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

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BENIN CITY, EDO STATE

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.

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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 ELEGON IREDIA MICHAEL with matriculation number LSC1906489, 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.

DR. S. I. OJEABURU (PROJECT SUPERVISOR)	DATE
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PROF. E. C. ONYENEKE (HEAD OF DEPARTMENT)	DATE

DEDICATION

This work is dedicated to the God Almighty for His unfailing love and grace to carry out this research. Also, to my parents and siblings for their prayers and financial support.

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ABSTRACT

Many therapeutic herbs have the ability to decrease cholesterol. In traditional medicine, activated charcoal is frequently used to cure poisonings, aches, and hangovers. The search for affordable medications is a response to the rising expense and adverse effects of cholesterol-lowering medications. In order to assess the anti-hyperlipidemic effects of the activated charcoal produced from the biochemical transformation of *Terminalia catappa* stem bark, a cholesterol-induced model was utilized in this study. Hypercholesterolemia was induced with 5% cholesterol dissolved in ratio 1:2 of Dimethyl sulfoxide (DMSO) and water given orally as vehicle to all experimental rats. In addition, 5kg of animal lard was mixed with 10kg of animal feed and given to rats for seven days. About 500g of the plant's stem bark were collected from the *Terminalia catappa* tree, dried in the sun, and then allowed to turn completely into charcoal and a highly porous material with a large surface area in an airtight Thermofitcher muffle furnace at 1450°C without oxygen for about an hour. After being reduced to a smooth powder, the resulting charcoal was analyzed. Animals were grouped in 4 which comprise of 3 rats each. All groups except group 1 (control group) were induced with 5% cholesterol orally with gastric gavage and fed with a diet rich in lard. Group 2 (negative control) was left untreated, group 3 and group 4 were treated with 200mg activated charcoal of *Terminalia catappa* and standard drug of 10mg Atorvastatin tablets respectively. GC-MS results show that activated charcoal of *Terminalia catappa* contains 2 compounds which are 93.24% of Methyl 6- beta galactopyranoside and 6.76% of Stigmastan 3,5-diene, which is a plant sterol. Lipid profile parameters except high-density lipoprotein cholesterol (HDL-C) evaluated revealed a significant decrease ($p \leq 0.05$) in low-density lipoprotein (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), total cholesterol (TC), triglyceride (TG) in group 3 when compared to group 2. Parameters evaluated revealed a significant increase ($p \leq 0.05$) in high-density lipoprotein cholesterol (HDL-C), total protein in group 3 when compared to group 2. Antioxidant parameters evaluated an increase in superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx) but the increase is not significant. Data from this study show that activated charcoal of *Terminalia catappa* contains phytochemical compounds that can reduce hypercholesterolemia by adsorbing bile acids.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

The use of medicinal plants for healing dates back as long as humanity. There is substantial proof of the long-standing human link to the hunt for pharmaceuticals in nature from various sources, including written records, preserved monuments, and even the original plant medicines. The many years of fighting diseases have taught us to seek out pharmaceuticals in the bark, seeds, fruit bodies, and other parts of plants. This has led to an awareness of the use of therapeutic plants. Modern research has recognized their potent effects and incorporated a variety of plant-based medications, which have been utilized for millennia by ancient societies, into contemporary pharmacotherapy. Pharmacists and doctors are better equipped to address the issues that have arisen with the expansion of professional services in the facilitation of human existence because they are aware of the notions that have developed around the use of medicinal plants and the evolution of awareness (Petrovska, 2012).

Since ancient times, medicinal plants have been utilized for therapeutic purposes; over the ages, their applications have changed to suit different geographical areas and cultural traditions. For instance, the prevalent medical theory in Central Europe was founded on the ancient Greek philosophical notion of an analogy between microcosm and macrocosm, which offered a structure for the methodical examination of complex illnesses (Dal Cero *et al.,* 2022).

Many therapeutic plants were employed in ancient Egypt and Mesopotamia. Examples include thyme, which was utilized for its expectorant and antiseptic properties, and the opium poppy, which produced morphine in the nineteenth century (Hosseinzadeh *et al.,* 2015). The Bible only

lists five species directly as therapeutic plants: mandrake (Mandragora officinarum), balm of Gilead (Commiphora gileadensis), fig (Ficus carica), and nard (Nardostachys jatamansi). Old Jewish post-Biblical texts describe not one but eighteen therapeutic plants in addition to those found in the Bible. Three of these fifteen plants are indigenous to Egypt, but the most are known throughout Mesopotamia and Egypt. A few medicinal plants, including myrtle, fig, nard, and hyssop, were utilized for a variety of purposes, such as healing sores and serving as an ingredient in anointing oils (Dafni and Bock, 2019).

In traditional Chinese medicine, botanical materials are utilized after being processed, like by stirring them or soaking them in vinegar or wine. A complex, frequently customized cure may be prescribed following a conventional diagnosis. Herbal medicine has a long history in India, where snakeroot is used for its calming properties (Hosseinzadeh *et al.,* 2015).

