischemic heart disease (IHD) is a leading cause of morbidity and mortality worldwide, representing a substantial public health challenge in both developed and developing nations

(World Health Organization [WHO], 2023). Globally, cardiovascular diseases—including IHD—accounted for nearly 19.8 million deaths in 2022, with IHD being the single largest contributor to these fatalities (Gao *et al.,* 2024). This high mortality is accompanied by significant morbidity, as millions of individuals live with chronic cardiac symptoms, functional limitations, and reduced quality of life due to ischemic injury (American Heart Association [AHA], 2025).

The global burden of IHD is not evenly distributed. High-income countries have observed a gradual decline in age-standardized mortality rates due to improved healthcare access, early diagnosis, and advanced interventions such as percutaneous coronary procedures and pharmacological therapies (Zhang *et al.,* 2025). In contrast, low- and middle-income countries, particularly in sub-Saharan Africa, are experiencing a rapid increase in both incidence and mortality due to epidemiological transition, urbanization, and the rising prevalence of risk factors such as hypertension, diabetes, obesity, and dyslipidemia (Adebayo *et al.,* 2022; Amoah and Kengne, 2023).

In Africa, deaths attributable to IHD have risen sharply, increasing by more than 50% between 1990 and 2019, reflecting a shift from predominantly infectious diseases to chronic non-communicable diseases (African Union Health Report, 2023). This trend is particularly pronounced in Nigeria, where hospital-based studies indicate an increasing number of admissions for acute coronary syndromes and higher case-fatality rates, highlighting the growing clinical and economic burden of IHD (Ogunmola *et al.,* 2021). The rising incidence of IHD in Nigeria is closely linked to lifestyle and behavioral factors, including sedentary habits, unhealthy diets, alcohol consumption, and tobacco use, as well

as inadequate awareness and control of traditional cardiovascular risk factors (Adebayo *et al.,* 2022).

Beyond mortality, IHD imposes a profound socioeconomic burden. Patients often experience chronic chest pain, fatigue, and reduced exercise tolerance, which limit daily functioning and workforce participation (WHO, 2023). Families bear additional caregiving responsibilities and financial strain from recurrent medical expenses, while communities face reduced productivity and increased dependency ratios, particularly in regions with limited healthcare resources (African Union Health Report, 2023). Economically, the global cost of cardiovascular disease, including direct healthcare costs and lost productivity, was estimated at US$417.9 billion in the United States alone between 2020 and 2021, illustrating the enormous financial implications of IHD (AHA, 2025).

The burden of IHD is further exacerbated by gender-specific factors. Women, especially those using hormonal contraceptives, may have elevated cardiovascular risk due to estrogen-induced prothrombotic effects, metabolic changes, and endothelial dysfunction (Lidegaard *et al.,* 2012; Okonofua *et al.,* 2019). In many African settings, limited access to specialized cardiac care, delayed presentation, and gaps in awareness contribute to higher morbidity and mortality, emphasizing the need for context-specific preventive and therapeutic strategies (Ogunmola *et al.,* 2021; Amoah and Kengne, 2023).

In summary, IHD represents a major and growing global health challenge. Its epidemiology demonstrates a dual burden: while high-income countries benefit from declining mortality, low- and middle-income nations face rising incidence and fatalities due to rapid urbanization, lifestyle changes, and healthcare disparities. Understanding these patterns is

critical for guiding public health interventions, optimizing resource allocation, and implementing strategies to reduce the human, social, and economic impact of IHD worldwide (WHO, 2023; Gao *et al.*, 2024).

2.1.3 Pathophysiology of Ischemic Heart Disease

Ischemic heart disease (IHD) develops when the heart's own blood supply becomes insufficient to meet its oxygen and nutrient demands. At the center of this condition is the slow, often silent buildup of **atherosclerosis**, which can start decades before a person experiences any symptoms (Gao *et al.*, 2024). In a healthy artery, blood flows freely through smooth, flexible walls, delivering oxygen and nutrients to the heart muscle. However, when risk factors such as high blood pressure, high cholesterol, smoking, obesity, or diabetes are present, tiny injuries occur to the inner lining of the arteries, called the endothelium. The body responds by sending immune cells and lipids to repair the damage, but over time, this healing process becomes maladaptive, leading to the formation of plaques that gradually narrow the coronary arteries (Amoah and Kengne, 2023).

As these plaques grow, they can remain stable for years or suddenly destabilize. Unstable plaques are dangerous because they can rupture, triggering the formation of blood clots that may completely block blood flow, leading to a heart attack. Even when plaques are stable, the narrowed arteries reduce oxygen delivery during periods of increased activity, causing chest pain, shortness of breath, or fatigue—symptoms that directly affect daily life and limit physical and social activities (Ogunmola *et al.*, 2021; Zhang *et al.*, 2025).

Oxidative stress plays a pivotal role in this process. Reactive oxygen species (ROS) are naturally produced in the body, but when they accumulate excessively, they damage the

cells lining the blood vessels and oxidize LDL cholesterol, creating highly atherogenic molecules. Oxidized LDL promotes inflammation, plaque formation, and further endothelial injury, creating a vicious cycle that accelerates disease progression (Vaziri *et al.,* 2020; Gao *et al.,* 2024). In practical terms, oxidative stress means the heart becomes more vulnerable to injury, and even routine physical exertion can trigger discomfort or fatigue in affected individuals.

Inflammation is closely intertwined with oxidative stress. The body's immune system, responding to arterial injury and oxidative signals, releases cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). While inflammation is normally protective, in IHD it becomes chronic and harmful. Macrophages in the arterial wall release enzymes that weaken the fibrous cap of plaques, making them prone to rupture. When a plaque ruptures, a blood clot can form instantly, potentially cutting off blood supply to part of the heart. This mechanism explains why heart attacks often occur suddenly, sometimes in people who felt healthy just hours before (Amoah and Kengne, 2023; Zhang *et al.,* 2025). Nigerian studies have documented higher inflammatory markers in patients with IHD, highlighting how lifestyle, diet, and healthcare access influence the inflammatory burden in African populations (Ogunmola *et al.,* 2021; Adebayo *et al.,* 2022).

Endothelial dysfunction is another critical contributor to IHD. The endothelium normally produces nitric oxide (NO), a molecule that relaxes blood vessels, prevents platelet aggregation, and suppresses inflammation. When the endothelium is damaged by oxidative stress or metabolic imbalances, NO production decreases. This leads to vasoconstriction, increased blood clotting, and heightened inflammation, which further limits the heart's

ability to receive adequate blood supply, especially during physical or emotional stress (Vaziri *et al.,* 2020; Gao *et al.,* 2024). In real life, this dysfunction translates into symptoms such as angina during exercise, cold intolerance, or even silent ischemia, where the heart suffers oxygen deprivation without the person realizing it.

The interplay between atherosclerosis, oxidative stress, inflammation, and endothelial dysfunction illustrates why IHD is so complex and unpredictable. Each mechanism amplifies the others, gradually reducing the heart's resilience. In countries like Nigeria, delayed diagnosis, limited access to cardiology services, and high prevalence of risk factors such as hypertension and diabetes mean that many people only seek care when the disease has already caused significant damage (Ogunmola *et al.,* 2021; African Union Health Report, 2023).

Understanding these pathophysiological processes is essential not only for medical treatment but also for preventive strategies. Interventions that reduce oxidative stress, control inflammation, and restore endothelial function—through lifestyle modification, pharmacological therapy, or complementary approaches such as garlic extract—hold promise in mitigating the impact of IHD and improving quality of life (Banerjee and Maulik, 2021; Ried *et al.,* 2016). Ultimately, this mechanistic understanding bridges the gap between cellular-level changes and the lived experiences of patients, highlighting why IHD remains a leading cause of morbidity and mortality worldwid

2.1.4 Risk Factors for Ischemic Heart Disease: Hormonal Influences in Females

Ischemic heart disease (IHD) arises from a combination of traditional cardiovascular risk factors and sex-specific influences that affect women differently than men. Globally

recognized risk factors, including hypertension, diabetes, obesity, dyslipidemia, smoking, and sedentary lifestyles, contribute substantially to the development of atherosclerosis and myocardial ischemia (Adebayo *et al.,* 2022; Gao *et al.,* 2024). These factors are often compounded in women by hormonal variations that influence vascular function, lipid metabolism, and coagulation profiles throughout life.

Estrogen plays a protective role in premenopausal women by promoting vasodilation, improving lipid profiles, and exerting antioxidant and anti-inflammatory effects on the cardiovascular system (Reckelhoff, 2018). This protection partly explains why IHD tends to manifest later in women than in men, typically after menopause when estrogen levels decline. Reduced estrogen leads to endothelial dysfunction, increased oxidative stress, and a shift toward a prothrombotic state, all of which elevate the risk of coronary artery disease (Zhang *et al.*, 2025).

However, exogenous hormonal exposure through oral contraceptives or hormone replacement therapy can modify this risk. Combined estrogen-progestin contraceptives have been associated with increased thrombotic risk, alterations in lipid metabolism, and mild elevations in blood pressure, which may contribute to a higher susceptibility to IHD in women already predisposed due to underlying cardiovascular risk factors (Lidegaard *et al.*, 2012; Okonofua *et al.*, 2019). Recent studies in African populations have highlighted that women using oral contraceptives—especially those with obesity, hypertension, or a family history of cardiovascular disease—may experience early endothelial dysfunction and subtle metabolic disturbances that increase long-term cardiac risk (Ogunmola *et al.*, 2021; Adebayo *et al.*, 2022).

Other hormonal influences, such as menopause and polycystic ovary syndrome (PCOS)**,** also contribute to elevated cardiovascular risk in women. Postmenopausal estrogen deficiency is associated with central obesity, dyslipidemia, insulin resistance, and increased systemic inflammation, all of which accelerate atherosclerosis and IHD progression (Reckelhoff, 2018; Zhang *et al.,* 2025). Similarly, women with PCOS often exhibit hyperandrogenism, insulin resistance, and low-grade inflammation, creating a proatherogenic milieu that increases susceptibility to myocardial ischemia even at younger ages (Amoah and Kengne, 2023).

Lifestyle factors intersect with hormonal influences, particularly in African contexts. Urbanization, dietary shifts toward high-calorie and high-fat foods, and reduced physical activity compound the effects of hormonal and metabolic changes, heightening IHD risk among women (Adebayo *et al.,* 2022). Additionally, limited access to preventive healthcare, lack of routine cardiovascular screening, and low awareness of sex-specific risk factors mean that many women in Nigeria and other sub-Saharan African countries present with advanced disease or complications when they first seek medical attention (Ogunmola *et al.,* 2021; African Union Health Report, 2023).

While traditional cardiovascular risk factors remain central to IHD development, hormonal influences in females—including natural fluctuations, menopause, and exogenous hormonal exposure—play a critical and sometimes underappreciated role. Recognizing these sex-specific risks is essential for timely intervention, personalized prevention strategies, and therapeutic approaches that address both metabolic and vascular health in women. Integrating these insights with broader lifestyle and genetic considerations

provides a more complete understanding of IHD risk in females and underscores the importance of targeted public health measures, especially in African populations (Lidegaard *et al.,* 2012; Amoah and Kengne, 2023).

2.2 Garlic (*Allium sativum*) Extract as a Therapeutic Intervention

Garlic (*Allium sativum*) has a long history of medicinal use across multiple cultures, valued for its therapeutic properties in the management of cardiovascular, metabolic, and infectious diseases (Ried *et al.,* 2016). Contemporary scientific investigations have confirmed that garlic is rich in bioactive sulfur-containing compounds such as allicin, S-allyl cysteine, diallyl disulfide, and ajoene—that exert antioxidant, anti-inflammatory, hypolipidemic, and vasodilatory effects (Banerjee and Maulik, 2021; Li *et al.*, 2022). These pharmacological properties make garlic a promising candidate for counteracting ischemic heart disease (IHD), which is primarily driven by oxidative stress, endothelial dysfunction, platelet aggregation, and atherogenesis.

Oxidative stress is a central factor in the pathophysiology of IHD. Excessive reactive oxygen species (ROS) damage endothelial cells, reduce nitric oxide bioavailability, and accelerate the formation of atherosclerotic plaques (Vaziri *et al.,* 2020). Garlic has been shown to enhance endogenous antioxidant defense mechanisms by increasing the activity of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, thereby mitigating oxidative injury in cardiac tissues (Ried *et al.,* 2016; Banerjee and Maulik, 2021). In addition, garlic's anti-inflammatory properties reduce the production of pro-inflammatory cytokines, which play a key role in the progression of endothelial dysfunction and myocardial damage (Li *et al.*, 2022).

Garlic also possesses anti-platelet and anti-thrombotic effects, which are critical for maintaining coronary circulation and preventing arterial occlusion, a major contributor to myocardial ischemia (Rahman and Lowe, 2020). The active compounds in garlic inhibit platelet aggregation, reduce fibrinogen levels, and prevent thrombus formation, thereby improving blood flow to the myocardium and reducing the risk of ischemic injury (Kim *et al.,* 2021). Additionally, garlic's vasodilatory effects, mediated through enhanced nitric oxide release and endothelial function, contribute to improved coronary perfusion and reduced cardiac workload (Banerjee and Maulik, 2021).

Experimental studies in animal models have consistently demonstrated garlic's cardioprotective properties. Rodents subjected to ischemia-reperfusion injury or hyperlipidemic diets showed significant improvements in cardiac enzyme profiles, reduced infarct size, and improved myocardial histology after treatment with garlic extract (Kim *et al.,* 2021; Rahman and Lowe, 2020). In particular, studies involving female rats exposed to hormonal disturbances, including oral contraceptives, have indicated that garlic can attenuate oxidative stress, normalize lipid profiles, and protect cardiac tissue from ischemic injury (Maiti *et al.,* 2021).

Human studies, although fewer, support these findings. Clinical trials have demonstrated that garlic supplementation can lower blood pressure, reduce serum cholesterol, improve lipid ratios, and enhance endothelial function in individuals at risk of cardiovascular disease (Ried *et al.,* 2016; Li *et al.,* 2022). Importantly, garlic is generally well-tolerated, with minimal side effects compared to conventional pharmacological agents, making it a

safe option for long-term use in both preventive and therapeutic settings (Banerjee and Maulik, 2021).

Women using combined oral contraceptives are at increased risk of cardiovascular complications, including ischemic events, due to estrogen-induced prothrombotic and endothelial changes (Lidegaard *et al.*, 2012; Okonofua *et al.*, 2019). Garlic's multi-targeted pharmacological actions—antioxidant, anti-inflammatory, anti-thrombotic, and vasodilatory—make it a particularly attractive intervention to counteract these effects. By reducing oxidative stress, inhibiting platelet aggregation, and improving coronary perfusion, garlic may mitigate the myocardial injury associated with oral contraceptive use, providing a natural and complementary therapeutic strategy (Maiti *et al.*, 2021; Rahman and Lowe, 2020).

Beyond its pharmacological effects, garlic is affordable, widely available, and culturally accepted in many regions, including Africa, which enhances its feasibility as a therapeutic intervention in resource-limited settings (Banerjee and Maulik, 2021). Its safety profile, low cost, and potential to target multiple pathways involved in IHD pathogenesis support further research into its application in experimental models, such as female rats exposed to oral contraceptives. Such preclinical studies can generate critical evidence for its efficacy, optimal dosing, and mechanisms of action, ultimately informing future translational and clinical research.

Garlic (*Allium sativum*) represents a promising natural therapeutic agent for the prevention and reversal of ischemic myocardial injury, particularly in scenarios where oxidative stress, thrombotic risk, and endothelial dysfunction are heightened, such as in oral contraceptive

users. Its multi-faceted cardioprotective properties, combined with accessibility and safety, provide a strong rationale for investigating its effects in preclinical models and, potentially, clinical applications.

Figure 2.2 Garlic (*Allium sativum*) **(Amoah and Kengne, 2023).**

2.2.1 Antioxidant and Anti-Inflammatory Theory of Garlic (Allium sativum)

Garlic (*Allium sativum*) has been used for centuries, not only as a culinary ingredient but also for its medicinal properties, particularly in the prevention and management of cardiovascular diseases. Modern scientific research has increasingly validated its antioxidant and anti-inflammatory potential, which provides a mechanistic explanation for its cardioprotective effects. Garlic contains a variety of bioactive compounds, including allicin, ajoene, diallyl sulfide, S-allyl cysteine, and flavonoids, which act synergistically to modulate oxidative stress, reduce inflammation, and preserve endothelial function (Banerjee and Maulik, 2021; Ried *et al.,* 2016).

