

**ANTI-HYPERLIPIDEMIC AND BIOCHEMICAL EFFECT OF ACTIVATED
CHARCOAL FROM *Terminalia catappa* ON CHOLESTEROL INDUCED WISTAR RATS**



BY

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CERTIFICATION

This is to certify that this thesis titled “ANTI-HYPERLIPIDEMIC AND BIOCHEMICAL EFFECT OF ACTIVATED CHARCOAL FROM *Terminalia catappa* ON CHOLESTEROL INDUCED WISTAR RATS” by EWANLENFOH OMOZOYA FLORA with matriculation number LSC1906504, of the department of Biochemistry, Faculty of Life Sciences, University of Benin met the requirement and regulation governing the award of Bachelor of Science (B.Sc.) degree in Biochemistry, in the University of Benin, and is approved for its contribution to knowledge and literacy presentation.

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DEDICATION

This work is dedicated to Almighty God and to my parent Mr and Mrs Ewanlenfoh.

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First and Foremost, my sincere appreciation goes to God Almighty for his unending grace and provision towards the successful completion of this phase of my life.

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ABSTRACT

Hypercholesterolemia is an important risk factor for atherosclerosis and the development of cardiovascular disease. The increasing concerns regarding the side effects and long-term safety of conventional cholesterol-lowering drugs brought about the search for alternative therapeutic agents. This study biochemically transformed the stem bark of *Terminalia catappa* to activated charcoal and evaluated its anti-hyperlipidemic effect on cholesterol-induced models. Stem bark was gotten fresh from the *Terminalia catappa* tree, dried under the sun and subjected to an air tight Thermofischer muffle furnace at 1450°C in the absence of oxygen for about an hour to allow all the stem bark turn into charcoal and highly porous material with a large surface area. Twelve rats were divided into four groups of three rats each. All groups except group 1 (control) were induced with 5% cholesterol dissolved in ratio 1:2 of DMSO (Dimethyl sulfoxide) orally with gastric gavage and fed with diet rich in lard. Group 2 (negative control) were left untreated, group 3 and 4 were treated with 200mg/kg body weight of Activated charcoal and 10mg/kg body weight of Atorvastatin (standard drug) respectively. GC-MS results shows that activated charcoal of *Terminalia catappa* contains two phytochemicals; Methyl-6-beta galactopyranoside (93.24%) and Stigmastan-3, 5-diene (6.76%) which is a plant sterol. The results revealed a significant increase ($p < 0.05$) in Sodium dismutase (SOD), Catalase (CAT), Glutathione reductase (GSH), Glutathione Peroxidase (GPx), Total protein and Alanine Transaminase (ALT) in group 3 and 4 when compared with group 2 which were induced but not treated. A significant decrease ($p < 0.05$) in Aspartate Transaminase (AST), Alkaline phosphatase (ALP), Total cholesterol, LDL-Cholesterol, Urea, Creatinine, Malondialdehyde (MDA), Potassium and Sodium in group 3 and 4 when compared to group 2 which were induced but not treated. Data from this study show that activated charcoal contains phytochemicals, a plant sterol which reduces cholesterol levels by inhibiting the absorption of cholesterol in the intestines by absorbing bile acids. Therefore, activated charcoal of *Terminalia catappa* has an anti-hyperlipidemic effect.

CHAPTER ONE

1.1 INTRODUCTION

Hypercholesterolemia, characterized by elevated levels of cholesterol in the bloodstream, stands as a significant risk factor for cardiovascular diseases, including atherosclerosis, coronary artery disease, and stroke (Grundy, *et al.*, 2019). Some of the components of hypercholesterolemia are increased total serum cholesterol level, high LDL cholesterol level and low HDL cholesterol levels. Hypercholesterolemia can be classified as primary when the lipid disorder has genetic influence, or secondary, caused by inadequate diet and lifestyle as well as medications. A search for alternative therapeutic agents is brought about due to the concerns regarding the side effects and long-term safety of conventional cholesterol-lowering drugs despite advances in pharmacological interventions.

Terminalia catappa, commonly known as Indian almond or tropical almond, has been traditionally used in various cultures for its medicinal properties (Pino, *et al.*, 2020). Its potential as a source of activated charcoal is of particular interest, which has been explored for its adsorptive properties in diverse applications, including toxin removal and gastrointestinal disorders (Bansal, *et al.*, 2019). Activated charcoal is a highly porous form of carbon with a surface area of 950 to 2000 mg that is capable of adsorbing poisons with a molecular weight of 100 to 1000 daltons.

This study seeks to investigate the effect of activated charcoal derived from *Terminalia catappa* on hypercholesterolemia. *Terminalia catappa* activated charcoal may be a safer and more natural option for controlling high cholesterol levels by utilizing its claimed cholesterol-lowering

capabilities. Furthermore, exploring the mechanisms underlying its hypocholesterolemic effects can provide valuable insights into its therapeutic potential.

This study intends to contribute to the expanding body of information on natural treatments for hypercholesterolemia by conducting extensive experimental investigations that include lipid profile evaluation and clarification of underlying mechanisms. Ultimately, the findings may have implications for the development of novel therapeutic strategies aimed at reducing the burden of cardiovascular diseases.

1.1.1 AIM OF EXPERIMENT

This research aims to investigate the therapeutic potential of activated charcoal derived from *Terminalia catappa* in mitigating hyperlipidemia.

1.1.2 OBJECTIVES OF EXPERIMENT

1. To assess the impact of *Terminalia catappa* activated charcoal on serum cholesterol levels in an animal model of hypercholesterolemia, following established protocols.
2. To analyze changes in lipid profile parameters, including LDL, HDL, and triglycerides, post-treatment with *Terminalia catappa* activated charcoal.
3. To elucidate potential mechanisms underlying the observed effects, such as cholesterol absorption inhibition or bile acid sequestration, through histological and biochemical analyses.
4. To compare the efficacy of *Terminalia catappa* activated charcoal with standard hypercholesterolemia treatments, referencing clinical trials and meta-analyses.

1.2 LITERATURE REVIEW

Hypercholesterolemia, characterized by elevated levels of cholesterol in the bloodstream, remains a significant risk factor for cardiovascular diseases (CVDs), contributing to morbidity and mortality worldwide (Grundy, *et al.*, 2019). Conventional pharmacological interventions, such as statins, are commonly prescribed for managing hypercholesterolemia but are associated with adverse effects and poor tolerability in some individuals (FERENCE, *et al.*, 2019). Thus, there is growing interest in exploring alternative therapeutic strategies, including natural compounds with hypocholesterolemic properties.

Activated charcoal, a porous carbon material with high surface area and adsorptive capacity, has been studied for its potential to lower cholesterol levels by adsorbing bile acids and cholesterol derivatives in the gastrointestinal tract (Bansal, *et al.*, 2019). *Terminalia catappa*, commonly known as Indian almond or tropical almond, is a tropical tree with various medicinal properties, including anti-inflammatory, antioxidant, and hepatoprotective effects (Pino, *et al.*, 2020). *Terminalia catappa* has been traditionally used in different cultures for the management of various ailments, including gastrointestinal disorders and cardiovascular diseases.

Several studies have investigated the potential cholesterol-lowering effects of activated charcoal derived from various sources. In an animal study by Singh, *et al.*, (2018), activated charcoal derived from coconut shells demonstrated significant reductions in serum cholesterol levels in hypercholesterolemic rats, possibly through bile acid sequestration. Similarly, a study by Guerra, *et al.*, (2019) evaluated the cholesterol-lowering effects of activated charcoal from bamboo in hypercholesterolemic hamsters, reporting significant improvements in lipid profiles and atherosclerotic lesions.

Terminalia catappa has also garnered attention for its potential therapeutic effects on hypercholesterolemia. Kumar, *et al.* (2023) provided an overview of the traditional uses,

phytochemistry, and pharmacological properties of *Terminalia catappa*, highlighting its potential as a natural remedy for various ailments, including hypercholesterolemia. Additionally, Agus, *et al.*, (2020) conducted a study investigating the hypolipidemic effects of *Terminalia catappa* leaf extract in hypercholesterolemic rats, demonstrating significant reductions in serum cholesterol, LDL, and triglyceride levels.

Despite the promising findings from individual studies on activated charcoal and *Terminalia catappa*, there is a notable gap in the literature regarding the specific effects of activated charcoal derived from *Terminalia catappa* on hypercholesterolemia. Therefore, this research seeks to address this gap by investigating the hypocholesterolemic potential of activated charcoal from *Terminalia catappa* and elucidating the underlying mechanisms

1.2.1 MEDICINAL PLANTS

Traditional medicine has been used by the majority of the world population for thousands of years (Seifu, *et al.*, 2004). Medicinal plants are any plant in which one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, *et al.*, 2013). They contain phytochemicals that may have therapeutic effects in humans and can be processed into medicines. Examples of medicinal include *Moringa oleifera* (Moringa), *Ocimum basilicum* (Scent leaf), *Terminalia catappa* (Indian Almond), *Vernonia amygdalina* (Bitter leaf) and *Hibiscus sabbdariffa* (Zobo).