De materia medica gained a great deal of new medicinal plant knowledge with the discovery of America, including cinamon, ipecacuanha, cacao, and ratanhia (Petrovska, 2012). Among all therapeutic systems, the African traditional healthcare system is arguably the oldest and most diverse due to its core usage of medicinal plants. Traditional healers who prescribe medicinal herbs to the local population are often the most easily accessible, reasonably priced, and often the only viable form of treatment in rural parts of Africa. However, current thorough compilations of potentially useful medicinal herbs from Africa are still few. Traditional African medicine is used to treat and preserve health as well as physical and mental ailments. It is based on the theories, beliefs, and experiences inherent to many cultures (Mahomoodally, 2013).

1.2 *TERMINALIA CATAPPA*

The leaves of the Combretaceous plant *Terminalia catappa* are frequently employed in Southeast Asian folk medicine to cure dermatosis and hepatitis. This species is found throughout the world,

ranging from Australia to Indo-Maleisa. The big, spreading tropical almond tree, *Terminalia catappa*, is currently found in coastal regions across the tropics. The tree can withstand somewhat high salinity in the root zone, strong winds, and salt spray. It grows best in sandy, well-aerated soils that drain readily. The species has historically played a significant role in coastal communities by offering a variety of non-wood goods and services (Mohale *et al.,* 2009). The tree is also well-known for its therapeutic properties, especially in Ayurvedic medicine, where the juice of fresh leaves is used to make a lotion that treats scabies and leprosy and is consumed orally to treat headaches and stomachaches (Mohale *et al.,* 2009; Anand, 2015).

Research on *T. catappa*'s medicinal properties started late, and studies that replicate data every ten years are still being published. To use the plant continuously for the best possible health care, a method must be developed (Anand, 2015). *T. catappa* possesses a variety of bioactive chemicals, including as flavonoids, carotenoids, and phenols, which are responsible for some of its therapeutic qualities, according to phytochemical studies. Its traditional applications have been supported by the discovery that the leaves possess anti-inflammatory, antidiabetic, antioxidant, hepatoprotective, and anticancer properties. It has been demonstrated that corilagin, a tannin present in the tree's leaves and bark, protects against liver damage, which is why *T. catappa* has liver-protective properties (Mohale *et al*., 2009).

The inclusion of many bioactive substances, such as tannins, flavonoids, and saponins, which have been demonstrated to impede the growth of diverse cancer cell lines, is responsible for *T. catappa*'s anticancer effect. Additionally, the plant's antimutagenic properties have been discovered, pointing to a possible function in preventing cancer. (Anand, 2015).

Figure 1: *Terminalia catappa* plant

1.2.1 BOTANICAL CLASSIFICATION OF *TERMINALIA CATAPPA*

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Myrtales

Family: Combretaceae

Genus: Terminalia

Species: catappa

1.3 ACTIVATED CHARCOAL

Activated charcoal is a highly porous carbon with a large surface area that contains an interior network of pores and gaps. As a result of these qualities, activated charcoal is an effective adsorbent that has the ability to physically bind to a wide range of substances.

Toxins that have been consumed are absorbed by the gastrointestinal system and are not absorbed systemically due to the adsorption of activated charcoal. Only toxins in the dissolved liquid phase can be directly absorbed by activated charcoal. When taken orally, activated charcoal operates in the gastrointestinal (GI) tract without being absorbed via the gastrointestinal lumen. When a toxin is recirculated into the gut lumen through enterohepatic recirculation, entero-enteric recirculation through active secretion, or passive diffusion, it comes into touch with activated charcoal if the medication has not yet been absorbed from the gastrointestinal lumen. The equilibrium between the free toxin and the activated charcoal/toxin complex is the basis for the adsorption of poisons using activated charcoal. It is possible for the toxin to desorb from the activated charcoal. However, the balance is changed in favor of the activated charcoal/toxin combination when sufficient amounts of activated charcoal are present (Silberman *et al.,* 2023).

Toxins are best adsorbed by activated charcoal when they are not ionized. Adsorption of polar, water-soluble compounds is comparatively less probable. Activated charcoal's pharmacologic properties allow it to effectively absorb nonpolar, poorly water-soluble organic poisons (Chacko and Peter, 2019). Keep in mind that because these materials are polar, activated charcoal cannot successfully adsorb alcohols, metals like iron and lithium, electrolytes like magnesium, potassium, or sodium, or acids or alkalis.

1.4 HYPERCHOLESTEROLEMIA

One type of lipid that is necessary for the body to function normally is cholesterol. It's a substance that looks like wax and is found in body cells and circulation. Cholesterol is necessary for several physiological processes, including the production of bile acid, vitamin D, steroid hormones (such as testosterone and estrogen), and membrane preservation. Meals derived from animals, such as meat, eggs, and dairy products, as well as food produced by the liver, are natural dietary sources of cholesterol. (Ten Kate *et al.,* 2015). It is believed that hypercholesterolemia, a term used to describe abnormally elevated levels of cholesterol in the blood, particularly low-density lipoprotein (LDL) cholesterol, poses a serious risk for the development of cardiovascular diseases (CVD).

As a result, it is the primary cause of death globally and a developing health concern. Plasma cholesterol levels are largely controlled by the metabolism of both endogenous (within the cell) and extracellular (exogenous) cholesterol. It is therefore advisable to avoid consuming too much cholesterol, stop its synthesis from starting, and promote its elimination in order to preserve normal body processes (Li and Chiang, 2009; Nishina and Freedland, 1990).