The antioxidant activity of garlic is multifaceted. Bioactive compounds in garlic can directly scavenge reactive oxygen species (ROS), including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, thereby reducing oxidative damage to cellular membranes, proteins, and nucleic acids (Agarwal, 2020). Beyond direct ROS scavenging, garlic has been shown to upregulate endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, enhancing the heart's intrinsic defense system against oxidative stress (Banerjee and Maulik, 2021). In the context of ischemic heart disease, where oxidative stress plays a pivotal role in myocardial injury, garlic's antioxidant properties can attenuate lipid peroxidation, prevent mitochondrial dysfunction, and protect cardiomyocytes from apoptosis and necrosis (Zhang *et al.*, 2025).

In addition to its antioxidant effects, garlic exhibits powerful anti-inflammatory properties, which further contribute to cardiovascular protection. Chronic low-grade inflammation is a central driver of atherosclerosis and ischemic events, as pro-inflammatory cytokines such

as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) promote endothelial dysfunction, plaque formation, and plaque instability (Kim *et al.*, 2018). Garlic compounds, particularly allicin and S-allyl cysteine, inhibit the activation of nuclear factor-kappa B (NF-κB) signaling, a master regulator of inflammatory gene expression, thereby reducing the synthesis of pro-inflammatory cytokines and adhesion molecules (Banerjee and Maulik, 2021). This dual antioxidant and anti-inflammatory action is particularly relevant for women exposed to oral contraceptives, as hormonal modulation can induce oxidative stress and mild inflammation that may predispose the myocardium to ischemic injury (Okonofua *et al.,* 2019).

Furthermore, experimental studies have demonstrated that garlic can improve endothelial function, a critical factor in maintaining vascular health. Endothelial cells regulate vascular tone, platelet aggregation, and leukocyte adhesion, all of which are disrupted during ischemia and inflammation. Garlic bioactive compounds enhance nitric oxide (NO) bioavailability, reduce oxidative degradation of NO, and suppress endothelial activation, thereby improving vasodilation, reducing thrombosis risk, and protecting the myocardium from ischemic insult (Ried *et al.,* 2016; Agarwal, 2020).

In African and Nigerian populations, where cardiovascular risk factors such as hypertension, obesity, and diabetes are on the rise, garlic's cardioprotective potential is particularly significant (Adebayo *et al.,* 2022). The combination of dietary accessibility, low cost, and well-documented safety profile makes garlic an attractive natural therapeutic intervention for mitigating oxidative stress, reducing inflammation, and potentially

reversing early ischemic changes in the myocardium, especially in women using oral contraceptives.

In summary, the Antioxidant and Anti-Inflammatory Theory of Garlic provides a compelling mechanistic framework for its cardioprotective effects. By scavenging ROS, enhancing endogenous antioxidant defenses, suppressing inflammatory pathways, and improving endothelial function, garlic offers a multi-targeted approach to protect the heart from ischemic injury. These properties support ongoing research into its potential use as an adjunctive therapy to reduce myocardial damage in experimental models, including female rats exposed to oral contraceptives, and ultimately in clinical settings.

2.2.2 Pharmacological and Therapeutic Roles of Garlic (*Allium sativum*)

Garlic (*Allium sativum*) is a bioactive medicinal plant widely recognized for its cardioprotective properties. Its therapeutic potential derives primarily from its rich composition of organosulfur compounds, with allicin being the most biologically active constituent formed when raw garlic cloves are crushed or chopped. Other important compounds include S-allyl cysteine, ajoene**,** diallyl sulfides, and vinyldithiins, each contributing to garlic's broad spectrum of pharmacological effects (Ried *et al.*, 2016; Banerjee and Maulik, 2021). These compounds exert synergistic actions that target multiple pathways implicated in cardiovascular dysfunction, making garlic a uniquely multifaceted agent.

2.2.2.1 Antioxidant Properties

One of the primary mechanisms through which garlic exerts cardioprotective effects is via **antioxidant activity**. Ischemic heart disease is characterized by an imbalance between

reactive oxygen species (ROS) and antioxidant defenses, resulting in oxidative stress, which damages lipids, proteins, and nucleic acids in cardiomyocytes (Zhang *et al.,* 2025). Garlic's organosulfur compounds neutralize ROS directly, while also upregulating endogenous antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, thus reducing oxidative injury to cardiac cells (Agarwal, 2020). This antioxidative action helps preserve mitochondrial integrity, prevent lipid peroxidation in myocardial membranes, and maintain cellular viability during ischemic stress (Banerjee and Maulik, 2021).

2.2.2.2 Anti-inflammatory Effects

Inflammation is another key factor in the pathogenesis of myocardial ischemia and atherosclerosis. Chronic low-grade inflammation promotes endothelial dysfunction, plaque instability, and eventual myocardial injury (Kim *et al.*, 2018). Garlic and its bioactive constituents inhibit pro-inflammatory signaling pathways, particularly nuclear factor-kappa B (NF-κB**)**, and suppress the production of cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP**)** (Banerjee and Maulik, 2021). By reducing inflammation, garlic can stabilize atherosclerotic plaques, improve vascular function, and limit the extent of ischemic myocardial damage, thus contributing to both prevention and recovery.

2.2.2.3 Hypolipidemic and Endothelial Protective Actions

Garlic positively influences lipid metabolism and endothelial function, which are critical in preventing atherosclerosis, a central component of IHD. Studies have shown that garlic reduces serum total cholesterol, low-density lipoprotein (LDL), and triglycerides, while

modestly increasing high-density lipoprotein (HDL) levels (Ried *et al.,* 2016). Furthermore, garlic enhances endothelial nitric oxide (NO) bioavailability, promoting vasodilation and improved coronary perfusion (Agarwal, 2020). This dual effect—improving lipid profile and endothelial function—reduces the likelihood of plaque formation and improves myocardial oxygen delivery, particularly important in conditions of ischemia.

2.2.2.4 Antihypertensive and Antithrombotic Properties

Garlic has been widely reported to exert blood pressure-lowering effects, likely through modulation of the renin-angiotensin-aldosterone system and increased NO-mediated vasodilation (Ried *et al.,* 2016). Hypertension is a significant risk factor for IHD, and garlic's antihypertensive effect helps reduce myocardial workload and oxygen demand. Additionally, garlic exhibits antithrombotic effects, inhibiting platelet aggregation and fibrinogen synthesis, which decreases the risk of clot formation in coronary arteries a key event in the progression of myocardial infarction (Agarwal, 2020).

2.2.2.5 Evidence from Experimental Models

Experimental studies in rodents and other animal models have consistently demonstrated garlic's cardioprotective and reparative effects. For instance, in models of ischemia-reperfusion injury, garlic extract administration significantly reduced infarct size, preserved myocardial enzyme activity, and improved cardiac contractility (Banerjee and Maulik, 2021; Ried *et al.,* 2016). In hyperlipidemic or hypertensive animal models, garlic has been shown to normalize lipid profiles**,** reduce oxidative stress markers, enhance

endothelial function, and attenuate inflammatory responses, suggesting its potential to reverse early pathological changes in the cardiovascular system.

2.2.3 Relevance in Oral Contraceptive-Induced Cardiovascular Risk

The use of hormonal contraceptives, particularly combined estrogen-progestin formulations, has been associated with increased oxidative stress, low-grade inflammation, endothelial dysfunction, and a prothrombotic state in females (Okonofua *et al.,* 2019; Zhang *et al.,* 2025). Garlic's multifaceted pharmacological properties directly counteract these mechanisms, making it a promising adjunctive therapy to prevent or reverse cardiac injury in women exposed to OCs. By simultaneously reducing oxidative damage, modulating lipid profiles, stabilizing endothelial function, and mitigating inflammation, garlic offers a holistic approach to cardiovascular protection, potentially lowering the risk of ischemic events associated with hormonal modulation. Garlic**'s** bioactive constituents, particularly allicin and other organosulfur compounds, confer antioxidant, anti-inflammatory, hypolipidemic, antihypertensive, and antithrombotic effects, all of which are mechanistically relevant to cardiovascular protection. Evidence from experimental models supports its potential to reverse myocardial injury and restore cardiac function, particularly in settings of oxidative and hormonal stress, providing a scientifically plausible basis for its investigation in female rats exposed to oral contraceptives.

2.3 Oral Contraceptives

Oral contraceptives (OCs) are among the most widely used methods of birth control globally and are particularly popular in many African countries due to their convenience, effectiveness, and reversibility (Okonofua *et al.,* 2019). These medications work primarily

by modulating hormonal levels to prevent ovulation, alter cervical mucus to hinder sperm movement, and change the endometrial lining to reduce the likelihood of implantation (Grimes *et al.,* 2018). While highly effective for family planning, oral contraceptives vary in their composition and hormonal strength, which can influence both their efficacy and potential side effects, including cardiovascular risks (Lidegaard *et al.,* 2012).

The most common type is the combined oral contraceptive (COC)**,** which contains both estrogen (usually ethinylestradiol) and a synthetic progestin. These pills work synergistically to inhibit ovulation and maintain regular menstrual cycles. COCs have been extensively studied and are generally safe for most healthy women, but they can increase the risk of venous thromboembolism, arterial thrombosis, and changes in blood pressure, particularly in women with predisposing factors such as obesity, hypertension, or smoking (Lidegaard *et al.,* 2012; Okonofua *et al.,* 2019).

Progestin-only pills (POPs)**,** also known as mini-pills, contain only a synthetic form of progesterone and are preferred in women who cannot take estrogen, including those with a history of thromboembolic events or certain cardiovascular risk factors. POPs primarily work by thickening cervical mucus and thinning the endometrium, with some suppression of ovulation. While they have a lower thrombotic risk than COCs, careful monitoring is still required, especially in populations with other metabolic or cardiovascular risk factors (Grimes *et al.,* 2018).

Oral contraceptives are also categorized by hormonal dose**.** High-dose formulations, which contain higher amounts of estrogen and/or progestin, were more common in earlier decades but are now rarely used due to a greater incidence of adverse cardiovascular and metabolic

effects. High-dose OCs can significantly increase the risk of blood clots, stroke, and myocardial ischemia, particularly in women over 35 years or those with other risk factors (Lidegaard *et al.,* 2012). In contrast, low-dose formulations contain reduced concentrations of estrogen and/or progestin, which minimize side effects while maintaining contraceptive efficacy. Low-dose OCs are currently favored because they balance effectiveness with a lower risk of thrombotic and hypertensive complications (Okonofua *et al.,* 2019).

In addition to these classifications, newer generations of OCs have been developed to optimize hormonal balance and reduce side effects. Third- and fourth-generation progestins, for example, have improved metabolic profiles and may be associated with lower androgenic effects, such as acne or hirsutism, but some studies suggest they may carry a slightly higher risk of venous thrombosis compared to earlier formulations (Lidegaard *et al.,* 2012). The diversity of oral contraceptive types and doses highlights the importance of individualized prescription, particularly for women with pre-existing cardiovascular risk factors, as hormonal exposure can directly influence endothelial function, coagulation pathways, and myocardial oxygen demand (Adebayo *et al.,* 2022).

Overall, understanding the types and hormonal composition of oral contraceptives is essential in assessing their potential **cardiovascular implications**, particularly in women who may already be predisposed to ischemic heart disease. This knowledge also underpins the rationale for exploring complementary interventions, such as natural compounds with cardioprotective properties, to mitigate the adverse effects of long-term hormonal exposure (Ried *et al.,* 2016; Banerjee and Maulik, 2021).

Figure 2.3 Oral Contraceptives (Ried *et al.*, 2016).

2.3.1 Types of Oral Contraceptives

Oral contraceptives (OCs) are broadly classified based on their hormonal composition, dosage, and intended mechanism of action. These classifications are important because the type and dose of hormones influence both contraceptive effectiveness and the risk of side effects, including cardiovascular complications (Lidegaard *et al.,* 2012; Okonofua *et al.*, 2019).

2.3.1.2 Combined Oral Contraceptives (COCs)

Combined oral contraceptives contain both estrogen and progestin hormones. They are the most commonly used form of OCs globally and function by suppressing ovulation, altering cervical mucus to prevent sperm penetration, and modifying the endometrial lining to reduce the likelihood of implantation (Grimes *et al.,* 2018). COCs are available in various formulations, including monophasic (constant hormone dose throughout the cycle), biphasic, and triphasic (hormone dose changes throughout the cycle) preparations. While highly effective, COCs carry certain cardiovascular risks, particularly related to the estrogen component, which can increase thrombotic events in susceptible women (Vandenbroucke *et al.,* 2011).

2.3.1.3 Progestin-Only Pills (POPs)

Progestin-only pills, often called “mini-pills,” contain only synthetic progestin without estrogen. They primarily work by thickening cervical mucus and thinning the endometrium, and in some cases, by suppressing ovulation. POPs are particularly useful for women who cannot tolerate estrogen due to pre-existing conditions such as hypertension, migraines, or a history of thromboembolic events (Okonofua *et al.,* 2019).

Although generally safer for cardiovascular risk, POPs may be less forgiving if doses are missed and require strict adherence to maintain effectiveness.

2.3.1.4 High-Dose Oral Contraceptives

High-dose OCs were among the first formulations developed and typically contain higher levels of estrogen. While effective in preventing pregnancy, these formulations have been linked to an increased risk of cardiovascular events, including thromboembolism, hypertension, and ischemic heart disease (Lidegaard *et al.*, 2012). High-dose pills are rarely used today due to these risks, having been largely replaced by lower-dose alternatives.

2.3.1.5 Low-Dose Oral Contraceptives

Low-dose OCs contain reduced amounts of estrogen and progestin, offering effective contraception with fewer adverse effects. By minimizing estrogen exposure, low-dose pills reduce the risk of thrombotic complications, blood pressure elevation, and other cardiovascular issues, while maintaining contraceptive efficacy (Grimes *et al.*, 2018). These formulations are now preferred for routine use, particularly in women with moderate cardiovascular risk factors or those requiring long-term contraception. These categories, modern oral contraceptives are further refined by the generation of progestin, which affects androgenic activity, lipid metabolism, and cardiovascular safety. Third- and fourth-generation progestins are designed to reduce androgenic side effects such as acne and hirsutism but may carry slightly higher thrombotic risk compared to earlier formulations (Vandenbroucke *et al.*, 2011). Understanding the type, dose, and hormonal composition of OCs is therefore essential for evaluating their safety profile, particularly regarding cardiovascular complications. The selection of an oral contraceptive should consider the

woman's age, cardiovascular risk factors, reproductive goals, and lifestyle**.** Proper counseling and individualized prescription are critical to maximize benefits while minimizing potential adverse outcomes, especially in populations with rising cardiovascular disease prevalence, such as women in Nigeria and other sub-Saharan African countries (Adebayo *et al.*, 2022).

2.3.2 Oral Contraceptive Use and Cardiovascular Complications

Oral contraceptives (OCs) are one of the most commonly used methods of reversible contraception worldwide, offering women effective birth control, regulation of menstrual cycles, and management of gynecological disorders such as polycystic ovarian syndrome and endometriosis (Lidegaard *et al.,* 2012; Vessey *et al.,* 2023). While their clinical benefits are well-documented, accumulating evidence suggests that OCs, particularly combined estrogen-progestin formulations, may predispose women to cardiovascular complications, including ischemic heart disease (IHD), venous thromboembolism (VTE), and arterial events such as myocardial infarction and ischemic stroke (Baillargeon *et al.*, 2005; Dinger *et al.*, 2016). These risks, although generally low in healthy young women, become more pronounced in individuals with pre-existing cardiovascular risk factors, including hypertension, obesity, diabetes, smoking, and dyslipidemia (Vinogradova *et al.*, 2015; Okonofua *et al.,* 2019).

Mechanistically, the estrogen component of OCs influences the cardiovascular system by altering hemostasis and vascular function. Estrogen increases plasma concentrations of procoagulant factors such as fibrinogen and factors VII, VIII, and X, while simultaneously reducing anticoagulant activity of protein S and antithrombin, leading to a prothrombotic

state (Dinger *et al.,* 2016). It also promotes endothelial dysfunction and enhances platelet aggregation, which can impair coronary blood flow and increase the risk of arterial thrombus formation (Lidegaard *et al.,* 2012; Baillargeon *et al.,* 2005). The progestin component, depending on its type and generation, may further modulate these effects, with certain formulations carrying higher thrombotic risks than others (Vinogradova *et al.,* 2015).