1.2.1 TERMINALIA CATAPPA

Terminalia catappa Linn (T. catappa) belongs to the *Combretaceae* family as a considerable (25–40 m height) deciduous tree with smooth gray bark and whorly branches that form a canopy in tropical and subtropical regions of Asia and Africa (Iheagwan, 2022). It is often found in coastal

vegetation, growing at the edges of mangrove swamps or on rocky shores. The tropical climate in Nigeria, particularly in the southern regions, is suitable for the growth and cultivation of *Terminalia catappa*. It is widely planted throughout the tropics as an ornamental tree for shade and edible nuts in Africa. The tree loses its leaves twice yearly in most places, turning a brilliant red to yellow before leaf shedding (Mininel, *et al.*,2014). The botanical classification of *Terminalia catappa* is as follows:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Myrtales

Family: Combretaceae

Genus: Terminalia

Species: *Terminalia catappa* L.

Terminalia catappa has traditionally been used In Asia to treat diarrhea and hepatitis. Dry leaves are used to treat fish pathogens as an alternative to antibiotics. The leaves possess antioxidant, anticlastogenic, and antihyperglycemic effects (Anand, 2015). The leaves and bark extracts of *T. catappa* are anticancer, anti-HIV reverse transcripts, hepato-protective, anti-inflammatory, antihepatitis, antidiabetic, and aphrodisiac (Dwevedi, 2016). The fallen leaves of *T. catappa* have

been used in the management of sickle cell disorders (Aimola, 2014).



Figure 1: Image of *Terminalia catappa* L.

1.2.2.1 PHYTOCHEMISTRY OF *TERMINALIA CATAPPA*

Several studies have been conducted to investigate the photochemistry of *Terminalia catappa*. The phytochemical screening of T. catappa fruit flesh extract revealed the presence of flavonoids, alkaloids, phenolic, terpenoid, and saponin components. The extract's UV-Vis spectrum revealed the presence of conjugated double bonds, which are characteristic of flavonoid compounds. The IR spectra also revealed the existence of a characteristic functional group of flavonoid chemicals (Senny, *et al.*, 2022). *Terminalia catappa* extract (TCE) has been found to have potential as a medicine material compound. The aqueous extract of *Terminalia catappa* leaves has been shown to have antioxidant activity, and the hydroalcoholic extract of TC leaves has been found to have

hepatoprotective activity. The leaves of *Terminalia catappa*, which have antibacterial properties have also been used in the preparation of silver nanoparticles (Rohaeti, *et al.*, 2017).

The bark of *Terminalia catappa* contains several bioactive compounds that have been shown to have anti-hypercholesterolemic effects. These compounds include flavonoids, alkaloids, triterpenes, and coumarins, which have been found to have anthelmintic activity (Katiki, *et al.*, 2017). The bark of *Terminalia catappa* has been used to treat dysentery by numerous cultures in Southeast Asian countries, and its leaves have also been utilized traditionally. The leaves have been discovered to have antioxidant and hepato-protective properties, and the tannin corilagin has been identified as one of the active components.

1.2.3 ACTIVATED CHARCOAL

Activated charcoal is a highly porous carbon material made from the pyrolysis (heating in the absence of oxygen) of carbonaceous materials, such as wood, coconut shells, or plant biomass (Marsh, *et al.*, 2006), which has garnered significant interest due to its versatile applications in various fields including medicine, industrial processes and environmental remediation.

Activated Charcoal is primarily known for its absorptive properties in medicine, which enables it to trap and remove toxins, drugs and other harmful substances from the gastrointestinal tract. The absorptive capacity of activated charcoal stems from its large surface area and microporous structure, the binding of molecules through van der Waals forces, hydrogen bonding and other non-covalent interactions is facilitated (Bansal, *et al.*, 2019). This property forms the basis of its use in the management of acute poisonings and drug overdoses, where rapid removal of toxins from the digestive system is crucial for preventing systemic absorption and toxicity (Chyka, *et al.*, 2017).



Figure 2: Image of an Activated charcoal

1.2.3.1 PLANT-DERIVED ACTIVATED CHARCOAL

Plant-derived activated charcoal is produced by the thermochemical degradation of biomass in the absence of oxygen, followed by chemical or physical activation. The feedstocks for activated charcoal can be derived from various vegetative sources, including nut shells, sugarcane bagasse, coconut husks, cotton, crop remnants, grain remnants, grass residues, wood chips, and tree bark. The production process involves carbonizing and activating organic substances, which may include sawdust, peat, and other plant-based materials (Chew, *et al.*, 2023).

The use of plant-derived activated charcoal is consistent with a growing interest in natural products and traditional medicinal plants as potential sources of innovative therapeutics. Researchers hope to produce effective and safe therapeutic interventions for a variety of illnesses, including pain management and inflammation, by combining activated charcoal's adsorptive characteristics with bioactive chemicals found in plant materials (Dey, *et al.*, 2022).

Overall, the potential medicinal benefits of activated charcoal, particularly when obtained from plant sources, have sparked widespread scientific attention. Its adsorption capabilities, together with potential anti-inflammatory and analgesic effects, make it an intriguing candidate for further research into novel and sustainable treatment techniques.

1.2.3.2 BENEFITS OF ACTIVATED CHARCOAL

Activated charcoal has been investigated for its potential therapeutic benefits in various gastrointestinal disorders, including diarrhea, flatulence, and irritable bowel syndrome (IBS) in addition to its role in acute poisoning (Anderson, *et al.*, 2019). Activated charcoal may help alleviate symptoms and improve overall gastrointestinal health by adsorbing excess gas, bacterial toxins, and other irritants in the gut.

Activated charcoal has been researched for its potential in lipid-lowering therapy and cholesterol management in addition to its effects on the gastrointestinal tract. Activated charcoal has been shown in numerous studies to have the capacity to adsorb bile acids and cholesterol derivatives in the colon, which lowers blood cholesterol levels (Singh, *et al.*, 2018; Guerra, *et al.*, 2019). This hypocholesterolemic effect has implications for the prevention and management of hypercholesterolemia and cardiovascular diseases, although further research is needed to elucidate its efficacy and safety profile in clinical settings.

1.2.3.3 SIDE EFFECTS OF ACTIVATED CHARCOAL

Activated charcoal is generally considered safe when used short-term, but it may cause unpleasant side effects like constipation, vomiting, and black stools. These side effects are due to the fact that activated charcoal binds to substances in the stomach and intestines, preventing their absorption into the body (Peter, 2023).

1.2.3.4 LIMITATIONS OF ACTIVATED CHARCOAL

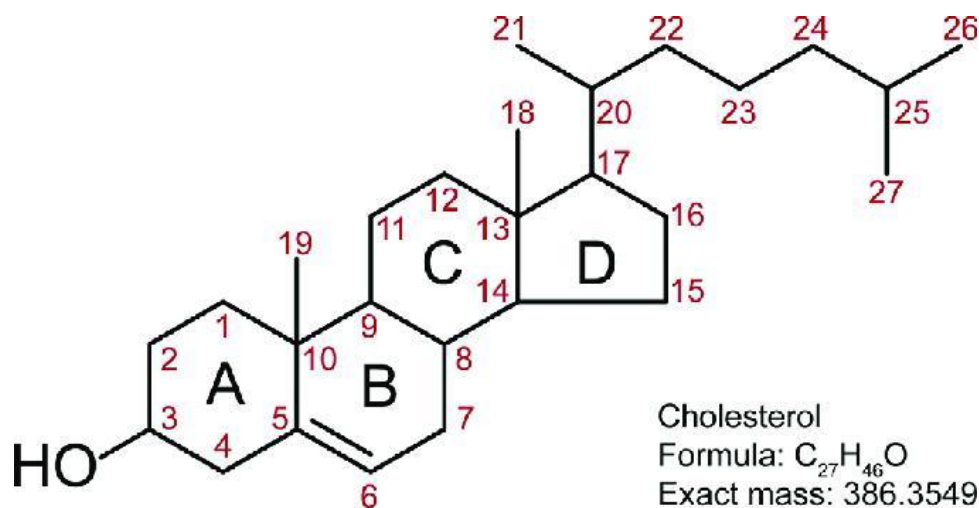
Activated charcoal is not without limitations, despite its numerous potential benefits. One concern is its non-specific adsorption, which can interfere with the absorption of medications and essential nutrients, leading to potential drug interactions and nutritional deficiencies (Krenzelok, 2016). Furthermore, several aspects including particle size, surface area, and the unique characteristics of the adsorbate might affect how effective activated charcoal is, which emphasizes the significance of standardized formulations and dosing regimens in clinical practice (Dart, *et al.*, 2019).

Activated charcoal remains a valuable tool in the management of acute poisonings and certain gastrointestinal disorders, offering rapid and effective toxin removal from the digestive system. Additionally, emerging evidence suggests potential benefits in cholesterol management and lipid-lowering therapy, although further research is needed to validate these findings and address existing limitations.

1.2.4 CHOLESTEROL

Cholesterol is a vital lipophilic compound, which is essential in the formation of cell membrane and also modulates its fluidity, in production of sex hormone (e.g. testosterone, estrogens and progesterone), in the production of steroid hormones (e.g. cortisol, aldosterone and adrenal androgens) and the formation of bile salts used in digestion to facilitate absorption of fat soluble vitamins A, D, E, and K) amongst other functions of the organism (Di Ciaula, 2017). It is a 27-carbon compound with a unique structure that includes a hydrocarbon tail, a central sterol nucleus made of four hydrocarbon rings, and a hydroxyl group. The hydrocarbon tail and the central ring are non-polar and therefore do not mix with water, so cholesterol (lipid) is packaged

together with apoproteins (protein) in order to be carried through the blood. It is transported through the blood, along with triglycerides, inside lipoprotein particles (HDL, IDL, LDL, VLDL, and chylomicrons). Humans can synthesize cholesterol de novo and can also obtain it from the diet. De Novo synthesis occurs in the liver and the intestines, each organ accounting for ~ 10% of total cholesterol in the body. The liver packages dietary triglycerides and cholesterol with Apo proteins before releasing them into the circulation. It can be found in its free form in animal fats.