1.5 JUSTIFICATION OF STUDY

The capacity of activated charcoal to adsorb cholesterol and bile acids containing lipid in the colon and limit their absorption provides a rationale for investigating its use in the treatment of elevated cholesterol levels.

In controlled experiments, activated charcoal has been demonstrated to lower total and LDL cholesterol levels; the maximum benefit was observed at a dose of 40 grams per day for three weeks. The binding of bile acids, which in turn promotes cholesterol utilization in fresh bile acid synthesis by the liver, is the mechanism by which activated charcoal lowers cholesterol levels.

Additionally, in animal models like wistar rat of hypercholesterolemia, activated charcoal has been demonstrated to enhance the histology of the aorta by reducing endothelial cell erosion and inflammatory cells. It has been established that activated charcoal lowers HDL levels in animal models of hypercholesterolemia; a dose of 4.950 mg/kg body weight is effective (Roosdiana *et al.,* 2019).

When treating hypercholesterolemia, activated charcoal is typically thought to be less expensive than statin medications. A family of drugs called statins is commonly used to treat high cholesterol because it prevents the liver from producing cholesterol. However, because statins can be costly, some patients might not be able to afford them (Husain *et al.,* 2022).

Conversely, activated charcoal is a naturally occurring material that has been utilized for centuries to treat a variety of health issues, including hypercholesterolemia. It is frequently less expensive than statin medications and is generally regarded as safe and efficient in lowering cholesterol levels (Sicras-Mainar *et al.,* 2018). In order to stop cholesterol from being absorbed into the bloodstream, activated charcoal binds to cholesterol in the gastrointestinal tract (Zodda *et al.,* 2018).

In research comparing the cost-effectiveness of medications for hypercholesterolemia in low- and middle-income nations, activated charcoal treatment shown to be more economical for primary prevention than statin-only therapy. Furthermore, a comprehensive analysis of the data about the financial viability of hypercholesterolemia medications in low- and middle-income nations revealed that polypill therapy—which may involve activated charcoal—is typically more economical than statin-only therapy (Husain *et al.,* 2022).

Since activated charcoal is expelled through feces rather than being absorbed by the body, it is typically regarded as safe and non-toxic (Roosdiana *et al.,* 2019). For patients who might be worried about the adverse effects of conventional cholesterol-lowering drugs, this makes it a more

desirable option. In the colon, activated charcoal can absorb cholesterol before it enters the bloodstream, protecting the aorta from injury.

1.6 AIM OF STUDY

The aim of this study is to evaluate the anti-hypercholesteric effect of activated charcoal of *Terminalia catappa* stem bark on cholesterol induced hypercholesterolemia.

1.7 OBJECTIVE OF STUDY

The objectives of the study include evaluation of

1. Superoxide dismutase (SOD) activity
2. Catalase activity
3. Total protein level
4. Glutathione (GSH) level
5. Glutathione peroxidase (GPx) activity
6. Malondialdehyde (MDA) level
7. Alanine amino transferase (ALT) level
8. Aspartate amino transferase (AST) level
9. Alkaline phosphatase (ALP) level
10. Creatinine level
11. Urea level
12. Sodium activity
13. Potassium activity

1.8 LITERATURE REVIEW

Numerous research has examined the possible anticholesteric effects of activated charcoal, indicating that it may be able to lower cholesterol and shield the aorta from injury in animal models of hypercholesterolemia (Zhang *et al*., 2022; Roosdiana *et al.,* 2019).

Cholesterol is a fat-like substance that is waxy and produced in the liver and present in all bodily cells as well as in the circulatory system which is important for optimal health. Cholesterol is a necessary building block of cell membranes and the starting point for the production of bile acids and steroid hormones. This chemical is partly synthesized in the membrane where the relevant enzymes, substrates, and products are frequently quite hydrophobic. Due to its link to cardiovascular diseases, which are among the world's most common causes of mortality, cholesterol has become more significant over the past 50 years. Given the present demand for novel medications that can regulate blood cholesterol levels, it's critical to comprehend the body's synthesis of cholesterol and recognize the key enzymes in this process (Cerqueira *et al.,* 2016).

1.9 METABOLISM OF HYPERCHOLESTEROL IN RAT

The intricate interaction of nutritional, metabolic, and genetic factors that affect the animals' cholesterol levels and metabolism results in hypercholesterolemia in rats. High-cholesterol meals have been used in studies to cause hypercholesterolemia in Wistar rats, offering important insights into the metabolic characteristics of this illness (Cunha *et al.,* 2021; Sheyla *et al.,* 2005).

Studies have indicated that the metabolism of cholesterol is compromised in older rats relative to their younger counterparts, as seen by the higher levels of blood cholesterol in these animals. Furthermore, studies have emphasized the role of cholesterol in cell membrane construction,

hormone production, and bile salt formation, and the need to comprehend cholesterol metabolism in humans in order to draw comparisons with animal models like rats.

Given that elevated cholesterol is a significant risk factor for disorders like atherosclerosis, understanding the pathobiology of hypercholesterolemia in rats is essential for researching cardiovascular diseases. Wistar rats especially are excellent models for studying hypercholesterolemia and investigating novel treatment modalities for this illness (Cunha *et al.,* 2021).