Epidemiological evidence consistently shows that OC users have an increased risk of venous thromboembolism, with reported rates ranging from 2 to 6 times higher than non-users, particularly during the first year of use or when high-dose estrogen formulations are employed (Lidegaard *et al.,* 2012; Vinogradova *et al.,* 2015). Arterial events, including myocardial infarction and ischemic stroke, though less frequent, have also been linked to OC use, especially in women over 35 years of age, smokers, or those with underlying metabolic syndrome (Baillargeon *et al.,* 2005; Dinger *et al.,* 2016). Studies conducted in African populations highlight similar trends: Nigerian women using OCs have been reported to exhibit elevated blood pressure, impaired lipid profiles, and a greater prevalence of cardiovascular events compared to non-users, underscoring the importance of cardiovascular risk assessment prior to contraceptive prescription (Okonofua *et al.,* 2019).

The cardiovascular risk associated with OCs is further compounded by lifestyle and environmental factors prevalent in developing countries. High rates of obesity, sedentary lifestyles, and limited access to healthcare services increase the baseline risk for IHD and related complications, making OC use in these populations more clinically significant

(Okonofua *et al.,* 2019; Vessey *et al.,* 2023). Animal studies have also provided evidence for the mechanistic basis of these effects. Rodent models exposed to estrogen-containing contraceptives exhibit endothelial dysfunction, increased oxidative stress, and heightened platelet aggregation, all of which mimic the pathophysiological changes seen in human IHD (Maiti *et al.,* 2021). These findings are particularly relevant for preclinical studies investigating interventions, such as garlic (*Allium sativum*) extract, that may reverse or mitigate the cardiovascular damage induced by hormonal contraceptives.

Despite these risks, modern low-dose OC formulations have significantly reduced the absolute incidence of thrombotic and ischemic events. However, caution remains essential, particularly for women with predisposing risk factors, and individualized assessment of cardiovascular risk is recommended before initiation (Lidegaard *et al.,* 2012; Dinger *et al.,* 2016). The intersection of oral contraceptive use and cardiovascular complications highlights an important clinical and public health concern, especially in settings with rising prevalence of metabolic and cardiovascular disorders. Understanding this relationship is critical for informing safer contraceptive practices, guiding preventive strategies, and identifying potential therapeutic interventions to mitigate OC-induced cardiovascular damage (Okonofua *et al.,* 2019; Vessey *et al.,* 2023).

2.3.2 Mechanism of Action of Oral Contraceptives

Oral contraceptives (OCs) prevent pregnancy through a combination of hormonal effects that target the reproductive system at multiple levels. These mechanisms differ slightly between combined oral contraceptives (COCs) containing both estrogen and progestin, and

progestin-only pills (POPs), but all aim to disrupt ovulation, fertilization, and implantation (Grimes *et al.,* 2018; Okonofua *et al.,* 2019).

1. **Suppression of Ovulation**

The primary mechanism of COCs is the inhibition of ovulation. Estrogen and progestin act synergistically to suppress the hypothalamic-pituitary-ovarian axis. Specifically, estrogen provides negative feedback to the hypothalamus and pituitary gland, reducing the release of gonadotropin-releasing hormone (GnRH) and subsequently follicle-stimulating hormone (FSH). Reduced FSH levels prevent the maturation of ovarian follicles. Progestin also contributes by suppressing luteinizing hormone (LH) surges, which are necessary for ovulation (Okonofua *et al.,* 2019). By preventing the release of a mature ovum, the risk of fertilization is effectively eliminated.

2. **Alteration of Cervical Mucus**

Progestin in both COCs and POPs thickens cervical mucus, creating a barrier that is hostile to sperm penetration. This effect reduces the likelihood of sperm reaching the ovum, further preventing fertilization. In women taking POPs, where ovulation suppression may be less consistent, this mechanism is particularly critical for maintaining contraceptive effectiveness (Grimes *et al.,* 2018).

3. **Endometrial Changes**

OCs induce structural and functional changes in the endometrium, making it less receptive to implantation. Estrogen and progestin regulate the proliferation and secretory

transformation of the endometrial lining. Under the influence of OCs, the endometrium remains thin and less vascularized, reducing the likelihood that a fertilized ovum can successfully implant and develop (Okonofua *et al.,* 2019).

4. Effects on Tubal Motility

OCs may also alter the contractility of the fallopian tubes, slowing the transport of the ovum. This delay can further reduce the chances of sperm meeting the ovum and contribute to the overall contraceptive efficacy of the pill (Reed *et al.,* 2016).

5. Additional Systemic Effects

Beyond reproductive targets, OCs exert systemic hormonal effects that influence lipid metabolism, coagulation, and vascular function. Estrogen increases hepatic production of clotting factors, such as fibrinogen and prothrombin, and can also modify lipid profiles by increasing HDL cholesterol and, in some cases, raising triglycerides (Vandenbroucke *et al.,* 2011; Lidegaard *et al.,* 2012). Progestins may counterbalance some of estrogen's effects on lipids but can vary by generation, dose, and androgenic activity. These systemic effects are critical to consider, as they contribute to the cardiovascular risk profile associated with OC use, especially in women with pre-existing risk factors or prolonged exposure (Okonofua *et al.,* 2019). Oral contraceptives exert a multi-level mechanism of action that effectively prevents pregnancy through ovulation suppression, cervical mucus alteration, endometrial modulation, and tubal motility change**s**. These reproductive effects are complemented by systemic hormonal influences, which while beneficial in some contexts, can also predispose users to cardiovascular complications. Understanding these

mechanisms provides the foundation for evaluating both the benefits and risks of OC use, particularly in populations with elevated metabolic or cardiovascular vulnerabilities, such as Nigerian women exposed to urbanized

2.3.3 Cardiovascular Effects Associated with Oral Contraceptive Use

Oral contraceptives (OCs) have been widely acclaimed for their effectiveness in family planning, yet their long-term use has been linked to several cardiovascular effects that require careful consideration, particularly in women with pre-existing risk factors or metabolic conditions. These effects are largely influenced by the hormonal composition, dose, and duration of OC use, as well as individual characteristics such as age, body mass index, and genetic predisposition (Lidegaard *et al.,* 2012; Okonofua *et al.,* 2019).

2.3.3.1 Thrombotic Events and Hypercoagulability

One of the most well-documented cardiovascular risks associated with OCs is the increased tendency for blood clot formation. Estrogen, a key component of combined oral contraceptives (COCs), enhances hepatic synthesis of clotting factors such as fibrinogen and prothrombin while reducing anticoagulant proteins like protein S. This creates a hypercoagulable state, increasing the likelihood of venous thromboembolism (VTE), deep vein thrombosis (DVT), pulmonary embolism, and arterial thrombosis, which may precipitate myocardial infarction or ischemic stroke (Vandenbroucke *et al.,* 2011; Lidegaard *et al.,* 2012). The risk is especially significant in women with additional factors such as obesity, hypertension, smoking, or a family history of thrombotic events, which are increasingly prevalent in urbanized African populations (Adebayo *et al.,* 2022).

2.3.3.2 Blood Pressure Elevation

OCs can also influence blood pressure regulation. Estrogen promotes sodium and water retention, while both estrogen and progestin may modulate the renin-angiotensin-aldosterone system. These hormonal effects can result in modest but clinically meaningful increases in blood pressure, particularly in women with baseline hypertension or salt-sensitive individuals (Reckelhoff, 2018; Ogunmola *et al.,* 2021). Chronic elevations in blood pressure over time can accelerate vascular damage, contributing to endothelial dysfunction, arterial stiffness, and increased risk of ischemic heart disease (Zhang *et al.*, 2025).

2.3.3.3 Lipid and Metabolic Alterations

The androgenic or anti-androgenic properties of progestins in OCs can affect lipid metabolism. Early-generation progestins with higher androgenic activity tend to raise low-density lipoprotein (LDL) cholesterol and reduce high-density lipoprotein (HDL) cholesterol, promoting atherogenesis. Modern low-dose and newer-generation progestins have improved lipid profiles but may still induce mild changes that cumulatively impact cardiovascular risk over time (Okonofua *et al.,* 2019). In populations with increasing rates of obesity, insulin resistance, and metabolic syndrome—such as women in Nigeria—these subtle metabolic effects may have significant long-term consequences.

2.3.3.4 Endothelial Dysfunction and Inflammation

OCs have been associated with changes in vascular function, including endothelial dysfunction and elevated markers of systemic inflammation. Endothelial cells, which normally maintain vascular homeostasis and prevent thrombosis, may become less

responsive due to oxidative stress, hormonal fluctuations, and altered nitric oxide bioavailability induced by estrogen and progestin. Additionally, pro-inflammatory cytokines may increase, contributing to vascular inflammation, plaque formation, and atherosclerotic progression (Ogunmola *et al.*, 2021; Zhang *et al.*, 2025).

2.3.3.5 Risk Stratification in African Women

In the African and Nigerian context, these cardiovascular effects are of particular concern. Rapid urbanization, dietary changes, sedentary lifestyles, and rising prevalence of non-communicable diseases like hypertension, diabetes, and obesity compound the baseline risk associated with OC use. Limited access to routine cardiovascular screening further exacerbates the problem, often resulting in women continuing hormonal contraceptives without early detection of adverse vascular changes (Ogunmola *et al.*, 2021; Adebayo *et al.*, 2022).

2.3.3.6 Dose, Duration, and Individual Susceptibility

The cardiovascular impact of OCs is dose-dependent and influenced by the duration of use. High-dose estrogen formulations are more strongly associated with myocardial infarction, stroke, and thromboembolic events, whereas low-dose preparations carry lower but not negligible risks. Long-term use, particularly in women over 35 or with coexisting risk factors, magnifies these effects. Individual susceptibility, including genetic thrombophilia, lipid profile, and lifestyle factors, plays a critical role in determining the extent of cardiovascular compromise (Lidegaard *et al.*, 2012). While oral contraceptives provide effective family planning, they carry measurable cardiovascular risks, including hypercoagulability, elevated blood pressure, adverse lipid changes, endothelial

dysfunction, and inflammation. These risks are modulated by hormone type, dose, and individual factors, emphasizing the importance of risk assessment, monitoring, and patient education before and during OC use. Understanding these mechanisms is essential for exploring therapeutic interventions, such as natural compounds like garlic (*Allium sativum*), which may offer protective cardiovascular effects in women exposed to hormonal contraceptives.

2.4 Hormonal Modulation Theory

The hormonal modulation theory provides a conceptual framework for understanding how exogenous hormones, such as those in oral contraceptives (OCs), influence cardiovascular physiology and the development of ischemic heart disease (IHD). This theory posits that hormones—particularly estrogen and progestin—modulate vascular tone, lipid metabolism, coagulation pathways, and endothelial function, which collectively affect cardiovascular risk (Reckelhoff, 2018; Okonofua *et al.,* 2019).

2.4.1 Estrogen and Vascular Function

Estrogen plays a dual role in cardiovascular health. At physiological levels, it has protective effects by promoting vasodilation, enhancing endothelial nitric oxide production, and improving lipid profiles through increased high-density lipoprotein (HDL) cholesterol and reduced low-density lipoprotein (LDL) cholesterol (Zhang *et al.,* 2025). However, when administered exogenously in OCs, particularly at higher doses, estrogen can paradoxically increase prothrombotic tendencies by enhancing hepatic synthesis of clotting factors and reducing anticoagulant proteins such as protein S, thereby elevating the risk of thrombosis and ischemic events (Vandenbroucke *et al.,* 2011; Lidegaard *et al.,* 2012).

2.4.2 Progestin and Metabolic Modulation

Progestins in OCs influence cardiovascular outcomes through their androgenic or anti-androgenic properties. Earlier-generation progestins with androgenic activity have been shown to adversely affect lipid metabolism, raising LDL cholesterol and lowering HDL cholesterol, which can accelerate atherosclerosis (Okonofua *et al.,* 2019). Newer-generation progestins are formulated to minimize these effects, yet they may still contribute modestly to pro-inflammatory and pro-thrombotic states. By modulating insulin sensitivity, vascular tone, and inflammatory responses, progestins interact with estrogen to collectively influence cardiovascular risk.

2.4.3 Hormonal Effects on Hemostasis and Coagulation

The hormonal modulation theory also emphasizes the role of exogenous hormones in altering hemostatic balance. Estrogen promotes the synthesis of clotting factors such as fibrinogen, prothrombin, and factors VII, VIII, and X, while reducing natural anticoagulants. Progestins further modulate platelet aggregation and fibrinolysis, creating a hormonal environment conducive to hypercoagulability (Vandenbroucke *et al.,* 2011). This explains why certain women using OCs, particularly those with underlying risk factors like obesity, smoking, or genetic thrombophilia, are more prone to cardiovascular events such as myocardial infarction or stroke.

2.4.4 Endothelial Dysfunction and Inflammation

Hormones in OCs influence endothelial cell function, which is crucial for maintaining vascular health. Estrogen and progestin modulate nitric oxide availability, reactive oxygen species generation, and inflammatory cytokine expression, all of which contribute to

endothelial integrity or dysfunction depending on the balance of hormonal and metabolic factors (Zhang *et al.,* 2025). Chronic endothelial stress induced by exogenous hormones can accelerate atherogenesis and impair myocardial perfusion, linking OC use with increased ischemic risk.

2.4.5 Implications for Women in African Settings

In African populations, including Nigerian women, the interplay between hormonal modulation and cardiovascular risk is particularly significant due to high prevalence of metabolic syndrome, hypertension, obesity, and limited access to routine cardiovascular screening (Adebayo *et al.,* 2022; Ogunmola *et al.,* 2021). The hormonal modulation theory thus provides a valuable lens through which to understand why OC use, while highly effective for family planning, may inadvertently exacerbate cardiovascular vulnerability in these populations. The hormonal modulation theory underscores how exogenous estrogen and progestin influence multiple cardiovascular pathways—ranging from lipid metabolism and endothelial function to coagulation and inflammation—thereby shaping the risk of IHD and thrombotic events. This theoretical perspective is fundamental for understanding the rationale behind exploring adjunctive therapies, such as garlic (*Allium sativum*) extract, which may counteract OC-induced oxidative stress, endothelial dysfunction, and hypercoagulability in experimental models.

2.5 Hormonal Balance and Cardiovascular Function

The cardiovascular system is highly sensitive to hormonal fluctuations, and the balance between estrogen and progestin plays a pivotal role in maintaining vascular health. **Estrogen** is generally considered cardioprotective when present at physiological levels. It

promotes vasodilation by enhancing endothelial nitric oxide synthase (eNOS) activity, increases HDL cholesterol while reducing LDL cholesterol, and exerts anti-inflammatory effects on vascular tissues (Reckelhoff, 2018; Zhang *et al.,* 2025). Estrogen also modulates vascular smooth muscle tone, thereby improving coronary perfusion and reducing the likelihood of ischemic events.

Progestins**,** on the other hand, have more complex effects that depend on their type and androgenic activity. Some progestins can counteract the beneficial lipid and vascular effects of estrogen by increasing LDL cholesterol and reducing HDL cholesterol, promoting mild vasoconstriction, and influencing coagulation pathways (Okonofua *et al.*, 2019). The interplay between estrogen and progestin determines whether the net cardiovascular effect is protective, neutral, or adverse. High estrogen relative to progestin may enhance prothrombotic states, while certain progestins may amplify lipid abnormalities and endothelial dysfunction. Oral contraceptives, the exogenous administration of estrogen and progestin alters this natural balance, often in ways that can predispose to cardiovascular compromise. Combined oral contraceptives (COCs), for example, deliver synthetic estrogen and progestin simultaneously, which may shift the hormonal milieu toward increased coagulation, endothelial stress, and subtle metabolic changes (Vandenbroucke *et al.,* 2011; Lidegaard *et al.,* 2012). These shifts are more pronounced in high-dose formulations and in women with pre-existing cardiovascular risk factors such as hypertension, obesity, diabetes, or smoking, which are increasingly common in urban African populations (Ogunmola *et al.,* 2021; Adebayo *et al.,* 2022).

2.6 Theoretical Basis for Oral Contraceptive-Induced Ischemic Changes

The theoretical basis for OC-induced ischemic changes is grounded in the hormonal modulation theory and integrates knowledge from vascular biology, hemostasis, and atherogenesis. Exogenous hormones influence cardiovascular function through multiple interrelated pathways:

1. **Prothrombotic Effects**: Estrogen increases the synthesis of clotting factors (fibrinogen, prothrombin, factors VII, VIII, and X) and reduces natural anticoagulants, creating a hypercoagulable state. Progestins can further modulate platelet aggregation and fibrinolysis, raising the risk of thrombus formation in coronary arteries (Vandenbroucke *et al.,* 2011). Arterial or venous occlusion from thrombi may precipitate ischemic events, such as myocardial infarction.
2. **Endothelial Dysfunction**: OCs can impair endothelial function by reducing nitric oxide availability, increasing oxidative stress, and promoting inflammation. Endothelial dysfunction diminishes the ability of coronary arteries to dilate appropriately in response to increased oxygen demand, setting the stage for ischemia (Zhang *et al.,* 2025).
3. **Lipid and Metabolic Dysregulation**: Certain progestins may worsen lipid profiles by increasing LDL and triglycerides while reducing HDL. This accelerates atherogenesis, narrowing coronary arteries and compromising blood flow to the myocardium (Okonofua *et al.,* 2019).
4. **Blood Pressure Modulation**: Estrogen-mediated sodium retention and progestin effects on vascular tone can elevate blood pressure, which increases shear stress on arterial walls and promotes atherosclerotic plaque development (Reckelhoff, 2018).