Its main food sources are egg yolk, milk and milk derivatives, shrimp, beef, bird skin and viscera.

Figure 3: Structure of Cholesterol

1.2.4.1 TYPES OF CHOLESTEROL

1. Low-Density Lipoprotein (LDL) Cholesterol: LDL cholesterol is often referred to as “bad” cholesterol because it can contribute to plaque buildup in the arteries, leading to atherosclerosis and cardiovascular diseases (Grundy, *et al.*, 2018).
2. High-Density Lipoprotein (HDL) Cholesterol: HDL cholesterol is often referred to as “good” cholesterol because it helps remove LDL cholesterol from the bloodstream, reducing the risk of cardiovascular diseases (Barter, *et al.*, 2007).

3. Very Low-Density Lipoprotein (VLDL) Cholesterol: VLDL cholesterol carries triglycerides from the liver to the tissues in the body. High levels of VLDL cholesterol are associated with an increased risk of cardiovascular diseases (Virani, *et al.*, 2020).

1.2.4.2 ABSORPTION OF CHOLESTEROL

Cholesterol absorption occurs in the intestines, where around half of the cholesterol supplied is normally absorbed, with individual variances ranging from 20% to 80%. Of the cholesterol absorbed, approximately 75% comes from bile, while only about 25% comes from the diet.

The absorbed cholesterol is delivered to the liver in the form of chylomicron remnants, which are then incorporated into very low-density lipoprotein (VLDL) particles, contributing to the production of atherogenic LDL particles in the plasma (Miettinen, *et al.*, 1988).

1.2.4.2.1 Factors Influencing Cholesterol Absorption:

Dietary cholesterol intake affects the efficiency of cholesterol absorption, with higher dietary cholesterol intake leading to lower rates of biliary secretion, fecal elimination, and cholesterol synthesis.

High serum levels of total, LDL, and HDL cholesterol are associated with high cholesterol absorption, while high HDL cholesterol levels are associated with low synthesis and fecal elimination of cholesterol (Miettinen, *et al.*, 1988)

1.2.4.3 ELIMINATION OF CHOLESTEROL

Cholesterol is broken down and eliminated primarily by the liver, which regulates the synthesis, secretion, and clearance of cholesterol-rich lipoproteins. The liver determines the amount of

cholesterol eliminated into bile, either as cholesterol or after conversion to bile acids, with the intestine serving as the site of cholesterol absorption and elimination (David, 2004).

1.2.5 HYPERCHOLESTEROLEMIA

Hypercholesterolemia is a condition characterized by high levels of cholesterol in the blood which can lead to the formation of plaques in the arteries, which can increase the risk of cardiovascular disease, particularly coronary heart disease. High cholesterol can be defined as a LDL-cholesterol greater than 190 mg/dL, greater than 160 mg/dL with one major risk factor, or greater than 130 mg/dL with two cardiovascular risk factors (Ibrahim, *et al.*, 2023). The important risk factors include:

- Age; male 45 years or older, female 55 years or older
- A positive family history of premature atherosclerotic cardiovascular disease (younger than 55 years in a male and younger than 65yrs in a female)
- Diabetes
- Smoking
- Hypertension
- Low HDL-cholesterol levels (less than 40 mg/dl in male and less than 55 mg/dl in a female).

1.2.5.1 CAUSES OF HYPERCHOLESTEROLEMIA

1. Genetic Cause

Genetic causes, such as mutations in the LDL receptor gene, can contribute to familial hypercholesterolemia, a disorder characterized by extremely high levels of LDL cholesterol from birth. This defect in the LDL receptor accounts for at least 85% of familial hypercholesterolemia.

The reduction in LDL receptor activity in the liver results in a reduced rate of clearance of LDL from the circulation. The plasma level of LDL increases to a level such that the rate of LDL production equals the rate of LDL clearance by residual LDL receptors as well as non-LDL receptor mechanisms. More than 1600 mutations have been reported in association with familial hypercholesterolemia. The elevated levels of LDL-Cholesterol in familial hypercholesterolemia are primarily due to a delayed removal of LDL from the blood (Mytilinaiou, *et al.*, 2018).

Other genetic causes of familial hypercholesterolemia includes defective apolipoprotein B (most common with a mutation at position 3500) resulting in a loss of ligand binding to the LDL receptor and a gain-of-function mutation in proprotein convertase subtilisin/kexin type 9 (PCSK9) gene leading to increased affinity of PCSK9 for the LDL-receptor which results in a more rapid clearance of the LDL-receptor by targeting it to the lysosome for degradation in the liver, thus resulting in high plasma LDL-Cholesterol (Ibrahim, *et al.*, 2023).

2. Acquired Cause

Hypercholesterolemia can also be caused by lifestyle factors, such as a diet high in saturated and trans fats, physical inactivity, obesity, smoking, and excessive alcohol consumption. Other medical conditions, such as diabetes, hypothyroidism, and kidney disease, can also contribute to the development of hypercholesterolemia (Ibrahim, *et al.*, 2023).

1.2.5.2 LABORATORY TEST USED TO EVALUATE HYPERCHOLESTEROLEMIA

Plasma lipid profile should be measured in all adults older than 40 years, preferably after a 10 to 12-hour overnight fast. The lipid profile reports the total cholesterol, triglycerides, and HDL-cholesterol, and calculates the LDL-cholesterol by the Friedewald Equation:

$$\text{LDL-C} = \text{Total Cholesterol} - \text{VLDL}(\text{TG}/5) - \text{HDL-C}$$

This formula (the Friedewald formula) is accurate if test results are obtained on fasting plasma and if the triglyceride level does not exceed 200 mg/dL. By convention, it cannot be used if the triglyceride level is greater than 400 mg/dL since high triglycerides alter the TG/5 or VLDL-C. Many methods can directly measure LDL-C. Secondary causes can be excluded by doing the following tests: TSH (hypothyroidism), glucose (diabetes), urinalysis and serum albumin (nephrotic syndrome), and bilirubin and alkaline phosphatase (cholestasis). Ideally, if there is an abnormal lipid profile (high cholesterol), the test should be repeated within 2 weeks to confirm the diagnosis before embarking on lifelong therapy.

1.2.5.3 TREATMENT AND MANAGEMENT OF HYPERCHOLESTEROLEMIA

The cornerstone of treatment of hypercholesterolemia is a healthy lifestyle, an optimum weight, no smoking, exercising for 150 minutes per week, and a diet low in saturated and trans-fatty acids and enriched in fiber, fruit, and vegetables and fatty fish. Plant sterols which are cholesterol-like compounds at a dose of 2 g/d can help reduce LDL-Cholesterol. The standard drug class of choice is the statin which can lower LDL-Cholesterol from 22% to 50%. Statins which are antihyperlipidemic agents are widely used in the treatment of atherosclerosis due to their excellent efficacy in reducing low-density lipoprotein levels. Statins competitively inhibit the 3-hydroxy-3-methylglutaryl coenzyme A reductase as catalysis of the rate-limiting step in cholesterol biosynthesis (Climent, 2022). Also, they have been shown to reduce cardiovascular events in both primary and secondary prevention trials.

1.2.5.4 SIDE EFFECTS OF HYPERCHOLESTEROLEMIA

The major side effects of hypercholesterolemia are elevated transaminases which includes Alanine transaminase and Aspartate transaminase, myalgia, and myopathy and new-onset diabetes. If transaminases exceed three times the upper limit of normal, the statin dose should be reduced, or a lower dose of another statin should be used. Myopathy is a serious problem since it can result in rhabdomyolysis and acute renal failure. Certain drugs in combination with statins increase this risk (Ibrahim, *et al.*, 2023).

1.2.6 BINDING OF CHOLESTEROL TO ACTIVATED CHARCOAL

Activated charcoal has been shown to bind to cholesterol and cholesterol-containing bile acids in the gut, preventing their absorption and potentially reducing cholesterol levels (Dilonardo, 2023).

A study published in the Journal of Physics: Conference Series found that activated charcoal can adsorb cholesterol, preventing its absorption in the intestine and reducing the damage to the aorta in hypercholesterolemia rat models. In the study, rats were given activated charcoal at a dose of 4.950 mg/kg body weight, which was found to be effective in preventing the decrease of HDL levels and improving the histopathology of the aorta (Roosdiana, *et al.*, 2019).

The adsorption mechanism of cholesterol by activated charcoal involves internal diffusion of cholesterol from a boundary layer to the surface of the adsorbent, with some of it adsorbed on the outer surface and most of it diffusing internally inside the adsorbent pore. Activated charcoal cannot be absorbed by the intestine or digestive tract and is excreted together with feces in the unchanged form, making it a potential preventive medication for hypercholesterolemia (Roosdiana, *et al.*, 2019).