1.9.1 ROLE OF LOW-DENSITY LIPOPROTEIN (LDL) AND HIGH-DENSITY LIPOPROTEIN (HDL) IN CHOLESTEROL METABOLISM IN RAT

Both high-density lipoproteins (HDL) and low-density lipoproteins (LDL) are essential for the metabolism of cholesterol in rats. HDL is recognized to provide protection against cardiovascular illnesses, but LDL is frequently linked to an increased risk of these conditions (Gwynne and Hess, 1980; Márcia *et al.,* 2002).

HDL's function in controlling the amount of cholesterol has been demonstrated in the context of cholesterol metabolism, where it has been demonstrated to be more successful than LDL in reducing cholesterol synthesis in rat adrenal glands (Gwynne and Hess, 1980). Furthermore, age-related alterations in the breakdown of plasma cholesterol in rats and primates indicate that HDL cholesterol rises while LDL cholesterol falls, pointing to a change in the equilibrium of cholesterol transport and metabolism in these species (Lacko and Davis, 1979).

Studies on the impact of dietary cholesterol on lipid metabolism in rats have also been conducted; elevated serum levels of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), together with disrupted triglyceride metabolism, have been observed in response to high cholesterol diets (Wang *et al*., 2010). This can lead to the development of hepatic steatosis, a form

of non-alcoholic fatty liver disease, suggesting the possible influence of cholesterol from food on rat lipid metabolism.

1.10 ROLE OF ACTIVATED CHARCOAL IN HYPERCHOLESTEROLEMIA TREATMENT

Carbon that has undergone treatment to enhance its surface area and porosity is known as activated charcoal. Toxins and substances in the gastrointestinal tract are frequently absorbed using it in medicine. Activated charcoal serves to treat hypercholesterolemia by binding to bile acids and cholesterol in the stomach and inhibiting their absorption into the bloodstream.

Activated charcoal works by attracting detrimental chemicals, such as cholesterol, to its surface and hindering their absorption from the digestive system. This is the basis of its method of action. Particle size, solubility, ionization, pH, and stomach contents of the material are some of the variables that affect this adsorption mechanism (Zellner *et al.,* 2019).

Activated charcoal at a dose of 4.950 mg/kg body weight was found to be beneficial in enhancing aortic histology and keeping HDL levels from declining in a study conducted on hypercholesterolemia rat models. Through internal diffusion, cholesterol moves from a boundary layer to the surface of the adsorbent, allowing activated charcoal to absorb cholesterol. An innocuous and efficient substance for preventing hypercholesterolemia, activated charcoal is expelled along with feces in their unaltered state because it is not absorbed by the colon or digestive tract (Roosdiana *et al.,* 2019).

In individuals with hypercholesterolemia, numerous studies have examined the dose-response relationship of activated charcoal in lowering cholesterol and contrasted its effects with those of other drugs, such as cholestyramine. The effects of activated charcoal on hypercholesterolemia were investigated in a study that was published in the European Journal of Clinical Pharmacology.

Patients in the cross-over research consumed different amounts of activated charcoal every day for three weeks. The results demonstrated a dose-dependent reduction in serum total and low-density lipoprotein cholesterol, with maximal reductions of 29% and 41%, respectively. Moreover, activated charcoal raised the ratio of HDL to LDL cholesterol, suggesting a positive change in cholesterol levels (Neuvonen *et al.,* 1989).

Additionally, a clinical study investigating the use of activated charcoal in the treatment of hypercholesterolemia was reported in The Lancet. Activated charcoal was administered to seven hypercholesterolemia patients at a dose of 8 g three times per day for a duration of four weeks. The results of the study showed that activated charcoal is effective in lowering cholesterol and offered important new information about its potential therapeutic uses for treating hypercholesterolemia (Kuusisto *et al.,* 1986). The effect was accompanied by a notable increase (up to 300–700%) in serum lathosterol and delta-8-lathosterol concentrations, as well as a modest elevation ($P < 0.05$) in serum squalene and desmosterol concentrations. Using activated charcoal had no effect on the levels of two plant sterols, campesterol and β-sitosterol, or just a minor reduction. Substantial negative correlations were found between the drop in blood cholesterol content and the levels of lathosterol and delta-8-lathosterol, while significant positive correlations were found between the levels of cholestanol and β-sitosterol, showing that the use of activated charcoal promoted the synthesis of cholesterol (Neuvonen *et al.,* 1989).

Research has indicated that activated charcoal possesses anticholesteric properties. It has been proven to lower cholesterol and shield the aorta from harm in animal models of high cholesterol. Activated charcoal's method of adsorption is regulated by multiple elements, and since it is not absorbed by the intestine or digestive tract, it is a safe and efficient material for minimizing hypercholesterolemia.