5. **Cumulative Risk Factors**: The likelihood of OC-induced ischemic changes is heightened when multiple risk factors converge, such as older age, prolonged OC use, obesity, smoking, sedentary lifestyle, and genetic predispositions. In African settings, these risk factors are increasingly prevalent, highlighting the clinical significance of understanding OC-induced cardiovascular changes (Ogunmola *et al.,* 2021; Adebayo *et al.,* 2022**).** Oral contraceptive-induced ischemia arises from a complex interplay of hormonal effects on coagulation, endothelial integrity, lipid metabolism, and vascular tone. The exogenous estrogen/progestin balance can shift cardiovascular homeostasis toward a state of hypercoagulability, atherogenesis, and reduced myocardial perfusion, ultimately increasing the risk of ischemic heart disease. Understanding these theoretical underpinnings provides a foundation for investigating protective interventions, including the cardioprotective potential of natural compounds like garlic (*Allium sativum*).

2.7 Oxidative Stress Theory in Cardiovascular Disease

Oxidative stress is a central and widely recognized mechanism underlying the development and progression of cardiovascular diseases, particularly ischemic heart disease (IHD). At its core, oxidative stress occurs when the delicate balance between reactive oxygen species (ROS) production and the body's endogenous antioxidant defenses is disrupted, leading to cellular and tissue damage (Madamanchi and Runge, 2022). ROS—including superoxide anions, hydrogen peroxide, and hydroxyl radicals—are natural byproducts of cellular metabolism, primarily generated within mitochondria during aerobic respiration. While physiological levels of ROS serve important roles in cell signaling, immune defense, and vascular tone regulation, excessive ROS accumulation overwhelms antioxidant systems

such as superoxide dismutase, catalase, and glutathione peroxidase, resulting in oxidative stress (Cadenas and Davies, 2020).

In the myocardium, oxidative stress has profound pathophysiological consequences. Excess ROS induce lipid peroxidation, damaging the phospholipid membranes of cardiomyocytes and endothelial cells, thereby compromising cell integrity and function (Zhang *et al.,* 2025). ROS also modify proteins and nucleic acids, disrupting enzymatic activity, gene expression, and mitochondrial function, which collectively impair myocardial energy metabolism and contractility (Madamanchi and Runge, 2022). During episodes of ischemia, when blood flow and oxygen supply to the heart are reduced, ROS production may initially decline; however, reperfusion—the sudden restoration of oxygen—triggers a dramatic surge in ROS, termed ischemia-reperfusion injury. This oxidative burst not only exacerbates cellular injury but also amplifies inflammatory responses, increases vascular permeability, and promotes microvascular dysfunction, all of which compound myocardial damage (Kalogeris *et al.*, 2016).

The link between oxidative stress and tissue necrosis is both direct and indirect. Lipid peroxidation destabilizes cell membranes, facilitating calcium overload within cardiomyocytes, which activates proteolytic enzymes such as calpains and caspases. These enzymatic cascades culminate in apoptosis and necrosis of cardiac cells (Zhang *et al.*, 2025). ROS further exacerbate mitochondrial dysfunction, impairing ATP synthesis and precipitating energy failure in myocardial tissue. Additionally, oxidative stress promotes atherogenesis by oxidizing low-density lipoprotein (LDL) cholesterol, enhancing endothelial adhesion molecule expression, and recruiting inflammatory cells, thereby

accelerating plaque formation and coronary artery narrowing (Cadenas and Davies, 2020). Collectively, these processes create a vicious cycle where oxidative stress both triggers ischemic injury and amplifies its severity.

From a clinical and epidemiological perspective, oxidative stress is particularly relevant in African populations, including Nigeria, where rising rates of hypertension, diabetes, obesity, and metabolic syndrome are increasingly prevalent due to urbanization, dietary transitions, and lifestyle changes (Adebayo *et al.,* 2022). Women using oral contraceptives (OCs) may experience subtle hormonal shifts that further influence oxidative balance. Estrogen and progestin modulate endothelial nitric oxide production, lipid metabolism, and inflammatory responses, and exogenous exposure through OCs can amplify ROS generation, impair antioxidant defenses, and exacerbate endothelial dysfunction (Okonofua *et al.,* 2019; Zhang *et al.,* 2025). This combination of hormonal modulation and oxidative stress increases susceptibility to ischemic events, particularly in women with additional cardiovascular risk factors.

The clinical consequences of oxidative stress in the heart are profound. Beyond myocardial necrosis, ROS contribute to arrhythmogenesis, impaired contractility, and adverse ventricular remodeling, which can lead to chronic heart failure if left unchecked. Moreover, oxidative stress interacts with other pathogenic mechanisms, including inflammation, endothelial dysfunction, and dyslipidemia, forming an interconnected network of cardiovascular injury pathways (Madamanchi and Runge, 2022).

Given the central role of oxidative stress in myocardial injury, therapeutic strategies targeting ROS have gained considerable attention. Natural compounds with antioxidant

properties, such as garlic **(***Allium sativum***)** extract**,** have demonstrated promising cardioprotective effects by scavenging free radicals, enhancing endogenous antioxidant activity, improving lipid profiles, and preserving endothelial function (Ried *et al.,* 2016; Banerjee and Maulik, 2021) Experimental studies indicate that such interventions may mitigate oxidative myocardial damage, reduce ischemia-reperfusion injury, and improve overall cardiac function, offering a potential adjunctive strategy to counteract OC-associated cardiovascular risks.

In conclusion, the oxidative stress theory provides a compelling framework for understanding the cellular and molecular mechanisms of myocardial ischemia. It emphasizes that excessive ROS production, overwhelmed antioxidant defenses, and subsequent tissue injury are central drivers of IHD pathophysiology. This understanding not only informs clinical management and preventive strategies but also underscores the rationale for exploring antioxidant therapies, including natural plant extracts like garlic, to reduce ischemic injury and improve cardiovascular outcomes, particularly in women exposed to hormonal contraceptives.

2.8 Garlic Extract in Myocardial Protection and Repair

Garlic (*Allium sativum*) has garnered significant attention for its cardioprotective properties**,** particularly in experimental models of myocardial ischemia and reperfusion (I/R) injury. Myocardial I/R injury occurs when blood supply to the heart is temporarily restricted and then restored, resulting in oxidative stress, inflammation, and cell death. Studies have shown that garlic extract can ameliorate the deleterious effects of I/R injury, preserving cardiac structure and function. For example, Banerjee and Maulik (2021)

demonstrated that garlic supplementation in rodent models reduced myocardial infarct size, decreased serum levels of cardiac enzymes such as creatine kinase-MB and lactate dehydrogenase, and improved left ventricular function, highlighting its role in cardiac repair mechanisms.

A key mechanism underlying garlic's cardioprotective action is its ability to modulate oxidative stress. Ischemic injury generates excessive reactive oxygen species (ROS), which damage lipids, proteins, and nucleic acids in myocardial cells. Garlic's organosulfur compounds, particularly allicin, scavenge free radicals and enhance endogenous antioxidant defenses. Experimental studies report that garlic treatment reduces malondialdehyde (MDA) levels**,** a marker of lipid peroxidation, while increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Ried *et al.,* 2016; Agarwal, 2020). By restoring redox balance, garlic protects cardiomyocytes from oxidative damage, reduces apoptosis, and promotes tissue repair following ischemic insult.

In addition to antioxidant effects, garlic exhibits anti-inflammatory and endothelial-protective actions**,** which further enhance myocardial recovery. Studies indicate that garlic downregulates pro-inflammatory cytokines (TNF-α, IL-6) and reduces the expression of adhesion molecules in endothelial cells, thereby limiting inflammatory infiltration and vascular injury during I/R events (Kim *et al.,* 2018). These combined effects help preserve myocardial microcirculation, maintain contractile efficiency, and accelerate recovery of cardiac function.

While much of the research has been conducted in male rodents, evidence in female or hormonally-influenced models is emerging, which is particularly relevant in the context of oral contraceptive use or estrogen/progestin modulation. In female rat models, garlic supplementation has been shown to improve antioxidant capacity, reduce oxidative damage, and enhance myocardial enzyme profiles**,** suggesting that its protective effects extend across sexes and may counteract hormonal influences that predispose to ischemic injury (Banerjee and Maulik, 2021; Zhang *et al.,* 2025). These findings provide a strong rationale for investigating garlic in female rats exposed to oral contraceptives, where hormonal modulation may exacerbate oxidative stress and endothelial dysfunction.

Furthermore, garlic's multi-targeted pharmacological profile makes it particularly attractive for myocardial repair. By simultaneously addressing **o**xidative stress, inflammation, endothelial dysfunction, and thrombosis**,** garlic provides a holistic protective effect that not only limits injury during ischemic events but also facilitates tissue repair and functional recovery, essential for mitigating long-term cardiovascular complications.

In summary, experimental studies consistently demonstrate that **g**arlic extract reduces myocardial ischemia and reperfusion injury, enhances antioxidant defense systems (MDA, SOD, CAT, GPx), and improves overall cardiac function. Evidence from female or hormonally-influenced models supports its potential role in protecting against cardiovascular insults associated with hormonal fluctuations, such as those induced by oral contraceptives. These findings establish a compelling foundation for further investigation into garlic as a natural therapeutic agent for myocardial protection and repair.

2.9 Interaction Between Garlic and Hormonal Contraceptive Effects

The use of hormonal contraceptives, particularly combined estrogen-progestin formulations, has been consistently associated with metabolic and cardiovascular perturbations. Prolonged exposure to exogenous hormones can induce oxidative stress, endothelial dysfunction, and low-grade inflammation, all of which contribute to increased susceptibility to ischemic heart disease (Okonofua *et al.,* 2019; Zhang *et al.,* 2025). Elevated reactive oxygen species (ROS) generated during hormonal modulation can damage myocardial cells, while progestin and estrogen imbalances may alter lipid metabolism, promoting dyslipidemia, atherosclerotic plaque formation, and subsequent cardiovascular complications (Adebayo *et al.,* 2022).

Garlic (*Allium sativum*) presents a promising adjunctive intervention due to its multifaceted antioxidant, anti-inflammatory, and hypolipidemic properties. Experimental evidence suggests that garlic can neutralize ROS and enhance endogenous antioxidant enzyme activity—including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—thereby mitigating oxidative damage caused by hormonal fluctuations (Ried *et al.,* 2016; Banerjee and Maulik, 2021). By reducing oxidative stress, garlic may protect cardiomyocytes from apoptosis and necrosis, potentially counteracting myocardial injury that can result from prolonged oral contraceptive use.

In addition to its antioxidant role, garlic has been shown to modulate lipid metabolism and improve cardiovascular homeostasis. Studies indicate that garlic reduces total cholesterol, low-density lipoprotein (LDL), and triglycerides**,** while modestly increasing high-density lipoprotein (HDL), thereby improving lipid profiles and reducing atherogenic risk (Ried *et*

al., 2016; Agarwal, 2020). These lipid-modulating effects are particularly relevant for women on hormonal contraceptives, as estrogen-progestin therapy may exacerbate dyslipidemia and accelerate atherosclerotic changes, increasing ischemic vulnerability (Okonofua *et al.,* 2019).

Furthermore, garlic exhibits anti-inflammatory effects, which may help counteract the systemic inflammation induced by hormonal contraceptives. Its bioactive compounds suppress pro-inflammatory cytokines such as TNF-α and IL-6 and reduce endothelial activation, which collectively stabilizes the vascular environment and prevents further ischemic insult (Kim *et al.,* 2018). By simultaneously addressing oxidative stress, lipid dysregulation, and inflammation, garlic provides a holistic protective effect that may mitigate cardiovascular risks associated with exogenous hormonal exposure.

Despite these promising mechanistic insights, gaps remain in the literature. Most studies on garlic's cardioprotective effects have been conducted in male or general animal models, with limited research specifically focusing on female or hormonally-influenced models. Very few studies directly investigate garlic's ability to counteract oral contraceptive-induced oxidative stress, dyslipidemia, or endothelial dysfunction, particularly in African or Nigerian populations where metabolic syndrome and cardiovascular risk factors are increasingly prevalent (Ogunmola *et al.,* 2021; Adebayo *et al.,* 2022). Additionally, the optimal dosage, duration, and form of garlic extract necessary to confer maximal cardioprotection in these contexts remain unclear, necessitating further experimental and translational research. Garlic's antioxidant, anti-inflammatory, and lipid-modulating properties provide a plausible mechanism by which it may mitigate the cardiovascular side

effects of hormonal contraceptive use. While experimental evidence supports its potential in myocardial protection and repair, the interaction between garlic and exogenous hormones remains underexplored, highlighting an important research gap. Investigating this interaction, particularly in female animal models exposed to oral contraceptives, offers an innovative avenue for developing natural therapeutic strategies to prevent or reverse contraceptive-induced cardiovascular dysfunction.

2.10 Histology of the Heart, Kidney, and Liver

2.10.1 Histology of the Heart

The heart is a specialized muscular organ that functions as a central pump in the circulatory system, delivering oxygenated blood to tissues and removing metabolic wastes. Its wall is composed of three distinct layers: the endocardium, myocardium, and epicardium, each with unique histological features that support its physiological roles (Ross and Pawlina, 2024).

The endocardium is the innermost layer that lines the chambers of the heart and the valves. It consists of a single layer of squamous endothelial cells resting on a thin layer of connective tissue. This arrangement provides a smooth, non-thrombogenic surface that facilitates unobstructed blood flow. In addition to the endothelial lining, the endocardium houses Purkinje fibers in the ventricles, which are specialized modified cardiac muscle cells responsible for conducting electrical impulses, ensuring synchronized ventricular contraction (Mescher, 2021).

The myocardium is the thick, middle layer of the heart wall and is primarily composed of cardiac muscle fibers. These fibers are striated, branched, and interconnected through

intercalated discs, which contain desmosomes for mechanical adhesion and gap junctions for electrical coupling (Young and Heath, 2023). This structure allows the myocardium to contract in a coordinated manner, producing effective pumping action. The myocardial cells also contain abundant mitochondria to meet the high energy demand required for continuous contractions. Within the myocardium, a dense network of capillaries and small blood vessels supplies oxygen and nutrients to cardiac cells, supporting their high metabolic activity (Ross and Pawlina, 2024).

The epicardium, or visceral pericardium, forms the outermost layer of the heart wall. It is composed of mesothelial cells supported by connective tissue that contains blood vessels, lymphatics, and adipose tissue. This layer provides mechanical protection, supports the coronary vessels, and reduces friction between the heart and surrounding structures during contractions (Mescher, 2021). Together, the organization of these three layers and the presence of specialized structures such as intercalated discs and Purkinje fibers enable the heart to function efficiently as a pump throughout life.

2.10.2 Histology of the Kidney

The kidney is a bean-shaped organ responsible for filtering blood, maintaining fluid and electrolyte balance, and excreting metabolic wastes. It is histologically organized into two main regions: the cortex and the medulla, each containing specialized structures that support renal function (Junqueira and Carneiro, 2022).

The renal cortex contains renal corpuscles and convoluted tubules. The renal corpuscle is composed of a glomerulus, which is a tuft of fenestrated capillaries, surrounded by Bowman’s capsule. The visceral layer of Bowman’s capsule is formed by podocytes,

specialized epithelial cells with foot processes that wrap around the capillaries and form part of the filtration barrier. This barrier permits the selective passage of water and small solutes from the blood into the renal tubules while retaining larger proteins and blood cells (Mescher, 2021).

The proximal convoluted tubules (PCTs), located in the cortex, are lined by cuboidal epithelial cells with a prominent brush border of microvilli, which greatly increases the surface area for reabsorption of water, glucose, amino acids, and electrolytes. In contrast, the distal convoluted tubules (DCTs) are also lined by cuboidal cells but with fewer microvilli, and they are primarily involved in the selective reabsorption of ions and secretion of waste products.