1.2.7 COMPARISON OF ACTIVATED CHARCOAL FROM *TERMINALIA CATAPPA* WITH OTHER CHOLESTEROL-LOWERING AGENTS

Activated charcoal has been researched for its efficacy in decreasing cholesterol levels in comparison to other cholesterol-reduction medications. Activated charcoal has been found in studies to lower serum cholesterol levels in people with hypercholesterolemia. In a study comparing activated charcoal and cholestyramine, both drugs were found to considerably reduce total and LDL-cholesterol levels, with activated charcoal exhibiting the highest reduction of 29% and 41% in total and LDL-cholesterol, respectively (Neuvonen, *et al.*, 1989). Another study examined superactivated charcoal with cholestyramine and found that both drugs had comparable ability to lower plasma cholesterol concentrations, with superactivated charcoal lowering total plasma cholesterol by 21.8% and cholestyramine by 16.2% (Park, *et al.*, 1988).

Furthermore, activated charcoal was discovered to prevent the fall of HDL levels and enhance aorta histology in hypercholesterolemic rats, suggesting its potential preventive effect on HDL levels and aorta health (Roosdiana, *et al.*, 2019).

These studies demonstrate the effectiveness of activated charcoal as a cholesterol-lowering agent, with outcomes comparable to standard drugs such as cholestyramine. According to the study, activated charcoal could be a feasible alternative for controlling hypercholesterolemia, providing a potentially effective, cost-effective and non-toxic method of lowering cholesterol levels.

1.2.8 ASPARTATE TRANSAMINASES IN HYPERCHOLESTEROLEMIA

Aspartate transaminases, also known as AST, are enzymes found in various tissues of the body, with the highest concentrations in the liver, muscles, heart, kidney, brain, and red blood cells (Burke, *et al.*, 2021). Elevated AST levels in the blood might suggest tissue damage, and liver

injury is a common cause. AST levels can rise due to injury in tissues where the enzyme is present. The AST test is widely used to check for liver disorders including hepatitis (Burke, *et al.*, 2021).

Hypercholesterolemia, a disorder defined by excessive cholesterol levels in the blood, has been connected to liver health and has the potential to affect AST levels. According to research, hypercholesterolemia can cause liver injury, which may result in high AST levels. The link between hypercholesterolemia and liver health emphasizes the need of monitoring AST levels in high-cholesterol individuals to determine liver function and possible damage.

1.2.9 ALANINE TRANSAMINASES IN HYPERCHOLESTEROLEMIA

Hypercholesterolemia is a condition marked by elevated cholesterol levels in the blood, which can contribute to the development of cardiovascular disease. Alanine transaminases (ALT) are enzymes present in the liver; increased ALT levels in the blood might indicate liver damage or disease. Recent research suggests that there may be a relationship between elevated ALT levels and cardiovascular risk factors, particularly hypercholesterolemia (Siddiqui, *et al.*, 2013).

The study also found that increasing levels of ALT and fasting hyperinsulinemia synergized with increasing levels of triglyceride, VLDL particles, LDL-P, sdLDL-C, and percentage of sdLDL-C. These findings suggest that elevated ALT levels may be a marker for increased cardiovascular risk in individuals with hypercholesterolemia.

Another study published in the Journal of the American Heart Association found that elevated ALT levels were associated with an increased risk of cardiovascular events in patients with type 2 diabetes (Park, *et al.*, 2005). The study found that each 10-unit increase in ALT was associated with a 12% increased risk of cardiovascular events, independent of traditional risk factors.

These studies suggest that elevated ALT levels may be a useful marker for increased cardiovascular risk in individuals with hypercholesterolemia. However, more research is needed to fully understand the relationship between ALT and cardiovascular risk in this population. Additionally, it is important to note that elevated ALT levels can also be a marker for liver damage or disease, and should be evaluated in the context of other clinical findings.

1.2.10 ALKALINE PHOSPHATASE IN HYPERCHOLESTEROLEMIA

Alkaline phosphatase (ALP) is an enzyme that has been associated with cardiovascular disease (CVD) risk, but the shape and independence of this association from established risk factors have not been fully characterized (Kunutsor, *et al.*, 2015). A study published in the Journal of Cardiovascular Development and Disease found that serum ALP activity was associated with atherosclerotic cardiovascular disease (ASCVD) in patients with dyslipidemia over a 6-year period (Adamidis, *et al.*, 2024). The study also discovered that each 10-unit increase in ALP was associated with a 12% higher risk of cardiovascular events, regardless of established risk variables.

These findings imply that raised ALP levels could be a valuable indicator of increased CVD risk in people with dyslipidemia and type 2 diabetes. However, further study is needed to completely understand the link between ALP and CVD risk in these populations. Furthermore, increased ALP values can indicate liver injury or disease and should be considered in conjunction with other clinical findings.

CHAPTER TWO

2.1 MATERIALS AND APPARATUS

2.1.1 MATERIALS

The materials used for this study are Terminalia catappa (Tropical Almond) wood activated charcoal, reagents and laboratory apparatus/equipment

2.1.2 APPARATUS

Beaker, Syringes (5ml), Cotton wool, Gastric gavage, Measuring cylinder (Pyrex, England), Spatula, Permanent marker, Test tubes/rack, Cheese cloth, Hand Gloves, Petri-dish, Cork borer (10mm), Bursen burner.

2.1.3 INSTRUMENT USED: AGILENT 720 ICP-OES

2.1.4 CHEMICALS AND REAGENTS

- Extracts/samples
- Distilled Water

2.2 METHODOLOGY

2.2.1 SAMPLE COLLECTION

The stem bark of Terminalia catappa were gotten fresh from trees of Terminalia catappa cut down at the open field of the Students Affairs Division, University of Benin, Benin City, Nigeria. The logs of wood were collected and identified by a recognized taxonomist of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State.

The specimens with Voucher numbers were deposited in the same Department, while their stem bark was dried to remove moisture content under the sun at 34-36°C for 14 days.

2.2.2 PREPARATION OF THE ACTIVATED CHARCOAL

Carbonation of chopped *Terminalia catappa* stem bark into charcoal was carried out in the laboratory with a Thermofischer muffle furnace at 1450 °C in the absence of oxygen for about an hour to allow all the stem bark turned charcoal and highly porous material with a large surface area. Charcoal was pulverized to smooth powder and then subjected to analysis for about an hour to allow all of the carbon present to develop lots of spores

2.2.3 GC-MS ANALYSIS OF ACTIVATED CHARCOAL DERIVED FROM *TERMINALIA CATAPPA*

About 100 g of activated charcoal of *Terminalia catappa* was dissolved in 100 mL distilled water, further diluted with water 1:1 and filtered through a 0.22 µm PVDF filter. GC-MS analysis of *Terminalia catappa* was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-I, fused silica capillary column (30 mm x 0.25 mm ID x 1 µMdf, composed of 100% Dimethylpolysiloxane). For GC-MS detection, an electron ionization system with ionizing energy in a single-phase ion mode of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min and an injection volume of 0.50 ml was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan-interval of 0.5 seconds and fragments from 45 to 450 Da. The total GC running time was 27 minutes. The relative % amount of each component was calculated by

comparing its average peak area to the total areas. Software adapted to handle mass spectra and chromatograms was a Turbo mass.

2.2.4 IDENTIFICATION OF COMPONENTS

Interpretation of mass spectrum from GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) and has more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known and authentic samples stored in the NIST library. Computer searches in an HP Mass Spectral Library NIST98 were also applied. The name, molecular weight, and structure of the components of the test materials were ascertained. Known and authentic samples stored in the NIST library. Computer searches in an HP Mass Spectral Library NIST98 were also applied. The name molecular weight and structure of the components of the test materials were ascertained.

2.3 ANIMAL MANAGEMENT AND EXPERIMENTAL DESIGN

2.3.1 ANIMAL MANAGEMENT

A total number 12 healthy male and female rats (Wistar strain), weighing between 170g and 240g, were obtained from the animal facility at the University of Benin's Department of Pharmacy. The rats were housed in cages with wire mesh floors, to prevent coprophagia. The animals were then acclimatized for one week. Water and commercial rat pellets (from top feed Ltd, Ibadan) were given ad libitum. All animals were handled according to the animal ethical guideline of the University of Benin.

2.3.2 EXPERIMENTAL DESIGN

Before acclimatization, the twelve (12) albino-wistar rats were divided into four groups;

GROUP	GROUP NAME	NUMBER OF RATS
Group 1	Positive control; normal rats	3
Group 2	Negative control; Induced but not treated	3
Group 3	Induced + treatment 200mg Activated Charcoal	3
Group 4	Induced + treatment 10 mg Atorvastatin	3

Table 1: Table showing the four groups of rats used for the experiment.

2.3.3 INDUCTION AND TREATMENT OF HYPERCHOLESTEROLEMIA

The rats were induced with hypercholesterolemia for seven (7) days, about 5kg of animal lard was mixed with 10kg of rat pellets and fed to the rats. All groups except group 1 (Control) were induced with 5% cholesterol dissolved in 1% of DMSO orally with gastric gavage.

Then, Group 2 was left untreated, Group 3 was treated with 200mg/kg of activated charcoal from *Terminalia catappa*, Group 4 was treated with 10mg of Advas-10, Atorvastatin, the standard drug. The treatment was administered for fourteen 14 days.

On the last day, the wistar rats were placed on an overnight fast and thereafter placed under chloroform.

2.3.4 COLLECTION AND PREPARATION OF SAMPLES

The experimental animals were sacrificed via cardiac puncture, blood was obtained and transferred to a heparin bottle and separated by centrifuging the blood at 3500rpm at 15mins to obtain plasma.

The liver, heart and kidney were excised, prepared and stored in formalin for histology.