Figure 2: Pictorial representation of activated charcoal

1.10.1 ANTIHYPERCHOLESTERIC EFFECT OF *TERMINALIA CATAPPA*

Researchers have looked into the medicinal plant *Terminalia catappa* for possible antihypercholesterolemic effects. Rats with diabetes-induced dyslipidemia have demonstrated reduced cholesterol levels when exposed to activated charcoal generated from *Terminalia catappa* (Iheagwam *et al.,* 2023). Researchers examined the mitigating effect of *Terminalia catappa* leaf aqueous extract (TCLAE) on diabetes-induced dyslipidemia in type 2 diabetic rats in a study that was published in gene expression. The results of the study showed that TCLAE lowered the levels of low-density lipoprotein-triglyceride, low-density lipoprotein-cholesterol, weight loss, plasma insulin, alanine transaminase, bilirubin, cholesterol, and triglycerides caused by diabetes. Additionally, aberrant cardiovascular indicators were reduced, and high-density lipoprotein cholesterol levels were dramatically raised (Iheagwam *et al.,* 2023).

In a different study, it was discovered that *Terminalia catappa* leaf extract increased antioxidant activity and lipid metabolism in a Caenorhabditis elegans model, hence extending lifetime (Kim *et al.,* 2023). This shows that *Terminalia catappa* could potentially be useful as a medication to lower blood cholesterol.

1.11 BIOMARKERS USED TO CHECK FOR HYPERCHOLESTEROL

A biological molecule that is present in blood or other bodily fluids or tissues and serves as an indicator of a disease, condition, or aberrant process is called a biomarker, according to the National Cancer Institute. To determine how well the body reacts to an illness or condition's therapy, a biomarker may be utilized. Also known as a signature molecule or molecular marker. A biomarker's basic definition is surprisingly straightforward: "A measurable trait that serves as a marker for pathogenic processes, normal biological processes, or reactions to an exposure or intervention (Califf, 2018).

The risk of stroke and cardiovascular disease can both be markedly increased by hypercholesterolemia. The use of biomarkers in the detection and tracking of hypercholesterolemia is crucial. The biomarkers used to analyze level of lipid to detect if there is high level of bad cholesterol includes low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), total protein, total cholesterol (TC). Other test can be used to detect the level of cholesterol in various organ of the body like liver function test which include aspartate amino transferase (AST), alanine amino transferase (ALT), total bilirubin. Kidney function test that involves analyzing biomarker like serum creatinine.

1.11.1 LOW-DENSITY LIPOPROTEIN (LDL) CHOLESTEROL

Hypercholesterolemia, a disorder marked by elevated blood levels of lipids and lipoproteins, is well-known to be biomarked by low-density lipoprotein cholesterol (LDL-C) (Babakr *et al.,* 2023). A substantial risk factor for heart disease, atherosclerosis, along with other pathological illnesses is hypercholesterolemia (Held *et al.,* 2022). In numerous earlier studies, the relationship between dyscholesterolemia and cardiovascular disease has been clearly established (Močnik and Marčun, 2023).

It has been proposed that oxidized low-density lipoproteins, or Ox-LDL-C, are atherogenic particles and are crucial in the onset of atherosclerosis. A prothrombotic state can arise as a result of endothelial dysfunction caused by oxidized low-density lipoprotein cholesterol (LDL-C), a pro-inflammatory pathogenic particle. Levels of oxidized LDL-C in plasma could be a valuable diagnostic for heart disease prognosis. According to studies, individuals with elevated cholesterol levels have higher blood levels of LDL-C, triglycerides, and cholesterol and are more susceptible to thrombosis. Prior research has demonstrated that prothrombotic states are facilitated by oxidized lipoproteins, including oxidized low-density lipoproteins. It has been demonstrated that oxidized low-density lipoprotein cholesterol (LDL-C) is a pro-inflammatory pathogenic particle that causes endothelial dysfunction, a condition linked to the onset of atherosclerosis.

Measuring low-density lipoprotein cholesterol (LDL-C) is essential for both hyperlipidemia diagnosis and treatment. All persons 20 years of age or older should have their LDL cholesterol levels measured at least once every four to six years, according to the American College of Cardiology and the American Heart Association. According to the National Cholesterol Education Program (NCEP) guidelines, people without a history of cardiovascular disease should have LDL cholesterol levels of no more than 100 mg/dL, while people with a history of diabetes or cardiovascular disease should have levels no more than 70 mg/dL (Babakr *et al.,* 2023).

1.11.2 HIGH-DENSITY LIPOPROTEIN CHOLESTEROL (HDL-C)

A vital biomarker for detecting hypercholesterolemia, a disorder marked by high blood levels of lipids and lipoproteins, is high-density lipoprotein cholesterol (HDL-C). Due to the fact it helps eliminate excess cholesterol from the bloodstream and prevents the accumulation of plaque in the arteries, HDL cholesterol is widely recognized for its preventive effect against cardiovascular disease. (Hafiane and Genest, 2015).

Studies have demonstrated an inverse relationship between HDL cholesterol (HDL-C) levels and the risk of atherosclerotic cardiovascular disease, particularly coronary artery disease (Močnik and Marčun, 2023). As a significant biomarker of cardiovascular risk, HDL cholesterol mass is important, but research indicates that HDL function may be a better treatment target because simply measuring HDL cholesterol may not be enough to prevent atherosclerosis (Hafiane and Genest, 2015).