The renal medulla **c**ontains the loops of Henle and collecting ducts, structures that play key roles in urine concentration. The thin segments of the loops of Henle are lined by simple squamous epithelium, while the thick segments are lined by cuboidal epithelium. The collecting ducts are lined by cuboidal to columnar epithelial cells and eventually merge to form the papillary ducts, which drain urine into the renal pelvis (Young and Heath, 2023). The kidney also contains the juxtaglomerular apparatus, a specialized structure near the glomerulus that regulates blood pressure and glomerular filtration through renin secretion. Overall, the histological organization of the kidney ensures efficient filtration, selective reabsorption, and homeostatic regulation of the body's fluid and electrolyte balance.

2.10.3 Histology of the Liver

The liver is a large glandular organ involved in metabolism, detoxification, protein synthesis, and bile production. Histologically, it is organized into hepatic lobules, the functional units of the liver, which are hexagonal structures centered around a central vein and surrounded by portal triads at each corner (Mescher, 2021).

Hepatocytes, the principal liver cells, are polygonal and arranged in cord**s** or plates radiating from the central vein. These cords are separated by **s**inusoids, specialized capillary channels lined by fenestrated endothelial cells, which allow the exchange of nutrients, metabolites, and plasma components between the blood and hepatocytes. Kupffer cells**,** resident macrophages within the sinusoids, phagocytose debris and pathogens, contributing to the liver's immune defense (Ross and Pawlina, 2024).

The portal triad consists of a branch of the hepatic artery, a branch of the portal vein, and a bile duct. The bile canaliculi, tiny channels formed between adjacent hepatocytes, collect bile produced by hepatocytes and drain it into the bile ducts of the portal triad. The space of Disse**,** located between the hepatocytes and sinusoidal endothelium, facilitates the exchange of plasma proteins, nutrients, and lipids (Junqueira and Carneiro, 2022). This architectural arrangement allows the liver to carry out its diverse metabolic, synthetic, and detoxifying functions efficiently. Hepatocytes also contain abundant glycogen granules and mitochondria, which support energy production, carbohydrate storage, and detoxification processes.

2.10.4 Heart Histology and Its Relevance to Ischemic Heart Disease

The heart's unique histological organization, particularly the myocardium and its cardiac muscle fibers, is central to understanding ischemic heart disease (IHD). The myocardium's intercalated discs enable coordinated contraction, but when coronary arteries are narrowed or obstructed due to atherosclerosis, oxygen delivery to these highly metabolic cells is compromised (Ross and Pawlina, 2024). Prolonged oxygen deprivation triggers myocyte necrosis and the replacement of functional muscle with fibrotic tissue, reducing contractile efficiency and cardiac output (Mescher, 2021). Endocardial structures, including Purkinje fibers, are also vulnerable to ischemia, potentially leading to conduction abnormalities and arrhythmias, which are common complications in IHD (Young and Heath, 2023). The dense capillary network of the myocardium, normally supporting high energy demands, cannot compensate when perfusion is inadequate, highlighting the critical relationship between histological architecture and disease susceptibility.

2.10.5 Kidney Histology and Its Role in Renal Dysfunction

The kidney's structural organization reflects its vulnerability to both acute and chronic insults. The glomerulus, with its fenestrated capillaries and podocytes, serves as the primary filtration barrier, but it is highly susceptible to damage from hypertension, diabetes, and ischemia (Junqueira and Carneiro, 2022). Injury to podocytes or thickening of the glomerular basement membrane impairs filtration, resulting in proteinuria, a hallmark of chronic kidney disease. Similarly, the proximal convoluted tubules (PCTs)**,** with their brush border of microvilli, are highly metabolically active and particularly sensitive to ischemic injury or nephrotoxic agents. Damage to PCTs can reduce reabsorption of glucose, electrolytes, and amino acids, exacerbating fluid and electrolyte

imbalance (Mescher, 2021). The juxtaglomerular apparatus, which regulates blood pressure through renin secretion, may also contribute to secondary hypertension when structural or functional damage occurs, creating a feedback loop that worsens renal injury (Young and Heath, 2023).

2.10.6 Liver Histology and Its Implications in Hepatic Dysfunction

The liver's lobular architecture and specialized cells are crucial for its metabolic and detoxifying roles. Hepatocytes, arranged in **cords** with interspersed sinusoids, allow efficient exchange of nutrients and metabolic byproducts, but this arrangement makes the liver sensitive to hypoxia, toxins, and inflammatory damage (Ross and Pawlina, 2024). Kupffer cells, as resident macrophages, mediate immune responses; overactivation during chronic injury can lead to **fibrosis**, impairing hepatic function (Mescher, 2021). The space of Disse facilitates the movement of plasma proteins and lipids, and disruption here can compromise nutrient exchange. Similarly, obstruction of bile canaliculi or portal triad injury can lead to cholestasis and accumulation of toxic metabolites, which may further damage hepatocytes. Understanding these histological structures is essential for interpreting biochemical markers of liver injury, such as elevated transaminases, in clinical practice (Junqueira and Carneiro, 2022).

Histological organization underpins organ function and informs susceptibility to disease. In the heart, the loss of cardiomyocytes following ischemia explains reduced contractile function in IHD. In the kidney, glomerular and tubular injury underlies impaired filtration and electrolyte imbalance. In the liver, disruption of hepatocyte-sinusoid architecture contributes to metabolic derangements and impaired detoxification. Together, these

examples illustrate how a detailed understanding of tissue histology provides a foundation for understanding the pathophysiology of major organ-specific diseases, guiding both diagnosis and therapeutic interventions (Mescher, 2021; Young and Heath, 2023; Ross and Pawlina, 2024).

2.11 Summary of Literature Gaps

Despite the growing body of research on ischemic heart disease (IHD), oral contraceptive use, and the cardioprotective effects of garlic (*Allium sativum*), several important gaps remain, particularly in the context of female-specific cardiovascular risk. One significant limitation is the scarcity of studies using female rat models to investigate oral contraceptive-induced IHD**.** Most experimental models have historically focused on male subjects or general rodent populations, leaving a knowledge gap regarding the unique hormonal influences in females, including estrogen and progestin fluctuations that may modulate cardiovascular susceptibility (Okonofua *et al.*, 2019; Zhang *et al.*, 2025).

Furthermore, while garlic has been extensively studied for its preventive cardiovascular benefits**,** there is limited research exploring its therapeutic potential as a reversing agent for established myocardial injury. Most studies focus on prophylactic administration, leaving uncertainty about whether garlic can effectively restore cardiac function, reduce oxidative damage, and repair ischemic tissue once injury has already occurred (Ried *et al.*, 2016; Banerjee and Maulik, 2021). This distinction is particularly important in populations where women may experience prolonged exposure to oral contraceptives without prior cardiovascular monitoring.

Another gap lies in the comprehensive evaluation of biochemical, histological, and functional outcomes**.** While many studies report improvements in oxidative stress markers or lipid profiles with garlic treatment, few integrate these findings with histopathological assessments of cardiac tissue or functional measures such as cardiac output, ejection fraction, or myocardial contractility. A holistic evaluation combining biochemical, structural, and functional parameters would provide stronger evidence for garlic's cardioreparative potential and its relevance in hormone-modulated ischemic injury.

Finally, there is a lack of comparative studies examining different types and doses of oral contraceptives in the context of IHD. Combined estrogen-progestin versus progestin-only formulations, as well as high-dose versus low-dose preparations, may have differential effects on cardiovascular function, oxidative stress, and lipid metabolism (Ogunmola *et al.*, 2021; Adebayo *et al.*, 2022). Understanding these differences is crucial for tailoring interventions, including garlic supplementation, to specific hormonal profiles and minimizing cardiovascular risk in women.

Collectively, these gaps underscore the urgent need for research that integrates female-specific models, investigates garlic as a therapeutic rather than purely preventive agent, employs multidimensional evaluation strategies, and considers the diversity of oral contraceptive formulations. Addressing these gaps would provide critical insights into safe contraceptive use, innovative natural therapies, and effective strategies to mitigate cardiovascular risk in women, particularly in African populations where metabolic and cardiovascular risk factors are rising.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The research was conducted at the University of Benin, Benin City, Edo State, Nigeria. Established in 1970 and granted full university status in 1971, the University of Benin (UNIBEN) is a leading institution in Nigeria offering diverse undergraduate and postgraduate programs, including medical sciences, natural sciences, engineering, social sciences, law, and humanities. Laboratory analyses, including haematological, biochemical, and molecular assays, will be performed within the Department of Pharmacology, which provides well-equipped facilities for animal housing, molecular biology, and biochemical studies. Edo State, located in Nigeria's South-South geopolitical zone, is bordered by Kogi State to the north, Anambra State to the east, Delta State to the south, and Ondo State to the west. The state has a tropical climate with two distinct seasons: a rainy season from April to October and a dry season from November to March. Annual rainfall ranges from 1,500 to 2,500 mm, with temperatures between 25°C and 28°C. The fertile soils and favorable climate support medicinal plant cultivation and agro-biodiversity (Adeyemi *et al.,* 2022).

3.2 Research Design

This study was an experimental laboratory-based animal study.

3.3 Ethical Considerations:

All animal handling and procedures were adhered to strictly to institutional guidelines for

the care and use of laboratory animals. Ethical approval was sought from the Edo State Ministry of Health, Benin City with protocol number: HA/737/25/C/10331005

3.4 Experimental design

The study was conducted using adult female albino Wistar rats of comparable age and weight, sourced from a recognized animal breeding facility within the University of Benin. The rats were housed in clean, well-ventilated cages under standard laboratory conditions with a 12-hour light/dark cycle and free access to food and water. After a one-week acclimatization period, the animals were randomly assigned into seven groups, each consisting of a minimum of eight rats (n =8), ensuring equal distribution and minimizing selection bias.

- **Group A**: Control (no contraceptives, no garlic)
- **Group B**: 0.33mg/kg of OC only (no garlic treatment)
- **Group C**: 0.66mg/kg of OC + 600mg/kg of garlic extract
- **Group D**: 0.99mg/kg of OC + 700mg/kg of garlic extract
- **Group E**: 1.32mg/kg of OC + 800mg/kg of garlic extract
- **Group F**: 1.65mg/kg of OC + 900mg/kg of garlic extract
- **Group G**:500mg/kg Garlic extract only (no contraceptives)

3.5 Sample Size

The sample size was determined using the resource equation method, which is suitable for animal studies and ensures ethical use of animals while maintaining statistical validity (Festing and Altman, 2002).

The formula is:

E=N−GE = N - GE=N−G

Where:

- E = degrees of freedom for error (acceptable range: 10–20)
- N = total number of animals
- G = total number of experimental groups

Calculate E Value

E=N−G=56−7=49

The resulting E = 49 is slightly above the recommended range of 10–20 but is acceptable for ensuring adequate power and variability detection in molecular and hematological studies.

Final Sample Size

A total of 56 adult female Wistar rats were used for the study. The animals were randomly divided into seven experimental groups, with eight (8) rats per group. Using the resource equation method, the error degrees of freedom (E) was calculated as 49, which exceeds the recommended range of 10–20. Although this value is higher than the optimal range, the increased sample size was intentionally adopted to enhance statistical power and accommodate variability inherent in molecular and hematological analyses. This approach remains consistent with ethical principles for animal research, as the number of animals

used was justified by the complexity of the experimental endpoints (Festing and Altman, 2002; National Research Council, 2011).

3.6 Oral Contraceptive Administration

To induce cardiovascular alterations in the experimental model, commonly used estrogen-progestin-based oral contraceptives were administered orally to the subjects for a period of four weeks**.** These contraceptives were selected because of their widespread clinical use and their well-documented effects on cardiovascular physiology, including influences on lipid metabolism and endothelial function (Croft *et al.,* 2019; Manson *et al.,* 2020).

Oral administration was chosen to closely mimic typical human consumption, ensuring that the systemic effects of the contraceptives would be comparable to real-life exposure. Throughout the treatment period, subjects were carefully monitored for behavioral changes, signs of toxicity, and other physiological responses, providing a controlled environment for studying the cardiovascular effects of these agents.

Observations and measurements recorded during and at the conclusion of the four-week period allowed for assessment of the changes induced by the oral contraceptives, supporting comparisons with protective or restorative treatments (Croft et al., 2019).

3.7 Garlic Extract Treatment

Fresh garlic cloves were purchased from Uselu Market, Egor LGA, Edo State. The plant was authenticated at the Department of Plant Biology and Biotechnology, University of Benin. The cloves were air-dried for two weeks and pulverized into powder. Distilled water was added and the mixture was soaked for 24 hours with intermittent stirring. The extract

was filtered using Whatman filter paper, and the filtrate was concentrated using a water bath at 37°C. Following exposure to oxidative stress-inducing agents (OC), the garlic extract was given orally to the experimental subjects for a four-week period. Multiple were tested to explore potential dose-dependent effects. These dosages were chosen based on previous toxicological and pharmacological studies to ensure the safety of the subjects while still allowing the extract to provide therapeutic benefits (Bayan *et al.*, 2014; Ried *et al.*, 2016).

Throughout the treatment period, subjects were closely observed for any behavioral changes, tolerance issues, or signs of toxicity. The low-dose group received a concentration designed to approximate typical dietary intake of garlic, whereas the high-dose group was given a concentration intended to test the maximal therapeutic effects of the extract. Oral administration was selected to reflect the natural way humans consume garlic, enabling the assessment of its systemic and organ-specific effects.

3.8 Methodology for Biochemical Parameters

3.8.1 Lipid Profile

Total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TG)**,** were determined using enzymatic colorimetric methods (Allain *et al.*, 1974; Bucolo and David, 1973).

Principle:

The enzymatic colorimetric method is based on the ability of specific enzymes to catalyze reactions that produce a colored compound proportional to the concentration of the lipid being measured. For total cholesterol, cholesterol esterase hydrolyzes cholesterol esters to

free cholesterol, which is then oxidized by cholesterol oxidase to produce hydrogen peroxide. Hydrogen peroxide reacts with a chromogen in the presence of peroxidase to yield a colored product, the intensity of which is measured spectrophotometrically (Allain et al., 1974). Triglycerides are hydrolyzed by lipase to glycerol and fatty acids; glycerol is then phosphorylated and oxidized to produce hydrogen peroxide, which reacts with a chromogen to give a measurable color (Bucolo & David, 1973). HDL and LDL concentrations are determined either by direct enzymatic methods or after selective precipitation of other lipoproteins.

Procedure:

Blood samples were collected into plain tubes and allowed to clot at room temperature. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at −20°C until analysis. Enzymatic reagents were prepared according to the manufacturer's instructions. Serum samples and standards were added to the respective reagent wells or tubes. The reaction mixtures were incubated at 37°C for the specified time. Absorbance was measured using a spectrophotometer at the recommended wavelength (usually 500 nm for TC and HDL; 505 nm for TG). Concentrations were calculated by comparing sample absorbance to that of the standard curve.

3.8.2 Troponin

Cardiac troponin I was quantified using enzyme-linked immunosorbent assay (ELISA) kits obtained from Elabscience (Wu et al., 2019), with catalog number E-EL-R1253. The assay has a sensitivity of 0.19 ng/mL, with intra-assay and inter-assay coefficients of variation reported to be <10% and <12%, respectively.

Principle:

The ELISA technique relies on the specific binding of antibodies to the target protein. In a sandwich ELISA**,** troponin in the serum binds to a pre-coated capture antibody on the microplate well. A second enzyme-linked detection antibody is then added, which binds to the captured troponin. Upon addition of the substrate, the enzyme catalyzes a color change proportional to the amount of troponin present in the sample. The intensity of the color is measured spectrophotometrically, typically at 450 nm, and compared to a standard curve to determine concentration (Lehmann et al., 2017).

Procedure:

Serum samples were thawed and brought to room temperature before assay. Standards, controls, and samples were added to the wells pre-coated with troponin-specific capture antibodies. The plate was incubated for the recommended period at 37°C to allow binding. Wells were washed multiple times to remove unbound substances. Detection antibody conjugated to horseradish peroxidase was added and incubated. After washing, substrate solution was added to develop color. The reaction was stopped using the stop solution, and absorbance was read at 450 nm using an ELISA reader. Troponin concentrations in the samples were calculated based on the standard curve.

3.9 Histological Processing of the Heart, Kidney, and Liver

At the conclusion of the experimental period, the animals were sacrificed humanely in accordance with ethical guidelines. The heart, kidneys, and liver were immediately excised to prevent tissue degradation, and excess connective tissue and fat were carefully removed.

The organs were gently rinsed in ice-cold normal saline to remove blood and debris, ensuring clean tissue samples for further processing.