2.4 BIOCHEMICAL ASSAYS

2.4.1 DETERMINATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY

Principle

Superoxide dismutase is an enzyme that plays a crucial role in catalyzing the dismutation of the superoxide (O_2^-) radical into ordinary molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). The activity of SOD was assessed based on the method of Misra and Fridovich (1972). Adrenaline auto-oxidizes rapidly in aqueous solution to adrenochrome whose concentration can be determined spectrophotometrically at 420 nm. The auto-oxidation depends on the presence of superoxide anions (O_2^-). Superoxide dismutase (SOD) inhibits this auto-oxidation by catalyzing the breakdown of superoxide anions. The degree of inhibition is thus a measure of SOD activity. The amount of enzyme producing 50 % inhibition is defined as one unit of the enzyme activity.

Assay Procedure

The organ homogenate (0.2 mL) was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) and allowed to equilibrate. The reaction was initiated by the addition of 0.3 mL of freshly prepared 0.03 mM adrenaline as substrate. The solution was mixed by inversion. The reference tube contained 2.7 mL of carbonate buffer and 0.3 mL of adrenaline, while the blank contained 2.5

mL of carbonate buffer, 0.2 mL of distilled water and 0.3 mL of 0.03 mM adrenaline. The increase in absorbance at 420 nm due to the formation of adrenochrome was monitored every 30 sec for 120 sec. One unit of SOD activity was taken as the amount of SOD necessary to cause 50 % inhibition of the oxidation of adrenaline to adrenochrome within 120 sec.

Calculation

Inhibited percent of standard were calculated using the formula below:

Change in absorbance of sample (ΔS) = Absorbance of sample (1 minute) – absorbance of the sample initial

Change in absorbance of blank (ΔC) = absorbance of blank (1 minute) – absorbance of blank (initial)

% inhibition of pyrogallol autoxidation = $\Delta S / \Delta C \times 100\%$

SOD activity (μ/ml) = (% inhibition of pyrogallol autoxidation) / (50%)

SOD activity in (μ/g wet tissue) = (SOD in μ/mL) / (Weight coefficient(γ))

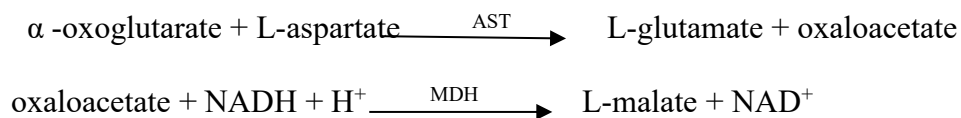
Where γ = (Volume of sample used (V_s) \times wet weight of tissue (W_s)g) / (Total volume of sample(mL))

SOD (μ/g wet tissues) = (SOD in μ/mL) / (Weight coefficient (γ))

A unit of SOD activity was taken as the amount of SOD required to cause 50 % inhibition of the auto-oxidation of adrenaline to adrenochrome per minute.

2.4.2 DETERMINATION OF ASPARTATE TRANSAMINASE ACTIVITY

The principle of the Aspartate Aminotransferase (AST) assay is based on the enzymatic reaction of AST, which catalyzes the reversible transamination of L-aspartate and α -ketoglutarate to oxaloacetate and L-glutamate. This was done in accordance with the method of the indicator reaction which utilizes the oxaloacetate for a kinetic determination of NADH consumption.



Assay Procedure

A volume of 100 μl of serum was pipetted into a test tube and mixed with 1 mL of working reagent. The initial absorbance was read after 1 min, and readings were taken at 1, 2 and 3 min respectively.

Calculation

To calculate for serum AST Concentration: $U/l = 1746 \times A_{340} \text{ nm/min}$

2.4.3 DETERMINATION OF ALKALINE PHOSPHATASE (ALP) ACTIVITY

Principle

Alkaline Phosphatase in serum was estimated by the method of Henry (1974). The reaction involves the catalytic hydrolysis of the colourless organic phosphate ester substrate, p-nitrophenylphosphate to the yellow-coloured product p-nitrophenol and phosphate. This reaction occurs in an alkaline pH of 10.3. The system monitors the rate of change in absorbance at 405 nm over a fixed-time interval. This rate of change in absorbance is directly proportional to the ALP activity in the serum.



Assay Procedure

Sample (serum) of 10 μL was added with working reagent of 500 μl , mixed and read at 405 nm.

The initial absorbance was read and readings were taken at an interval of 1 min to 3 min

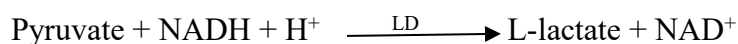
Calculation

Manual Calculation of ALP: $U/l = 2760 \times \text{Absorbance at } 405 \text{ nm/min.}$

2.4.4 DETERMINATION OF ALANINE TRANSAMINASE

Principle

In the reaction, the ALT catalyzes the reversible transamination of L-alanine and α -oxoglutarate to pyruvate and L-glutamate as described by the method of Reitman and Frankel (1957). The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase with the concurrent oxidation of NADH to NAD^+ . The rate of change at an absorbance of 340 nm over a fixed-time interval is monitored. The rate of change in absorbance is directly proportional to ALT activity in the sample.



Procedure

A volume of 100 μl of sample (serum) was pipetted into a test tube and mixed with 1 mL of working reagent. The initial absorbance was read immediately and after that, readings were taken at 1, 2 and 3 min respectively.

Calculation

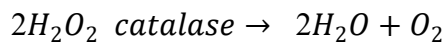
To calculate for serum ALT Concentration: $U/l = 1746 \times \text{Absorbance at } 340 \text{ nm/mi}$

2.4.5 DETERMINATION OF CATALASE ACTIVITY

Principle

Catalase is employed to remove H₂O₂, when it is produced in significant amounts. Catalase estimation was done using Cohen et al.'s approach from 1970. By monitoring the rate of hydrogen peroxide oxidation or disappearance following the addition of the substance containing the enzyme, catalase was identified.

The concentration of the enzyme in the sample immediately correlates with the amount of hydrogen peroxide that is decomposed. In order to quantify the product of hydrogen peroxide, it is first reacted with excess potassium permanganate (vi), KMnO₄ and the remaining kmno₄ is then quantified spectrophotometrically.



Assay Procedure

Sample (0.5 ml) was added to ice cold test tube and 0.5 ml of distilled water was added into another ice-cold tube as blank. The reaction was initiated by sequentially adding at fixed intervals 5 ml of cold 30 mm H₂O₂ phosphate buffer (PH7.4). They were thoroughly mixed by inversion. After exactly 3 minutes, the reaction was stopped sequentially at the same fixed interval by rapidly adding 1ml of 6M H₂SO₄ and mixing quickly by inversion. 7ml of 0.01m KMnO₄ was added to the test and blank tubes one at a time and were mixed twice by inversion. The absorbance was read at 480 nm within 30 to 60 seconds of mixing. The reference was

prepared by adding 7ml of 0.01M KMnO_4 , to a mixture of 5.0ml phosphate buffer (pH 7.4) and 1ml of 6M H_2SO_4 solution. The spectrophotometer was zeroed with the distilled water.

Calculations

The catalase activity was determined by using:

$$\text{Cat (k) (unit/ml)} = \log \log \left(\frac{S_0}{S_{30}} \right) \times \text{sod (unit/g tissue)}$$

$$\times 10\% \frac{2.3}{t}$$

$$\text{Cat (k) (unit/g wet tissue)} = \frac{\text{catk(unit/ml)}}{\gamma(\text{wet weight coefficient})}$$

$$\Gamma = \frac{\text{Volume of sample used} \times \text{weight of wet tissue}}{\text{total volume of sample aliquot}}$$

2.4.6 DETERMINATION OF TOTAL PROTEIN

Principle

This colorimetric method is a Biuret method which was described by Tietz (1995). Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex.

Assay Procedure

Sample (serum) of 20 μL was added to 1000 μL of Biuret reagent (sodium hydroxide 100 mmol/l Na-K-tartrate 16 mmol/l potassium iodide 15 mmol/l cupric sulphate) and mixed thoroughly. After which, it was left to incubate for 30 min at +25°C and the mixture was read at 546 nm.

Calculation

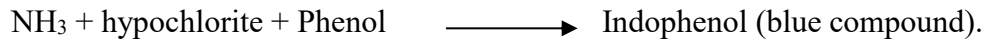
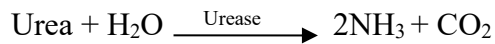
$$\text{Total Protein Conc.} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Standard conc. (mg/dL)}$$

Astandard

2.4.7 DETERMINATION OF UREA ACTIVITY

Principle:

Tietz's description of the Urease-Berthelot approach was followed. In the presence of urease, serum urea is hydrolyzed to ammonia. The resulting ammonia will subsequently combine with hypochlorite and a phenolic chromogen to generate a green complex. This complex can be detected photometrically, and the intensity of the color formed is directly related to the amount of urea in the sample.



Assay Procedure:

The reaction was initiated with 10 μL of sample (serum) and standard, which were combined with 1000 μL of urea working reagent (a mixture of tris-buffer 150 mmol/l, pH 7.6 R1b), the urea assay process was started.

Enzyme Reagent Urease ≥ 10 U/ml GLDH ≥ 2 U/ml NADH 0.26 mmol/l Adenosine-5-diphosphate 3 mmol/l I-oxoglutarate 14 mmol/l). After the combination was combined, it was read at 340 nm for 30 seconds, then again at 1, 2, and 3 minutes.