HDL-C is involved in more than only the transportation of cholesterol; it also has anti-inflammatory, antioxidant, and antithrombotic qualities. The evaluation of HDL-C function is difficult due to the heterogeneity of HDL-C particles, which differ in size, lipid and protein contents, and surface charge. Determining the most efficient clinical techniques for measuring HDL-C activity is essential to comprehending HDL-C's function as a cardiovascular risk biomarker. For clinical usage, laboratory assays that detect HDL-C function accurately need to be designed, validated, and scaled up to a high throughput. The development of a single measurement is difficult due to the complicated pleiotropic effects of HDL-C, which emphasizes the need for reliable and straightforward analytical techniques to precisely evaluate HDL-C activity. (Navab *et al.,* 2009).

1.11.3 TOTAL CHOLESTEROL (TC)

An accepted biomarker for determining the risk of atherosclerosis and cardiovascular disorders is total cholesterol. It is frequently used in clinical practice to track the lipid profile and assess how well cholesterol-lowering therapies are working (Chou *et al.,* 2020). Total cholesterol is a crucial biomarker in the diagnosis and treatment of hypercholesterolemia since high levels are linked to an increased risk of atherosclerosis and cardiovascular diseases (Babakr *et al.,* 2023).

A different study that was published in the Journal of Clinical Lipidology examined how various cholesterol-lowering medications affected the overall cholesterol levels of hypercholesterolemia patients. The results of the trial showed that while statins like simvastatin had a little impact on total cholesterol levels, ezetimibe, an inhibitor of cholesterol absorption, considerably lowered total cholesterol levels when compared to a placebo (Gaggini *et al.,* 2022).

Total cholesterol levels have been used as a biomarker to assess the efficacy of different medications in the context of treating a high level of cholesterol. For instance, among patients with hypercholesterolemia, ezetimibe plus simvastatin significantly lowered total cholesterol levels when compared to simvastatin monotherapy, according to research published in the Journal of the American College of Cardiology Močnik and Marčun, 2023).

1.11.4 TRIGLYCERIDES (TG)

Triglycerides (TGs) are a kind of fat which are transported by chylomicrons and very low-density lipoproteins (VLDLs) in the blood. Elevated levels of TG are linked to a higher chance of atherosclerosis and cardiovascular disorders (CVD) (Talayero and Sacks, 2011).

According to epidemiologic and clinical research, high TG levels are a biomarker of the risk of CVD (Budoff, 2016). For many years, hypertriglyceridemia, or elevated TG levels, has been linked to an increased risk of cardiovascular disease in people. Only lately, however, have data from observational and genetic studies, along with data from fundamental research, pointed to triglyceride-rich lipoproteins (TRLs) as potential causative agents of atherosclerotic cardiovascular disease (CVD). Remarkable discoveries emphasizing the significance of TRL-derived lipolytic products (also known as remnant lipoprotein particles, or "RLPs"), as opposed to plasma TG levels, have surfaced as crucial elements in the onset and advancement of atherosclerosis (Padro *et al.,* 2021).

Pharmacological approaches to lower plasma TG levels have centered on lowering hepatic VLDL synthesis and raising TRL clearance through LPL activity regulation. In order to lower TG levels, the 2019 ESC/EAS Guidelines for Management of Dyslipidemias advise considering the use of fibrates and omega-3 fatty acids. It's possible that there is more complexity involved in the association between TG level variations and decreased CVD risk. The effectiveness of Omega-3 Fatty Acids in lowering CVD occurrences has been demonstrated by the recently completed multicenter placebo-control trial REDUCE-IT, which involved 8179 high-risk patients based on documented CVD or the presence of CVD risk factors. It was not possible to establish a statistical correlation between the decline in occurrences and the decline in triglyceride levels (Padro *et al.,* 2021). Therefore, in order to more clearly determine if reducing remnant lipoprotein particles (RLPs) and triglycerides lowers the risk of CVD, more extensive clinical intervention trials are required.

CHAPTER TWO

2.1 MATERIALS AND METHODS

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 EQUIPMENTS

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), Bunsen burner.

2.1.3 INSTRUMENT USED

AGILENT 720 ICP-OES

2.1.4 CHEMICALS AND REAGENTS

- Extracts/samples
- Distilled Water

2.2 METHOD USED

2.2.1 SAMPLE COLLECTION

The stem bark of *Terminalia catappa* were be 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 specimen with Voucher number were deposited in the same Department, while their stem bark was dried under sun at 34-36°C for 14 days.

2.2.2 PREPARATION OF CHARCOAL (CABONIZATION PROCESS)

Carbonation of chopped *Terminalia catappa* stem bark into charcoal was carried out in the laboratory with a Thermo fisher 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

2.3 GC-MS ANALYSIS

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 1D 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.

2.3.1 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.

2.4 ANIMALS

Wistar-Albino rats (University of Benin, Benin city, Edo state, Nigeria) weighing 170 - 220g, were used. The animals were housed at room temperature (20-25^{0}C), with normal rat feed and water. The rats were grouped into four different groups which are;

GROUP	GROUP NAME	NUMBER OF RATS
1	Positive control (normal rat)	3
2	Negative control (induced with 5% cholesterol + not treated)	3
3	Induced with 5% cholesterol + treated with 200mg/activated charcoal of *Terminalia catappa*	3
4	Induced with 5% cholesterol + treated with 10mg Atorvastatin Tablets	3

Table 1: Grouping of albino-wistar rat used in carrying out the experiment.