The tissues were promptly fixed in 10% neutral buffered formalin for 24–48 hours at room temperature to preserve cellular and structural integrity. Following fixation, the tissues were dehydrated through graded ethanol concentrations (70%, 80%, 90%, 95%, and 100%), cleared in xylene, and embedded in molten paraffin wax. The paraffin blocks were sectioned at 4–6 μm thickness using a microtome. Sections were floated on a warm water bath, mounted on glass slides, deparaffinized in xylene, and rehydrated through descending grades of ethanol to water.

The sections were stained with hematoxylin and eosin (H&E) for general histological evaluation. After staining, the sections were dehydrated, cleared, and mounted with a coverslip using a suitable mounting medium. The slides were examined under a light microscope, and photomicrographs were captured using a digital imaging system.

To minimize subjective interpretation, a semi-quantitative histopathological grading system was employed. Tissue alterations in the heart, liver, and kidneys were assessed based on the severity of observed lesions, including cellular degeneration, necrosis, inflammatory infiltration, vascular congestion, and structural distortion. Each parameter was scored as follows: 0 = no visible lesion (normal histology), 1 = mild changes (slight alteration affecting <25% of the tissue), 2 = moderate changes (lesions affecting 25–50% of the tissue), and 3 = severe changes (extensive damage affecting >50% of the tissue). The scoring was performed in a blinded manner to reduce observer bias, and the mean score for each group was calculated for statistical analysis.

3.10 Data Analysis

Data were expressed as mean ± standard deviation. Normality was assessed using Shapiro–Wilk test. Statistical comparisons were performed using one-way ANOVA followed by appropriate post hoc tests. Significance set at $p < 0.05$. Data was analyzed using SPSS version 26.0.

CHAPTER FOUR

RESULTS

Mortality Observed

One animal death was recorded in Group E (1.32 mg/kg COC + 800 mg/kg garlic). Mortality rate = 1/8 (12.5%).

Table 4.1 shows the lipid profile analysis. Total cholesterol levels did not differ significantly among the experimental groups (P = 0.360). Triglyceride levels varied significantly across the groups (P = 0.001). Group B (0.33 mg/kg COC) showed a significant reduction in triglyceride levels, whereas Group F (1.65 mg/kg COC combined with 900 mg/kg garlic) had significantly higher triglyceride levels compared with the control. Triglyceride levels in the remaining treatment groups were not significantly different from those of the control group. HDL cholesterol levels also showed a significant overall difference among groups (P = 0.001). Notably, Groups B and C exhibited significantly higher HDL levels than the control group, suggesting that lower doses of COC, either alone or combined with garlic, may improve HDL cholesterol. No significant changes in HDL were observed in the other treatment groups. Similarly, LDL cholesterol levels differed significantly among the groups (P = 0.004). Groups B and C demonstrated significantly lower LDL levels compared with the control, while higher-dose COC–garlic combinations and garlic alone did not produce significant changes in LDL levels. Overall, these results suggest that lower doses of COC, whether administered alone or in combination with garlic, are associated with improved lipid profiles, characterized by reduced triglyceride and LDL levels and increased HDL levels. In contrast, higher-dose

COC–garlic combinations did not consistently produce beneficial effects on lipid parameters.

Table 4.1: Effect of COC and Garlic on Lipid Profile (Mean ± SEM)

Parameter	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Group A (Control)	89.50 ± 1.08	72.00 ± 1.22	28.00 ± 1.20	40.00 ± 1.41
Group B (0.33 mg/kg COC)	87.50 ± 1.39	68.00 ± 1.22	33.25 ± 1.16	32.25 ± 1.83
Group C (0.66 mg/kg COC + 600 mg/kg Garlic)	91.25 ± 1.62	72.50 ± 1.51	32.25 ± 1.39	34.25 ± 1.37
Group D (0.99 mg/kg COC + 700 mg/kg Garlic)	90.00 ± 1.33	74.00 ± 2.00	30.50 ± 1.64	36.25 ± 1.63
Group E (1.32 mg/kg COC + 800 mg/kg Garlic)	88.75 ± 1.30	70.25 ± 1.54	31.25 ± 1.39	33.75 ± 1.39
Group F (1.65 mg/kg COC + 900 mg/kg Garlic)	92.25 ± 1.47	78.25 ± 2.25	29.50 ± 1.46	41.25 ± 1.50
Group G (500 mg/kg Garlic)	90.88 ± 1.16	71.88 ± 1.39	30.38 ± 0.86	38.50 ± 1.25
F-value	1.135	4.439	4.431	3.810
P-value	0.360	**0.001**	**0.001**	**0.004**
Post Hoc (A vs B)	0.790	**0.028**	**0.031**	**0.019**
Post Hoc (A vs C)	0.681	0.223	**0.041**	**0.037**
Post Hoc (A vs D)	0.748	0.175	0.114	0.086
Post Hoc (A vs E)	0.812	0.347	0.268	0.241
Post Hoc (A vs F)	0.592	**0.001**	0.072	0.064
Post Hoc (A vs G)	0.764	0.125	0.188	0.133

Values are expressed as Mean ± SEM (n = 8**)**. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. P-values < 0.05 were considered statistically significant.

The results shown in Table 4.2 indicate that cardiac troponin I levels did not differ significantly among the experimental groups ($F = 2.329$, $P = 0.051$). Although some variation in mean values was observed, none of the treatment groups showed a statistically significant difference compared with the control group. Compared with the control value (0.330 ± 0.012 ng/mL), Group B (0.33 mg/kg COC) exhibited a slightly lower cardiac troponin I level. Similarly, Groups C and D, which received moderate doses of COC in combination with garlic, also showed reduced mean troponin I levels, with Group C recording the lowest value. Group E had a troponin I level that was comparable to, but still marginally lower than, that of the control. In contrast, Group F (1.65 mg/kg COC combined with 900 mg/kg garlic) displayed a higher mean cardiac troponin I level than the control group; however, this increase did not reach statistical significance (post hoc $P = 0.073$). Group G, which received garlic alone, had a troponin I level that was essentially the same as the control. Overall, these findings suggest that low to moderate doses of COC, particularly when administered in combination with garlic, tended to lower cardiac troponin I levels, whereas the highest dose of COC combined with garlic showed a tendency toward increased troponin I. Importantly, none of these observed changes were statistically significant.

Table 4.2: Effect of COC and Garlic on Cardiac Troponin I (Mean ± SEM)

Parameter	Cardiac Troponin I (ng/mL)
Group A (Control)	0.330 ± 0.012
Group B (0.33 mg/kg COC)	0.320 ± 0.012
Group C (0.66 mg/kg COC + 600 mg/kg Garlic)	0.290 ± 0.015
Group D (0.99 mg/kg COC + 700 mg/kg Garlic)	0.300 ± 0.010
Group E (1.32 mg/kg COC + 800 mg/kg Garlic)	0.310 ± 0.012
Group F (1.65 mg/kg COC + 900 mg/kg Garlic)	0.360 ± 0.016
Group G (500 mg/kg Garlic)	0.330 ± 0.014
F-value	2.329
P-value	0.051
Post Hoc (A vs B)	0.812
Post Hoc (A vs C)	0.246
Post Hoc (A vs D)	0.381
Post Hoc (A vs E)	0.594
Post Hoc (A vs F)	0.073
Post Hoc (A vs G)	0.999

Values are expressed as Mean ± SEM (n = 8)**.** Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test**.** P-values < 0.05 were considered statistically significant.

Figure 4.1 the effect of COC and Garlic on Initial and Final Body Weight Across Experimental Groups. the weight profile shows that **a**ll groups exhibited only minimal changes between initial and final body weights over the experimental period. Across Groups A–G, the final weights were generally comparable to the initial weights, with no marked weight loss or excessive weight gain observed. The control group (Group A) maintained a relatively stable body weight from the beginning to the end of the study. Similarly, Groups B, C, D, and E, which received varying doses of COC alone or in combination with garlic, showed slight increases or maintenance of body weight. Groups F and G, which received the highest COC–garlic combination and garlic alone respectively, also demonstrated stable weight patterns, comparable to the control group, with only minor fluctuations. The results indicate that administration of COC, garlic, or their combinations did not significantly alter body weight, suggesting that the treatments were well tolerated and did not negatively impact general body growth or nutritional status during the study period.

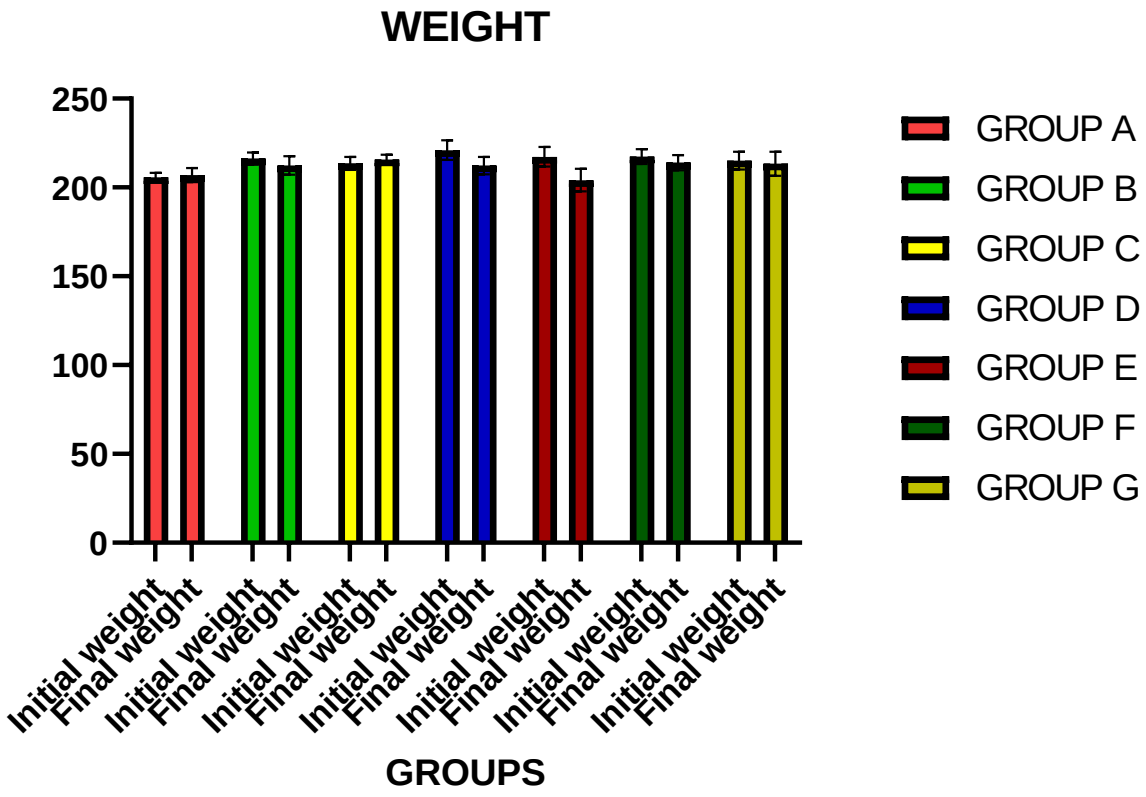


Figure 4.1: Effect of COC and Garlic on Initial and Final Body Weight Across Experimental Groups.

Histological Findings

After the administration of the (combined oral contraceptives)

GROUP F1 LIVER X100 GROUP F1 LIVER X400

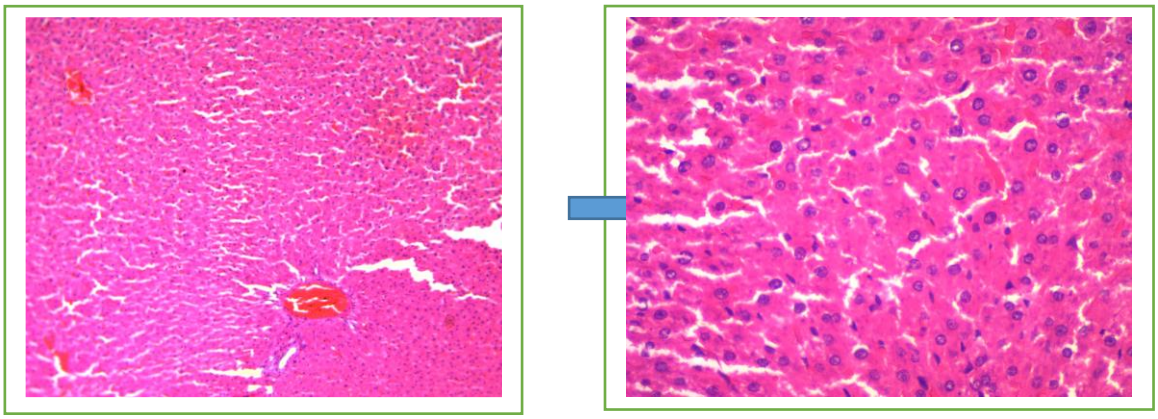
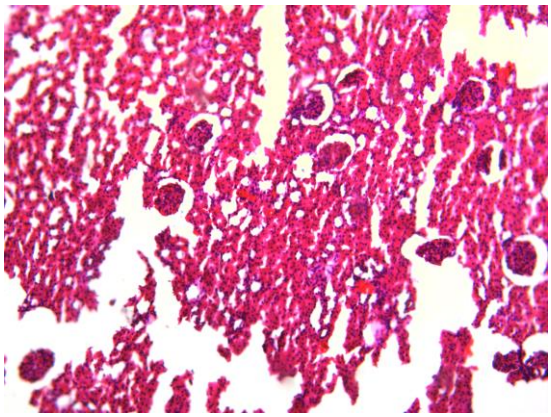


Plate 4.1 Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. Features in keeping with normal hepatocytes

GROUP F1 KIDNEY X100



GROUP F1 KIDNEY X400

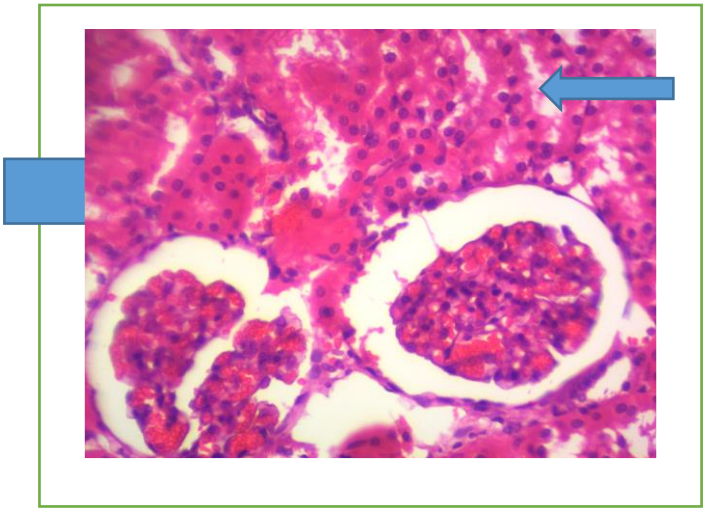
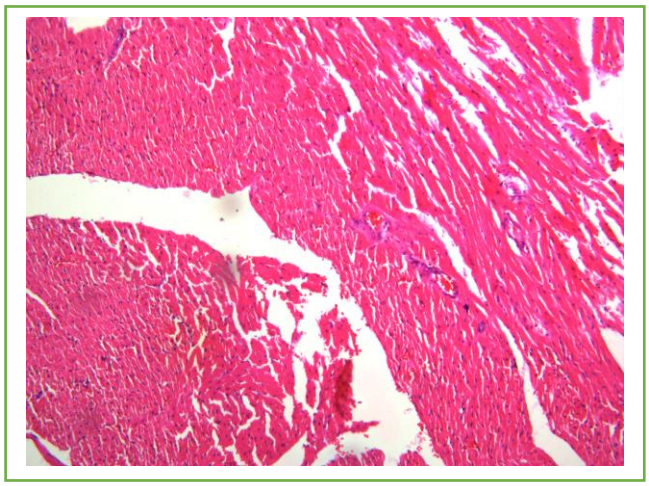


Plate 4.2 Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with normal kidney