Calculation

$$\text{Urea conc. (g/dl)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Concentration of standard}$$

2.4.8 DETERMINATION OF CREATININE ACTIVITY

Principle

The estimation of creatinine in serum was done by the method described by Bartels and Bohmer (1972). Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration, present in the serum.

Assay Procedure

Sample (serum) 100 μ L was added to 1 mL of working reagent (picric acid 35 mmol/l R1b. sodium hydroxide 0.32 mol/l), the mixture was mixed and after 30 sec the absorbance A1 of the standard and sample were read. Exactly 2 min later, the absorbance of A2 of standard and sample was read.

The serum's creatinine level was determined to be: $\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times \text{Standard conc. (mg/dl)}$

2.5 DATA ANALYSIS

Experimental data were analyzed using International Business Machines Statistical Package for the Social Science (SPSS) statistics 23 software for windows. The data were presented in means \pm standard error of mean (SEM). One way ANOVA was used in comparing the means followed by Duncan's Multiple Range (DMRT) Post Hoe Test. Student's t test were used to compare means when only two means were involved. $P \leq 0.05$ was taken as statistically significant

CHAPTER THREE

3.1 RESULTS OF THE GC-MS ANALYSIS OF ACTIVATED CHARCOAL OF *TERMINALIA CATAPPA*

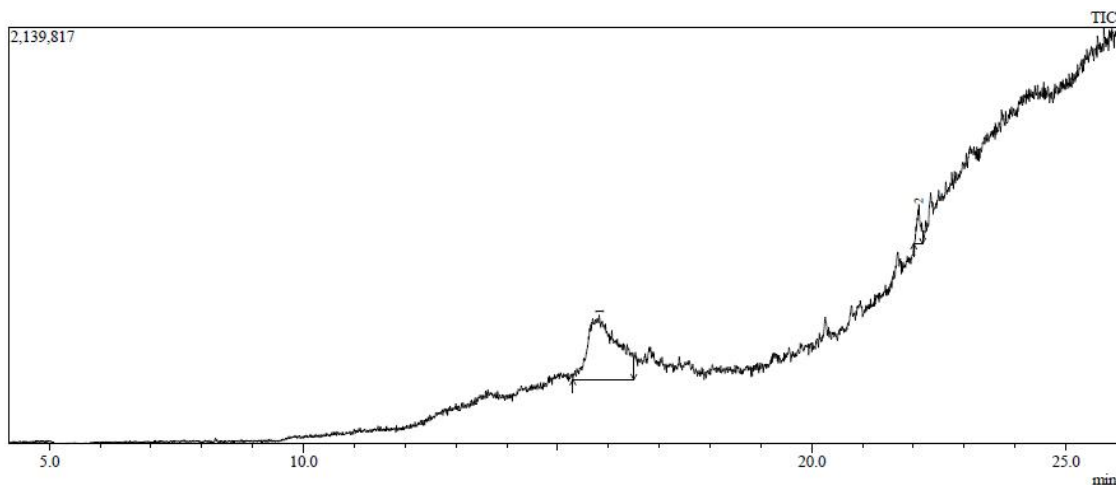


Figure 4: Chromatogram of the GC/MS analysis of the activated charcoal of *Terminalia catappa* showing two distinct peaks.

Each peak represents a distinct compound. The area covered by each peak correlates with the intensities of ions at specific mass to charge m/z values which corresponds to the relative abundance (concentration) of that compound. The higher the ion intensities the larger the peak area, which in turn contributes to a higher area percentage. The identified compound corresponding to each peak with its area % value is presented in Table 2. The total analysis time per injection on the GC-MS system as shown in the chromatogram was 25 minutes.

peak	Retention time	Area	Area%	Height	Height%	A/H	Name
1	15.818	13099460	93.24	331628	62.11	39.50	Methyl 6- beta galactopyranoside
2	22.108	950118	6.76	202277	37.81	4.70	Stigmastan 3,5- diene

Table 2: Phytochemicals detected in activated charcoal of Terminalia catappa by GC-MS

Shows compounds identified with their retention time, area-to-height ratio, and area percentage (%), which corresponds to the relative abundance of a compound. Methyl 6- beta galactopyranoside and Stigmastan 3,5-diene (93.24%, 6.76 %) were identified as the most predominant compounds.

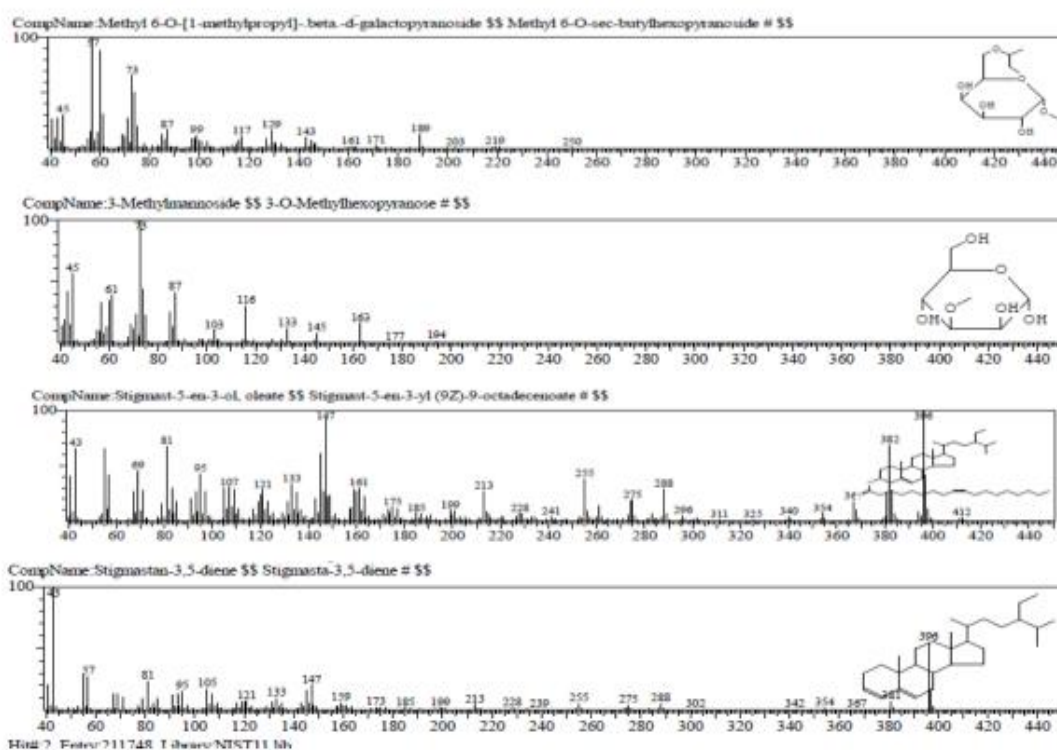
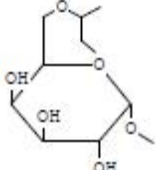


Figure 5: Mass spectrometry of the identified compounds in Activated charcoal

Compound name	Chemical structure	Molecular formula/weight	Biological activity	Reference	Concentration (Area %)
Methyl 6- beta galactopyranoside		$C_{11}H_{22}O_6$ 250	Anti-cancer, anti-viral and anti-diabetes		93.24

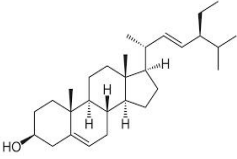
Stigmastan diene	3,5-		C₄₇H₈₂O₂ 396	Anti-diabetic potential. Anti- osteoarthritis, anticancer, anti- inflammatory effect.	Nualkaew, <i>et</i> <i>al.</i>, 2015.	6.76
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Table 3: Biological activity of activated charcoal of Terminalia catappa

The blood drawn from the rat was examined and various test were carried out which includes liver function test, kidney function test, lipid profiling, test for antioxidant activity, test for electrolyte.

3.2 RESULTS OF THE BIOCHEMICAL ASSAY

PARAMETERS	GROUPS			
	1	2	3	4
GPx (U/mg protein)	3.494 ± 0.570	3.206 ± 0.4093	3.232 ± 0.07358	3.408 ± 0.1400
T. Protein (g/dL)	78.650 ± 3.814	75.450 ± 2.405	80.470 ± 8.141	86.590 ± 7.800
GSH (mmol/L)	0.730 ± 0.059	0.655 ± 0.023	0.677 ± 0.03924	0.787 ± 0.039
MDA (mole/mg protein)	0.267 ± 0.044	0.3153 ± 0.028	0.2623 ± 0.019	0.2987 ± 0.029
SOD (U/mg protein)	0.137 ± 0.022	0.126 ± 0.016	0.127 ± 0.003	0.133 ± 0.006
Catalase (U/mg protein)	0.7750 ± 0.126	0.7112 ± 0.091	0.7168 ± 0.016	0.7559 ± 0.031
ALT (U/L)	92.28 ± 2.830	87.31 ± 3.124	90.21 ± 5.232	104.9 ± 5.134
AST (U/L)*10²	2.716 ± 0.232	311.6 ± 0.190	2.725 ± 0.110	3.388 ± 0.319

ALP (U/L)	19.58 ± 0.853	22.59 ± 2.679	22.22 ± 1.738	20.87 ± 0.033
T.CHOL (mmol/L)*10²	2.318 ± 0.1090	3.098 ± 0.079*	2.546 ± 0.044*	2.829 ± 0.015*
HDL (mmol/L)*10²	1.168 ± 0.041	0.634 ± 0.042*	0.859 ± 0.035*	0.612 ± 0.038*
UREA (mmol/L)	51.65 ± 3.964	41.24 ± 4.279	25.50 ± 4.043*	22.57 ± 1.046*
CREA (mmol/L)	85.23 ± 6.540	68.04 ± 7.060	42.07 ± 6.671*	37.24 ± 1.726*
POTASSIUM (mmol/L)	13.19 ± 0.546	10.15 ± 1.124	8.855 ± 0.809*	6.983 ± 1.227*
SODIUM (mmol/L)	21.77 ± 0.9017	16.75 ± 1.854	14.61 ± 1.335	11.52 ± 2.025

Table 4: Activities of the various biochemical parameters assayed.