2.5 HYPERCHOLESTEROL INDUCTION AND TREATMENT PROTOCOL

The control group were given normal rat feed and observed under normal conditions. All groups except group 1 (normal rat) were all fed with 5kg of animal lard mixed with 10kg of animal feed to increase their cholesterol levels, and they were also induced with 5% cholesterol dissolved in distilled water and transported with the aid of a vehicle known as dimethyl sulfoxide (DMSO) using a gastric gavage for seven days. Group 1was given only the vehicle solution (Dimethyl sulfoxide and distilled water). Right after the induction, the mixing of lard with the feed was also stopped, treatment was administered. Group 3 and 4 were treated and group 2 was left untreated. Group 3 was treated with 200mg/ activated charcoal of *Terminalia catappa* while group 4 was treated with 10mg/ Atorvastatin Tablets. The treatment lasted for fourteen days.

2.5.1 ASSESSMENT OF HYPERCHOLESTEROL EFFECT

Rats were sacrificed after 24 hours following the treatment that lasted for fourteen days in the two groups. Under chloroform, the rats were sacrificed by cervical decapitation. Blood was drawn from the heart. The kidney, liver and heart were also harvested. The harvested organs were stored in formalin for histological examination. Two sets of blood were drawn one for hematological examination stored in EDTA tube and the other blood stored in heparin tube.

2.6 BIOLOGICAL ASSAYS

As directed by the producers, the following bioassays were performed utilizing specialist kits and analytical grade chemicals/reagents.

2.6.1 LIVER FUNCTION TEST

The impact of the compounded diets on the liver was evaluated using the following tests. These are:

2.6.1.1 ESTIMATION OF ALKALINE PHOSPHATASE (ALP) ACTIVITY

PRINCIPLE:

The Henry method was used to calculate the amount of alkaline phosphatase in serum. P-nitrophenylphosphate, the colorless organic phosphate ester substrate, is catalytically hydrolyzed in this reaction to produce p-nitrophenol and phosphate, which have a yellow color. An alkaline pH of 10.3 is required for this process. Over a predetermined period of time, the system tracks the rate of change in absorbance at 405 nm. The pace at which absorbance changes is directly proportional with serum ALP activity.

P-nitrophenylphosphate + $H_2O \xrightarrow{ALP}$ Phosphate + P-nitrophenol.

PROCEDURE:

A 10 µL sample (serum) was combined with 500 µl of working reagent, mixed, and the reading was taken at 405 nm. Readings were conducted every one to three minutes after the first absorbance was determined.

Manual Calculation of ALP: U/l = 2760 x $\triangle$Absorbance at 405 nm/min.

2.6.1.2 ESTIMATION OF ALANINE AMINOTRANSFERASE (ALT) ACTIVITY

PRINCIPLE:

Reitman and Frankel's technique describes how the ALT catalyzes the reversible transamination of L-alanine and α-oxoglutarate to L-glutamate and pyruvate in the process. After that, pyruvate is converted to lactate in the presence of lactate dehydrogenase, and NADH is simultaneously oxidized to NAD^+. Monitoring is done at a defined time interval to see how quickly the absorbance changes at 340 nm. The sample's ALT activity is directly correlated with the rate at which absorbance changes.

α-oxoglutarate + L-alanine $\xrightarrow{ALT}$ L-glutamate + Pyruvate

Pyruvate + NADH + H^+ $\xrightarrow{LD}$ L-lactate + NAD^+

PROCEDURE

1 mL of working reagent was combined with 100 µl of sample (serum) that was pipetted into a test tube. Readings were obtained after 1, 2, and 3 minutes, respectively, after the initial absorbance was determined instantly.

To calculate for serum ALT Concentration: U/l = 1746 x △Absorbance at 340 nm/min

2.6.1.3 ESTIMATION OF ASPARTATE AMINOTRANSFERASE (AST) ACTIVITY

PRINCIPLE

In the presence of AST, α-oxoglutarate combines with L-aspartate to generate L-glutamate and oxaloacetate. This was carried out in compliance with the indicator reaction approach, which uses oxaloacetate to determine NADH consumption kinetically.

α -oxoglutarate + L-aspartate $\xrightarrow{AST}$ L-glutamate + oxaloacetate

oxaloacetate + NADH + H^+ $\xrightarrow{MDH}$ L-malate + NAD^+

PROCEDURE:

Pipetting 100 µl of serum into a test tube, combine it with 1 mL of working reagent. After one minute, the initial absorbance was measured. Subsequent readings were made at one, two, and three minutes.

To calculate for serum AST Concentration: U/l = 1746 x△ A 340 nm/min

2.6.1.4 TOTAL PROTEIN

PRINCIPLE

Tietz described the Biuret method, which is this colorimetric technique. An alkaline medium containing cupric ions interacts with protein peptide links to generate a colored complex.

PROCEDURE:

After adding 20 µL of the sample (serum) to 1000 µL of the Biuret reagent (potassium iodide, 15 mmol/l cupric sulphate, and sodium hydroxide, 100 mmol/l Na-K-tartrate), the whole thing was well mixed. After that, the mixture was measured at 546 nm and it was allowed to incubate for 30 minutes at +25°C.

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

2.6.2 KIDNEY FUNCTION TEST

The kidney's functioning was determined by measuring creatinine and urea.