GROUP F1 HEART X100



GROUP F1 HEART X400

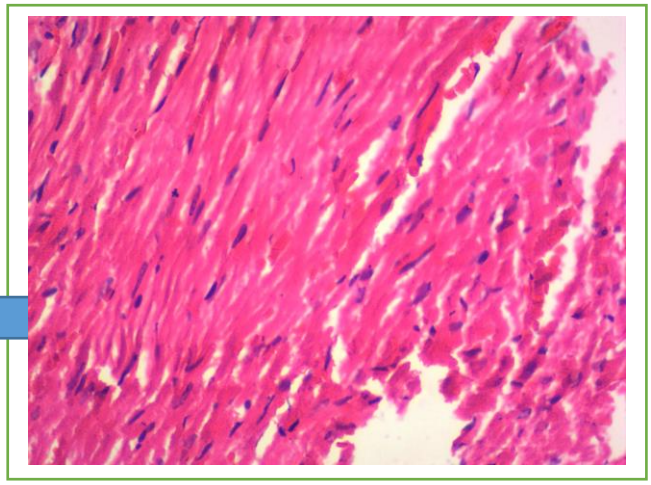
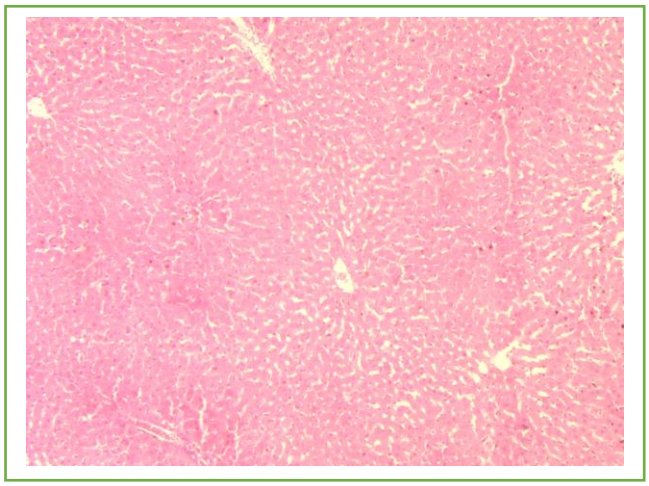


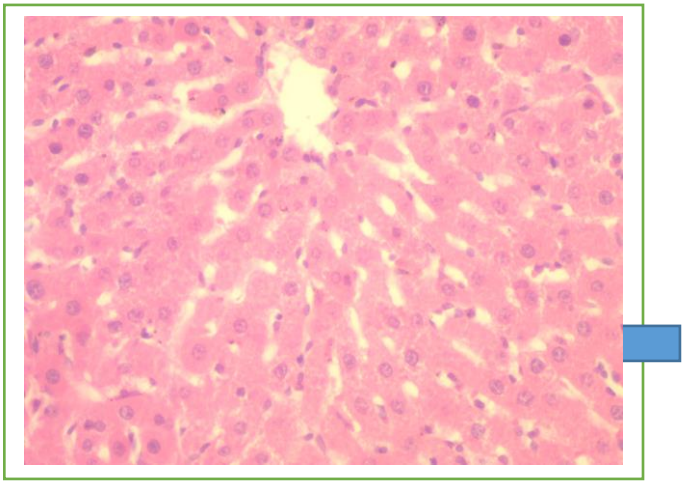
Plate 4.3 Section of cardiac muscle shows myocytes (arrow) with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with normal myocytes

AFTER TREATMENT WITH GARLIC EXTRACT

GROUP A LIVER X100

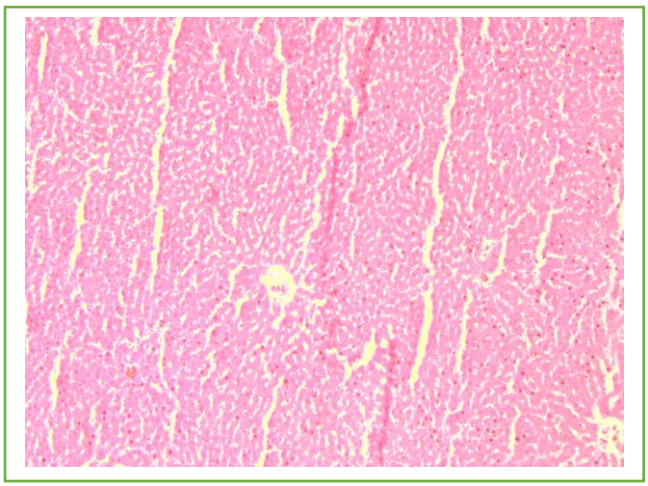


GROUP A LIVER X400



Plates 4.4: Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. Features in keeping with normal hepatocytes

GROUP B LIVER X100



GROUP B LIVER X400

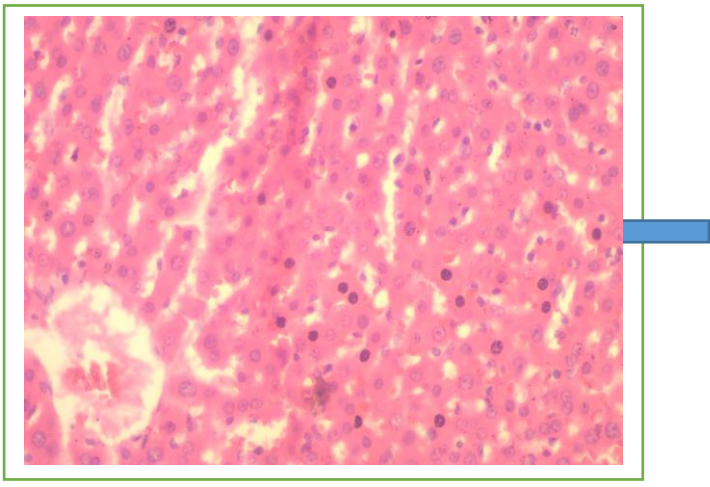


Plate 4.5 Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. Features in keeping with normal hepatocytes

GROUP F2 LIVER X100 GROUP F2 LIVER X400

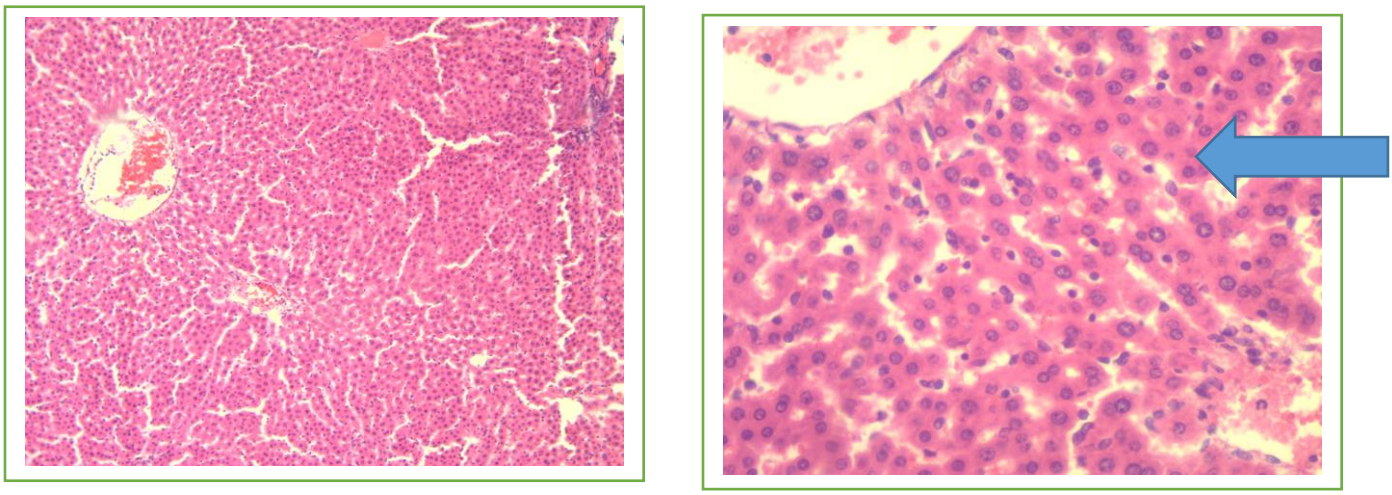


Plate 4.6 Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. Features in keeping with normal hepatocytes

GROUP A KIDNEY X100 GROUP A KIDNEY X400

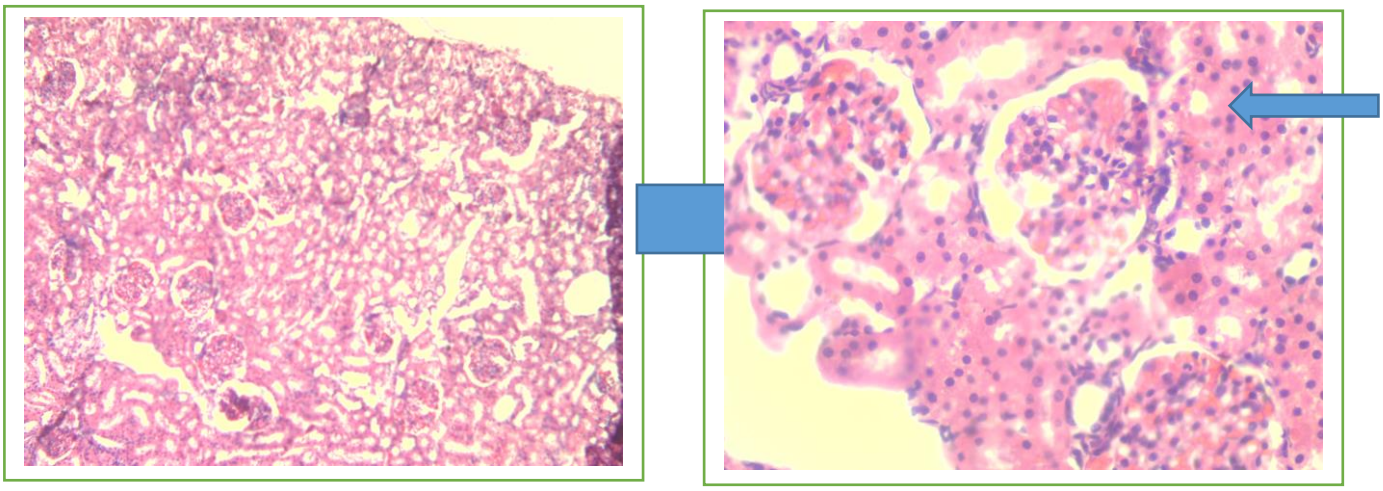
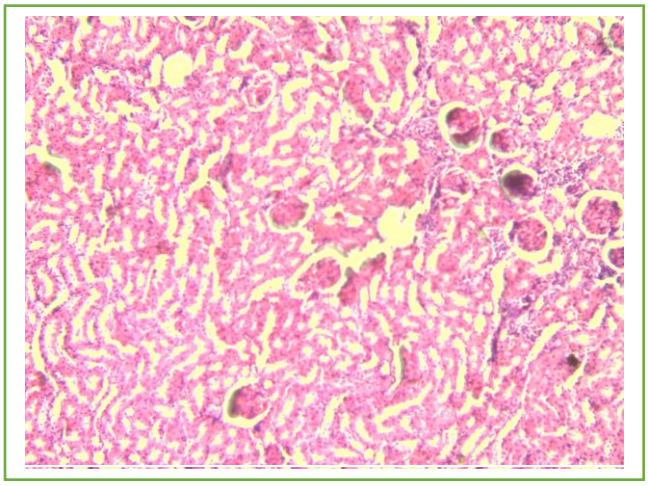


Plate 4.7 Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

GROUP B KIDNEY X100



GROUP B KIDNEY X400

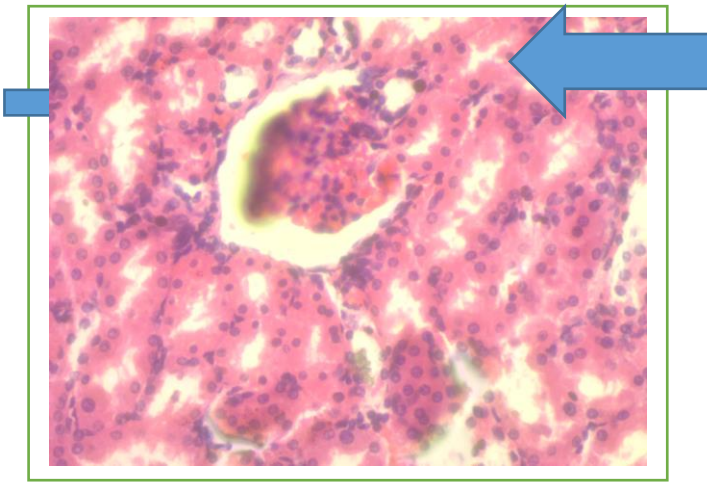
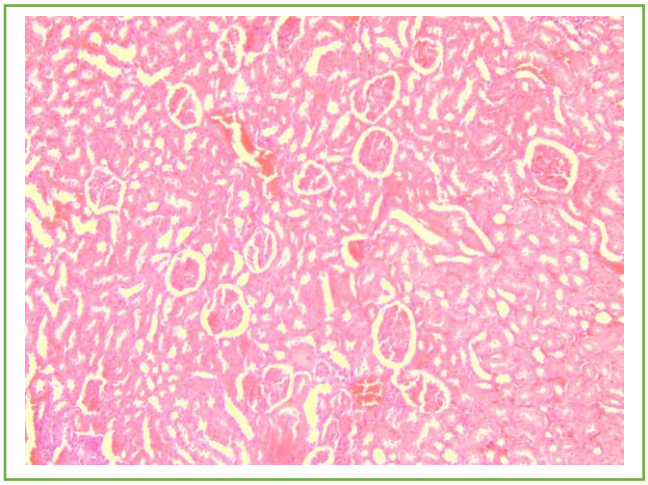


Plate 4.8 Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

GROUP F2 KIDNEY X100



GROUP F2 KIDNEY X400

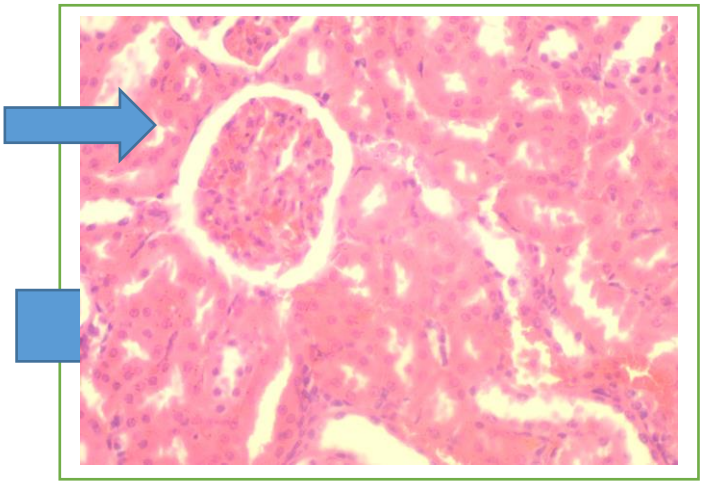
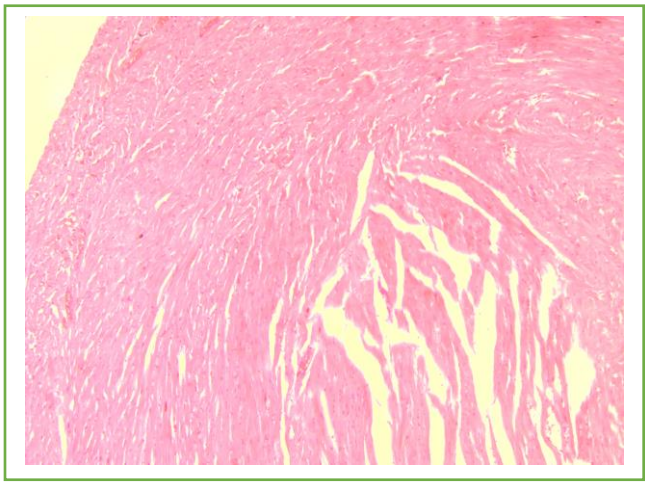


Plate 4.9 Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

GROUP A HEART X100



GROUP A HEART X400

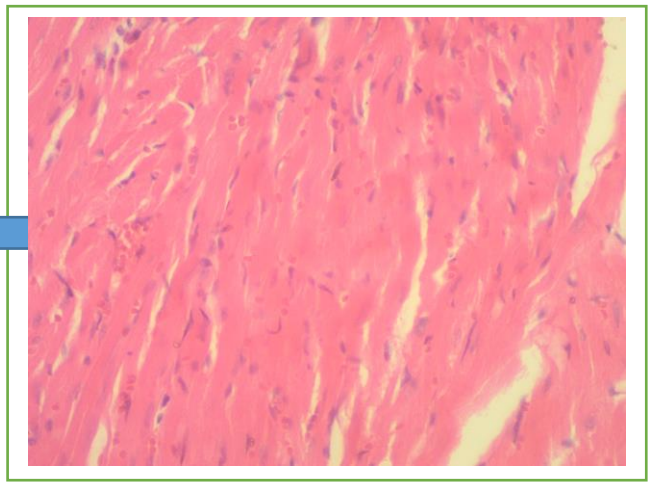
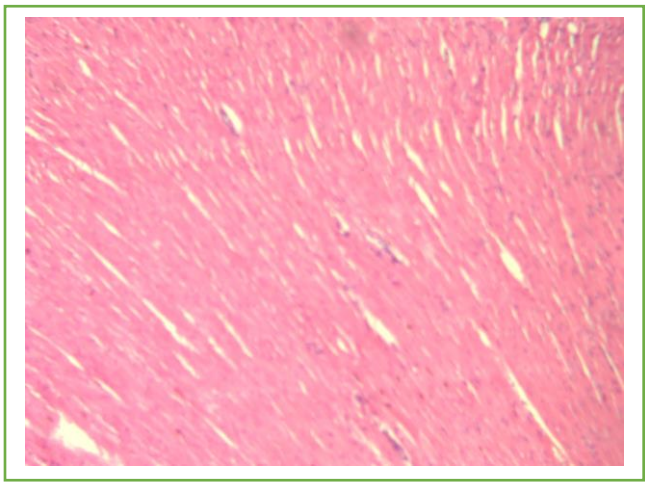


Plate 4.10 Section of cardiac muscle shows myocytes (arrow) with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with normal myocytes.