Values are oxidative stress indices and are expressed as mean ± SEM. Values with asterick (*) are significantly different when compared with group 1 (p < 0.05).

3.2.1 RESULTS OF THE LIVER FUNCTION TEST

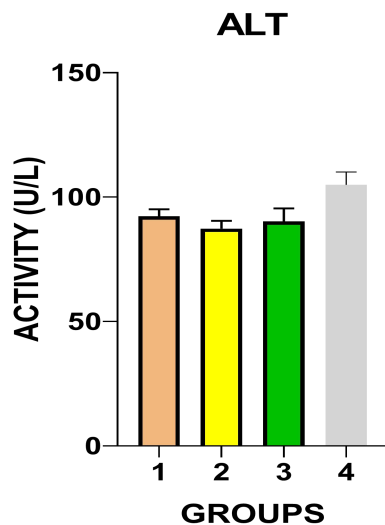


Figure 6: Statistical analysis of the level of ALT.

Rats in Group 3 and 4 (treated with 200mg of Activated Charcoal and 10mg of Atorvastatin Tablet respectively), showed an increase when compared to Rats in Group 2 (induced but not treated). Each column represents the mean \pm SEM values of ALT level.

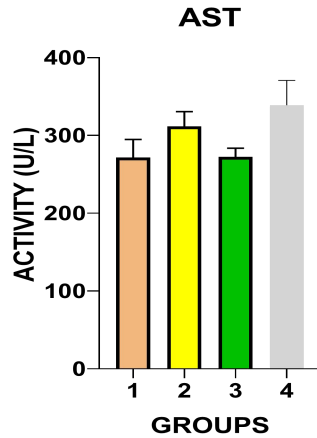


Figure 7: Statistical analysis of the level of AST.

Rats in group 3 (treated with 200mg of Activated Charcoal) showed a decrease when compared to group 2 (induced but not treated) and group 4 (treated with 10mg of Atorvastatin Tablet), which showed an increase in AST level. Each column represents the mean \pm SEM values of AST level.

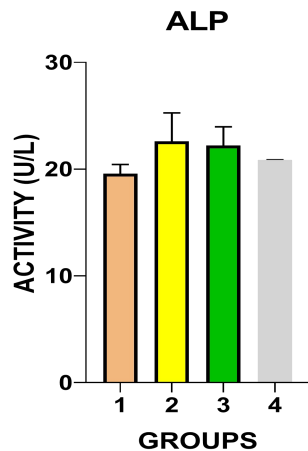


Figure 8: Statistical analysis of the level of ALP

Rats in group 3 (treated with 200mg of Activated Charcoal) and group 4 (treated with 10mg of Atorvastatin Tablet) showed a decrease when compared with group 2 (induced but not treated). Each column represents the mean \pm SEM values of ALP level.

3.2.2 RESULTS OF KIDNEY FUNCTION TEST

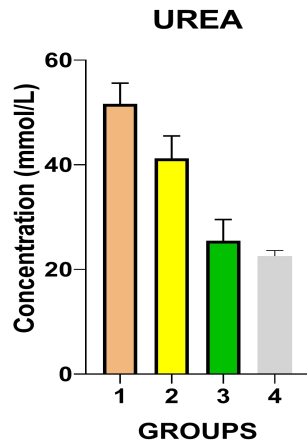


Figure 9: Statistical analysis of the level of Urea.

Rats in group 3 (treated with 200mg of Activated Charcoal) and group 4 (treated with 10mg of Atorvastatin Tablet) showed a significant decrease ($p < 0.05$) in urea level compared to group 2 (induced but not treated). Each column represents the mean \pm SEM values of Urea levels.

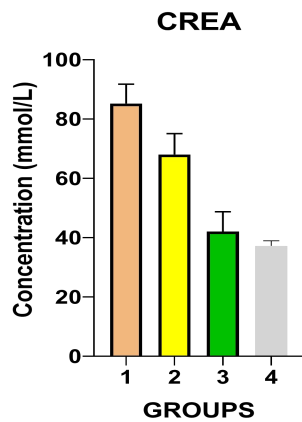


Figure 10: Statistical analysis of the activity of Creatinine.

Rats in group 3 (treated with 200mg of Activated Charcoal) and group 4 (treated with 10mg of Atorvastatin Tablet) showed a significant decrease ($p < 0.05$) in Creatinine activity when compared to group 2 (induced but not treated). Each column represents the mean \pm SEM values of Creatinine activity.

3.2.3 RESULTS OF ELECTROLYTES ACTIVITY

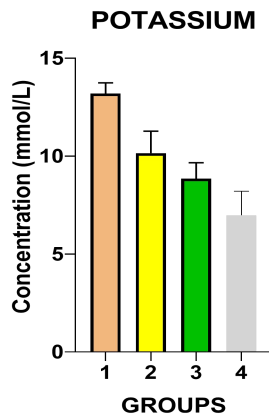


Figure 11: Statistical analysis of the level of Potassium.

Rats in group 3 (treated with 200mg of Activated Charcoal) and group 4 (treated with 10mg of Atorvastatin Tablet) showed a significant decrease ($p < 0.05$) in potassium level when compared to group 2 (induced but not treated). Each column represents the mean \pm SEM values of Potassium levels.

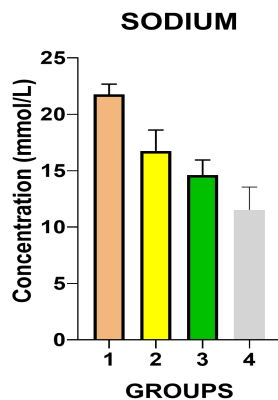


Figure 12: Statistical analysis of the level of Sodium.

Rats in group 3 and 4 (treated with 200mg of Activated charcoal y10mg of Atorvastatin Tablet respectively) showed a significant decrease ($p < 0.05$) in sodium levels when compared to group 2 (induced but not treated). Each column represents the mean \pm SEM values of Sodium levels.

3.2.4 RESULTS OF ANTIOXIDANTS ACTIVITY

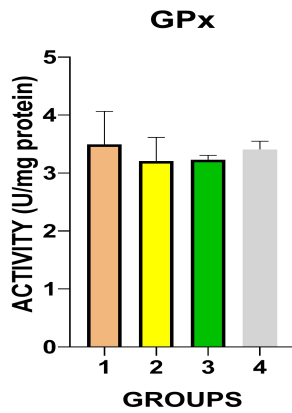


Figure 13: Statistical analysis of the Glutathione Peroxidase (GPx).

Rats in group 3 (treated with 200mg of Activated charcoal) shows a slight increase in Glutathione Peroxidase activity when compared to group 2 (induced but not treated), while group 4 (treated with 10mg of Atorvastatin Tablet) shows an increase in Glutathione Peroxidase activity when compared to group 2 (induced but not treated). Each column represents the mean \pm SEM values of Glutathione Peroxidase activity.

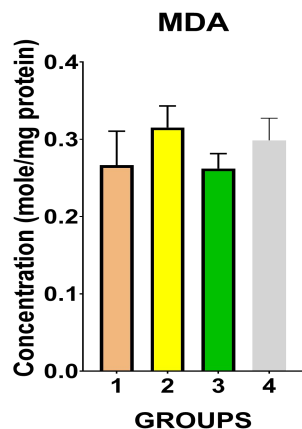


Figure 14: Statistical analysis of the level Malondialdehyde (MDA).

Rats in group 3 (treated with 200mg of Activated charcoal) shows a significant decrease in Malondialdehyde activity when compared to group 2 (induced but not treated), while group 4 (treated with 10mg of Atorvastatin tablet) shows a slight decrease in Malondialdehyde activity when compared to group 2 (induced but not treated). Each column represents the mean \pm SEM values of Malondialdehyde activity.

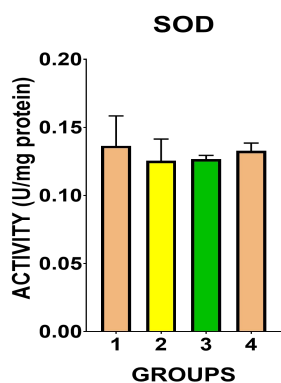


Figure 15: Statistical analysis of the level of Sodium Dismutase (SOD).

Rats in group 3 (treated with 200mg of Activated charcoal) shows almost the level of activity of SOD as group 2 (induced but not treated), while group 4 (treated with 10mg of Atorvastatin tablet) shows a slight increase in SOD when compared to group 2 (induced but not treated). Each column represents the mean \pm SEM values of Sodium dismutase activity.

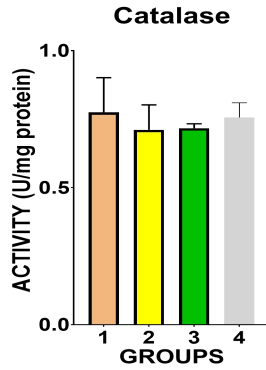


Figure 16: Statistical analysis of the level of Catalase.