GROUP B HEART X100



GROUP B HEART X400

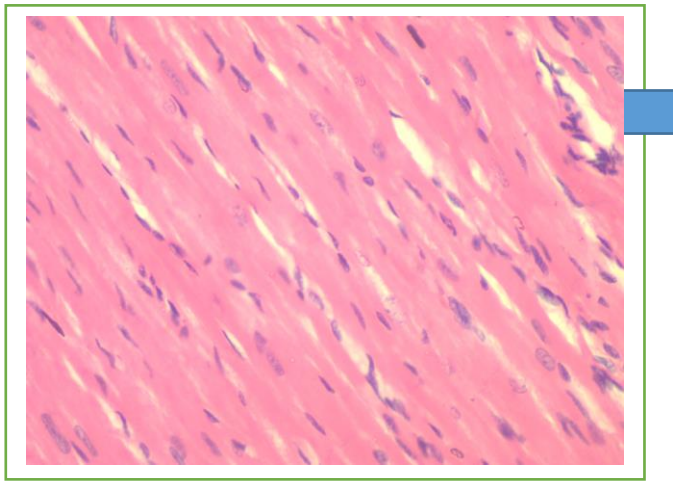
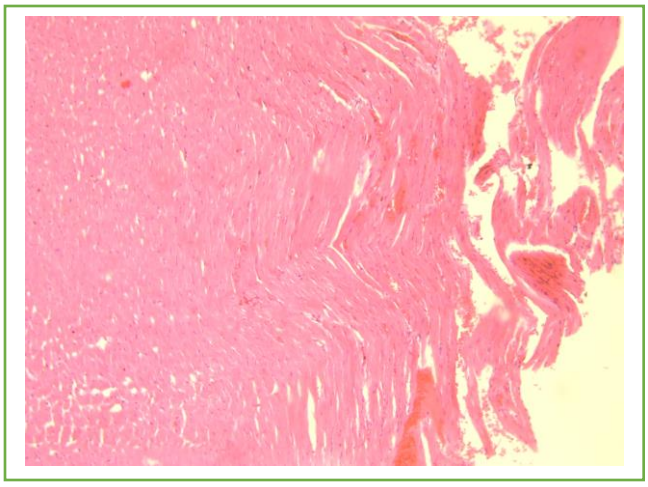


Plate 4.11 Section of cardiac muscle shows myocytes (arrow) with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with normal myocytes

GROUP F2 HEART X100



GROUP F2 HEART X400

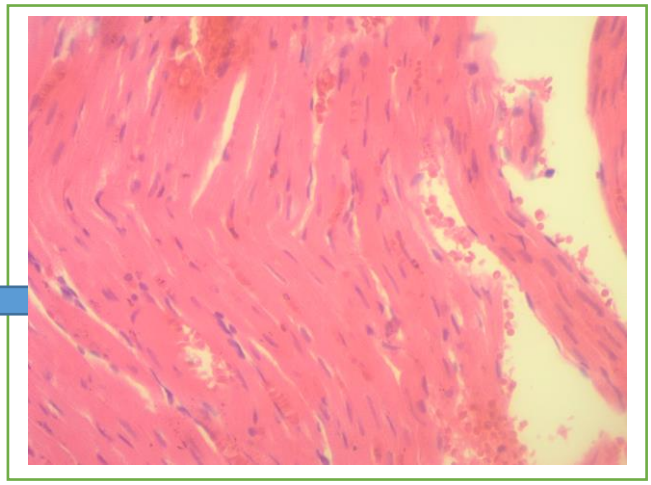


Plate 4.12 Section of cardiac muscle shows myocytes (arrow) with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with normal myocytes

CHAPTER FIVE

CONCLUSION, DISCUSSION AND RECOMMENDATIONS

5.1 Discussion

Garlic (*Allium sativum*) extract is phytochemically rich, with organosulfur compounds **as its** principal bioactive constituents**,** supported by flavonoids, phenolic acids, saponins, alkaloids, vitamins, and minerals. Together, these compounds underpin garlic's cardioprotective, hypolipidemic, antioxidant, and anti-ischemic effects, making it relevant in the management of ischemic heart disease (Banerjee *et al.*, 2021)

Findings of this study show that total cholesterol levels did not differ significantly across all experimental groups. This suggests that exposure to combined oral contraceptives (COC), garlic, or their combinations did not markedly influence overall cholesterol synthesis or clearance within the study duration. This finding is consistent with previous studies reporting that short-term exposure to estrogen-containing oral contraceptives may not significantly alter total cholesterol levels, especially when the duration of exposure is limited (Owiredu *et al.*, 2020).

However, significant changes were observed in triglyceride, HDL, and LDL cholesterol levels. In the present study, rats administered low-dose COC (0.33mg/kg of OC only) showed a significant reduction in triglyceride levels, while those receiving the highest COC–garlic combination (1.65mg/kg of OC + 900mg/kg of garlic extract) exhibited significantly elevated triglycerides. This biphasic response suggests a dose-dependent metabolic effect of COC exposure. Estrogen-containing contraceptives have been shown to increase hepatic triglyceride synthesis by stimulating very-low-density lipoprotein

(VLDL) production, particularly at higher doses (Oyelola *et al.*, 2019). The elevated triglyceride levels observed in Group F may therefore reflect an overriding estrogenic effect that surpassed the lipid-lowering capacity of garlic at high hormonal doses.

In contrast, 0.33mg/kg of OC and 0.66mg/kg of OC + 600mg/kg of garlic extract demonstrated significantly increased HDL cholesterol and reduced LDL cholesterol levels. These findings suggest that low-dose COC, either alone or combined with moderate garlic supplementation, may confer a cardioprotective lipid profile. Similar results have been reported by Zhang *et al.* (2024), who demonstrated that garlic supplementation significantly increased HDL levels while reducing LDL and triglycerides in experimental and clinical models. The improvement in HDL levels may be attributed to estrogen-mediated upregulation of apolipoprotein A-I synthesis, while garlic's sulfur-containing compounds, such as allicin, enhance reverse cholesterol transport and inhibit cholesterol biosynthesis (Banerjee and Maulik, 2021).

The absence of lipid improvement in higher COC–garlic groups contrasts with studies where garlic alone consistently improved lipid profiles (Pérez-Torres *et al.*, 2016). This disparity highlights the interactive effect of hormonal exposure, suggesting that excessive estrogen may negate or blunt the beneficial metabolic actions of garlic. Therefore, the present findings extend existing literature by demonstrating that garlic's cardioprotective potential is dose- and context-dependent, particularly in hormonally altered states.

Cardiac troponin I is a highly sensitive biomarker of myocardial injury and ischemia. In this study, cardiac troponin I levels did not differ significantly among experimental groups, although a downward trend was observed in low- and moderate-dose COC–garlic groups.

These findings indicate that while overt myocardial injury was not induced, garlic may exert subtle protective effects against subclinical ischemic stress.

Previous work by Nelson et al. (2024) reported a significant reduction in troponin I levels in female rats exposed to COCs and treated with garlic extract. The difference between their findings and the present study may be attributed to variations in duration of exposure, ischemic severity, garlic extract concentration, or extraction method. It is also possible that the ischemic insult in the present study was mild, limiting the extent of troponin elevation and thus reducing the likelihood of detecting statistically significant differences.

Interestingly, the highest COC–garlic combination showed a tendency toward increased troponin I levels, although this was not statistically significant. This observation aligns with reports that prolonged or high-dose estrogen exposure can increase cardiovascular risk by promoting oxidative stress, endothelial dysfunction, and hypercoagulability (Raps *et al.,* 2019). Garlic's antioxidant capacity may have been insufficient to fully counteract these effects at the highest hormonal dose.

The troponin findings suggest that low to moderate garlic supplementation may attenuate early myocardial **stress**, whereas excessive hormonal exposure may diminish this benefit.

The absence of significant changes in body weight across all groups indicates that administration of COC, garlic, or their combinations did not adversely affect general growth or nutritional status. This finding is consistent with earlier animal studies reporting minimal effects of garlic supplementation on body weight despite improvements in metabolic parameters (Zhang *et al.,* 2024).

Similarly, oral contraceptive-related weight gain remains controversial, with several studies reporting no significant association between COC use and body weight changes, particularly in controlled experimental settings (Gallo *et al.,* 2018). The stability of body weight observed in this study therefore suggests good tolerability of the administered doses and supports the safety of garlic as a complementary intervention.

Histological examination revealed preserved architecture of the liver, kidney, and heart across all assessed groups. Hepatocytes displayed normal morphology with intact nuclei, indicating absence of hepatocellular injury. This finding is noteworthy given the hepatic metabolism of oral contraceptives and supports previous reports that garlic possesses hepatoprotective properties through enhancement of antioxidant defense mechanisms (Abd El-Motteleb *et al.,* 2021).

Renal histology also showed normal glomerular and tubular structures, suggesting that neither COC nor garlic induced nephrotoxicity. This observation aligns with studies demonstrating that garlic extract mitigates oxidative renal damage and preserves glomerular integrity in experimental models (Nasri and Shirzad, 2019).

Cardiac muscle sections revealed normal myocyte morphology across all groups, corroborating the biochemical findings of non-significant troponin I levels. Previous studies have shown that garlic improves myocardial antioxidant enzyme activity and reduces lipid peroxidation, thereby preserving cardiac tissue structure in ischemic conditions (Banerjee and Maulik, 2021). The present findings support these observations and suggest that garlic may offer structural cardioprotection even in hormonally stressed states.

5.2 Conclusion

Based on the findings of this study, it can be concluded that garlic (*Allium sativum*) extract exhibits cardioprotective and lipid-modulating effects in female rats exposed to combined oral contraceptive**s**, particularly at low to moderate doses. Garlic supplementation was associated with improved lipid profiles, which are critical determinants of ischemic heart disease risk.

While garlic demonstrated a tendency to attenuate cardiac stress, as reflected by lower mean cardiac troponin I levels in some treatment groups, these effects were not statistically significant, indicating that the degree of myocardial injury induced in this model may have been mild or subclinical. Importantly, the absence of adverse histological changes in the heart, liver, and kidney confirms the relative safety of garlic extract when administered alone or in combination with COCs within the studied dose range.

However, the findings also indicate that higher doses of COC may diminish the cardioprotective benefits of garlic, emphasizing the role of dosage and hormonal burden in modulating cardiovascular outcomes. This study supports the potential use of garlic as a complementary intervention to reduce cardiovascular risk associated with oral contraceptive use, while highlighting the need for careful consideration of hormonal dosage.

5.3 Recommendations

Based on the outcomes of this study, the following are recommended:

1. **Therapeutic Application**

   Garlic extract may be considered as a complementary dietary or therapeutic agent for reducing cardiovascular risk factors, particularly dyslipidemia, in individuals using combined oral contraceptives. However, its use should be guided by appropriate dosing to maximize benefits.

2. **Public Health and Clinical Awareness**

   Healthcare providers should be aware of the potential cardiovascular effects of long-term or high-dose oral contraceptive use and the possible benefits of dietary interventions such as garlic supplementation in mitigating these risks.

3. **Further Experimental Studies**

   Future studies should employ longer exposure durations and higher ischemic challenge models to better elucidate the cardioprotective effects of garlic on myocardial injury markers, including additional biomarkers such as creatine kinase-MB, lactate dehydrogenase, and oxidative stress indices.

4. **Mechanistic Investigations**

   Further research should explore the molecular mechanisms underlying garlic's cardioprotective effects, particularly its influence on oxidative stress pathways, inflammatory mediators, endothelial function, and nitric oxide bioavailability in hormonally altered states.

5. **Clinical Translation**

   Clinical trials involving women using oral contraceptives are recommended to validate the translational relevance of these findings and to establish safe and effective garlic supplementation protocols.

6. **Dose Optimization Studies**

   Additional studies should investigate optimal dose combinations of garlic and oral contraceptives to identify thresholds beyond which beneficial effects may be reduced or lost.

5.4 Contribution to Knowledge

1. This study provides experimental evidence that garlic (*Allium sativum*) extract can modulate cardiovascular risk markers in female rats exposed to combined oral contraceptives, addressing a gap in research on female-specific and hormonally influenced ischemic heart disease.
2. The findings demonstrate a dose-dependent interaction between garlic and oral contraceptives, showing that cardioprotective benefits are more pronounced at low to moderate contraceptive doses but reduced at higher doses.
3. By integrating cardiac troponin I analysis with histological assessment, the study highlights the presence of subclinical cardiac changes associated with oral contraceptive exposure, even in the absence of overt myocardial damage.
4. The study establishes the organ safety profile of garlic when co-administered with oral contraceptives, as evidenced by preserved liver, kidney, and cardiac tissue architecture.

5. This research provides a validated experimental framework for future studies investigating herbal or dietary interventions for reducing cardiovascular risk in women using hormonal contraceptives.

5.5 Limitation to the Study

During the study, a single mortality was recorded in Group E (1.32 mg/kg COC + 800 mg/kg garlic). The death occurred without preceding signs of distress, and all other animals in the group completed the study normally. No mortality was observed in the control group or any other treatment groups. Body weight trends and biochemical parameters in Group E were comparable to the control, suggesting that the death was an isolated event rather than a treatment-related effect. Nevertheless, individual variability in response to COC–garlic co-administration cannot be completely ruled out.

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APPENDIX

EDO STATE MINISTRY OF HEALTH
HEALTH RESEARCH ETHICS COMMITTEE

PROTOCOL NUMBER	**HA/737/25/C/10311005 (PLEASE QUOTE IN ALL ENQUIRIES)**
APPROVAL NUMBER	**HA/737/25/C/11031005**
TITLE OF RESEARCH PROPOSAL	**USE OF GARLIC (*Allium Sativum*) EXTRACT TO REVERSE ISCHEMIC HEART DISEASE IN FEMALE RATS EXPOSED TO VARIOUS ORAL CONTRACEPTIVES**
PRINCIPAL INVESTIGATOR (S)	**ILUSEMITI SARAH ASITONKE**
DATE CONSIDERED	**3RD NOVEMBER, 2025.**
DECISION OF THE COMMITTEE	**APPROVED**

THIS APPROVAL DATES 03/11/2025 TO 03/11/2026. IF THERE IS A DELAY IN STARTING THE RESEARCH, PLEASE INFORM THE HREC EDO SMoH SO THAT THE DATES OF APPROVAL CAN BE ADJUSTED ACCORDINGLY

REMARK: Please kindly note that the HREC Edo SMoH seal authenticates this approval

DR (MRS) Omonyemen B. BELLO
(MBBS, MPH, FPHCM) *(CHAIRMAN)*

SIGNATURE & DATE.....4/11/2025.....

SUPERVISOR(S) .....

.....

ATTESTATION BY INVESTIGATOR(S)

No participant accrual or activity related to this research may be conducted outside of the approval dates. All informed consent forms used in this study must carry the Edo SMoH HREC-assigned number and duration of your research. No changes are permitted in the research without prior approval of the Edo SMoH HREC except in circumstances outlined in the Code. The Edo SMoH HREC reserves the right to conduct compliance visits to your research site without previous notification.

EDO STATE MINISTRY OF HEALTH
SECRETARIAT
DATE 4/11/2025
SIGN
DATE 4/11/2025
RESEARCH & ETHICS COMMITTEE

Signature & Date.....

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