Rats in group 3 (treated with 200mg of Activated charcoal) shows almost the same level of activity of Catalase as group 2 (induced but not treated), while group 4 (treated with 10mg of Atorvastatin tablet) shows a slight increase in the level of catalase activity when compared with group 2 (induced but not treated). Each column represents the mean \pm SEM values of catalase activity.

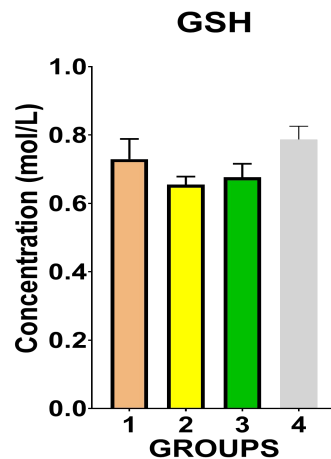


Figure 17: Statistical analysis showing the level of Glutathione reductase (GSH).

Rats in group 3 (treated with 200mg of activated charcoal) showed a slight increase in the level of Glutathione reductase when compared to group 2 (induced but not treated), while group 4

(treated with 10mg of Atorvastatin tablet) showed a significant increase in the level of Glutathione reductase when compared with group 2 (induced but not treated). Each column represents the mean \pm SEM. Values of glutathione reductase level.

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3.2.5 RESULTS OF LIPID PROFILE TEST

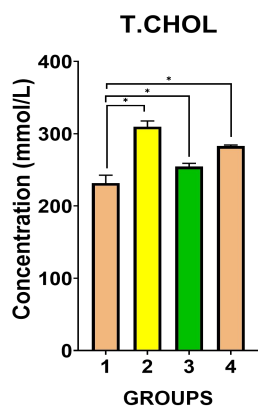


Figure 18: Statistical analysis showing the level of Total cholesterol.

Rats in group 3 and 4 (treated with 200mg of activated charcoal and 10mg of Atorvastatin tablet respectively) showed a significant decrease when compared to group 2 (induced but not treated). Each column represents the mean \pm SEM. Values of total cholesterol level.

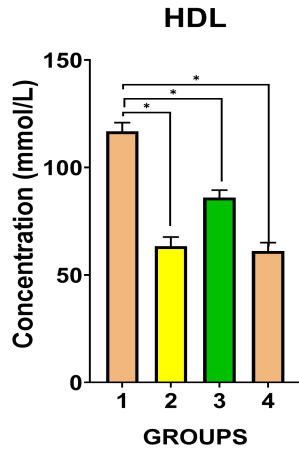


Figure 19: Statistical analysis showing the level of High-density lipoprotein cholesterol (HDL-C).

Rats in group 3 (treated with 200mg of activated charcoal) showed a significant increase in HDL cholesterol level when compared with group 2 (induced but not treated), while group 4 (treated with 10mg of Atorvastatin tablet) show a slight decrease in HDL cholesterol level when compared with group 2 (induced but not treated). Each column represents the mean \pm SEM. Values of high-density lipoprotein cholesterol level.

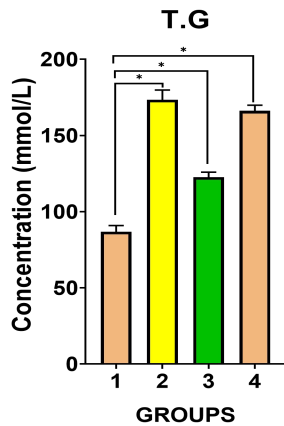


Figure 20: Statistical analysis showing the level of Triglyceride (TG).

Rats in group 3 (treated with 200mg of activated charcoal) showed a significant decrease in triglyceride level when compared with group 2 (induced but not treated), while group 4 (treated

with 10mg of Atorvastatin) showed a significant increase in triglyceride level when compared to group 2 (induced but not treated). Each column represents the mean \pm SEM. Values of triglyceride level.

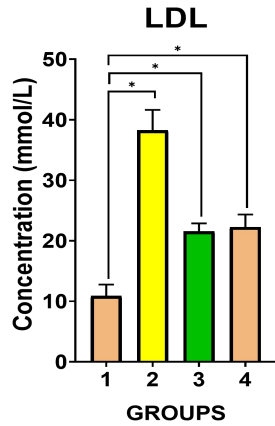


Figure 21: Statistical analysis showing the level of Low-density lipoprotein cholesterol (LDL-C).

Rats in group 3 and 4 (treated with 200mg of activated charcoal and 10mg of Atorvastatin tablet respectively) showed a significant decrease in LDL cholesterol level when compared to group 2 (induced but not treated). Each column represents the mean \pm SEM. Values of low-density lipoprotein cholesterol level.

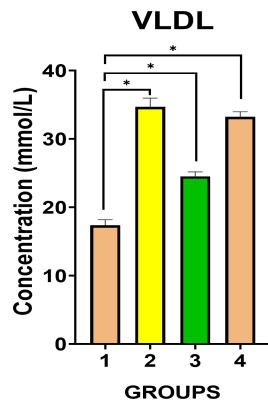


Figure 22: Statistical analysis showing the level of Very low-density lipoprotein (VLDL) cholesterol.

Rats in group 3 (treated with 200mg of activated charcoal) showed a significant decrease in VLDL cholesterol level when compared to group 2 (induced but not treated) while group 4 (treated with 10mg of Atorvastatin tablet) showed a significant increase in VLDL cholesterol when compared to group 2 (induced but not treated). Each column represents the mean \pm SEM. Values of very low-density lipoprotein cholesterol level.

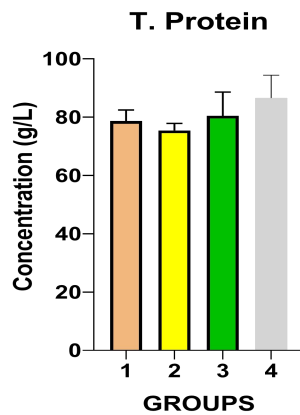


Figure 23: Statistical analysis showing the level of Total protein in group 1, 2, 3, and 4.

Rats in group 3 and 4 (treated with 200mg of activated charcoal and 10mg of Atorvastatin tablet respectively) showed an increase in the level of Total protein when compared to group 2 (induced but not treated). Each column represents the mean \pm SEM. Values of total protein level.

CHAPTER FOUR

4.1 DISCUSSION

This study gives insight on the relationship between hypercholesterolemia and elevated levels of liver enzymes such as Aspartate transaminase (AST), Alanine transaminase (ALT), and Alkaline phosphatase (ALP) and how various treatment of hypercholesterolemia affects the activities of the liver enzymes.

Aspartate transaminase (AST) is an enzyme found in various tissues in the body which includes, the liver, heart, kidney, skeletal muscle and brain. AST plays an important role in amino acid metabolism by catalyzing the reversible transfer of α -amino group between Aspartate and alpha-ketoglutarate, which entails the inter-conversion of Aspartate and α -ketoglutarate to oxaloacetate and glutamate which is essential in both amino acid degradation and biosynthesis processes.

Alanine transaminase (ALT) is an enzyme found in various tissues but its highest concentration is in the liver. ALT catalyzes the transfer of an amino group from L-alanine to alpha-ketoglutarate producing pyruvate and L-glutamate

Alkaline phosphatase (ALP) is an enzyme found in the blood, liver and bones that helps break down proteins. It plays a role in numerous processes including bone metabolism and bile secretion.

In clinical, settings, AST and ALT levels are commonly measured and their ratio (AST/ALT ratio) are used as biomarkers for liver health. Elevated ALP levels can indicate liver damage or bone disorder. Causes of elevated ALP include biliary obstruction due to stones, tumors or liver diseases (Verma, *et al.*, 2012). Elevated AST and ALT levels in the blood may indicate liver

damage or disease with conditions such as hepatitis, cirrhosis and mononucleosis, heart attack, muscle injury and drug overdose.

For AST and ALP activity, rats in group 3 and 4 which were treated with 200mg/kg body weight of activated charcoal and 10mg/kg body weight of Atorvastatin respectively showed a decrease in the levels of AST and ALP when compared with group 2 (negative control) which were induced but not treated

For ALT activity, rats in group 3 showed a slight increase in ALT levels when compared with group 2, also, group 4 showed a significant increase when compared with group 2, this might due to the side effects of the Atorvastatin drug.

Form the results, gotten from the study, it can be inferred that activated charcoal of *Terminalia catappa* has an anti-hyperlipidemic effect; the treatment with activated charcoal reduces the high levels of liver enzymes which were elevated due to hypercholesterolemia.

CONCLUSION

The level of activities of the liver enzymes which Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) was elevated due to the effects of hypercholesterolemia. Treatments with activated charcoal of *Terminalia catappa* showed a decrease in the level AST, ALP and ALT in the blood serum. Other parameters levels like total protein, HDL cholesterol, Sodium dismutase, Catalase, Glutathione Peroxidase, Glutathione reductase showed a significant increase ($p < 0.05$) suggesting the anti-oxidant and anti-hyperlipidemic effect of activated charcoal of *Terminalia catappa*. The level of triglycerides, LDL cholesterol also showed a significant decrease ($p < 0.05$). Results from this study shows that the plant sterol Stigmastan-3, 5-diene from the activated charcoal has an anti-hyperlipidemic effect.

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