2.6.2.1 UREA

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.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{hypochlorite} + \text{Phenol} \longrightarrow \text{Indophenol (blue compound).}$$

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.

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

2.6.2.2 CREATININE

PRINCIPLE:

The technique outlined by Bartels and Bohmer was used to estimate the creatinine in serum. A colored complex is formed when creatinine and picric acid combine in an alkaline solution. The concentration of creatinine in the serum is directly proportional with the amount of complex that forms.

PROCEDURE:

After mixing 1 mL of working reagent (sodium hydroxide, 0.32 mol/l, and picric acid, 35 mmol/l R1b) with 100 µL of sample (serum), the mixture was left for 30 seconds, and the absorbance A1 of the sample and standard were measured. Two minutes later, the absorbance of A2 in the sample and standard was measured.

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

2.6.3 TEST FOR ANTIOXIDANT

The antioxidant activity was tested by analyzing the activity of catalase and superoxide dismutase.

2.6.3.1 CATALASE

PRINCIPLE

The catalase activities were calculated using the Cohen method. When considerable quantities of H_2O_2 are generated, catalase is used to eliminate it. Catalase estimate was performed with the 1970 method by Cohen. Catalase was discovered by measuring the rate at which hydrogen peroxide oxidized or vanished after the enzyme-containing material was added. There is an immediate correlation between the amount of hydrogen peroxide that decomposes and the enzyme concentration in the sample. The hydrogen peroxide product is first quantified by reacting it with excess potassium permanganate (vi) ($KMnO_4$), and then measuring the remaining $KMnO_4$ using spectrophotometry.

$$2H_2O_2 \ \ catalase \rightarrow \ 2H_2O + O_2$$

PROCEDURE

A test tube was filled with 0.5 ml of the sample, and another ice-cold tube was filled with 0.5 ml of distilled water to serve as a blank. The reaction was started by adding 5 ml of cold 30 mm H_2O_2 phosphate buffer (pH 7.4) successively at predetermined intervals. By inversion, they were mixed thoroughly. The reaction was successively halted at the same predetermined interval after precisely three minutes by swiftly adding 1 ml of 6M H_2SO_4 and fast mixing by inversion. One by one, the test and blank tubes were filled with 7 ml of 0.01 m $KMnO_4$, which was then mixed twice by inversion. Upon mixing, the absorbance was measured in 30 to 60 seconds at 480 nm. The reference was made by combining 1 milliliter of 6M H2SO4 solution with 5.0 milliliters of phosphate buffer (pH 7.4) and 7 milliliters of 0.01 milliliters of $KMNO_4$. The distilled water was used to zero the spectrophotometer.

CALCULATIONS:

The catalase activity was determined by using:

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

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

2.6.3.2 SUPEROXIDE DISMUTASE (SOD)

PRINCIPLES

Superoxide dismutases are metalloproteins that help convert the free radical superoxide into H_2O_2 and molecular oxygen. Glutathione peroxidase, the catalase enzyme, subsequently reduces hydrogen peroxide to water.

Both prokaryotes and eukaryotes have these SODs. They are present in several cellular sites, including the external membrane (Cu, Zn-SOD or SOD3), the mitochondrial matrix and inner membrane (Mn-SOD or SOD2), the cytoplasm, and the mitochondrial intermembrane (Cu, Zn-SOD or SOD2).

PROCEDURES

Before equilibrating, 0.2 ml of liver homogenate was combined with 2.5 ml of 0.05 m carbonate buffer (PH 10.2). To initiate the reaction, 0.3 ml of freshly prepared 0.03 mm epinephrine was introduced as a substrate. The solution was mixed by inversion. The reference tube included 2.7 ml of carbonate buffer and 0.3 ml of epinephrine, while the blank tube contained 2.5 ml of carbonate buffer, 0.2 ml of distilled water, and 0.3 ml of 0.03 mm epinephrine. The increase in absorbance at 420 mm brought on by adrenochrome production was observed

every 30 seconds for 120 seconds. It was shown that one unit of SOD activity was needed to stop 50% of adrenaline from oxidizing to adrenochrome in less than 120 seconds.

The absorbance was measured at 420 mm on a spectrophotometer for a period of three minutes after the reaction as duplicate samples and for thirty seconds after xanthine oxidase was added as the start reagent.

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 = $\frac{\Delta s}{\Delta c} \times 100\%$

SOD activity (µ/ml) = $\frac{\%\ inhibition\ of\ pyrogallol\ autoxidation}{50\%}$

SOD activity in (µ/g wet tissue) = $\frac{SOD\ in\ \mu/mL}{Weight\ coefficient(\gamma)}$

Where γ = $\frac{Volume\ of\ sample\ used\ (Vs) \times wet\ weight\ of\ tissue\ (Ws)g}{Total\ volume\ of\ sample(mL)}$

SOD (µ/g wet tissues) = $\frac{SOD\ in\ \mu/mL}{Weight\ coefficient\ (\gamma)}$

2.7 DATA ANALYSIS

A statistical analysis was performed to determine the significance of the variances between the experimental group and the control group. The study's results were provided as (mean ± sd).

CHAPTER THREE

3.0 RESULT

The GC-MS done to analyze the component of the activated charcoal of *Terminalia catappa* gave the result in figure 4 below.

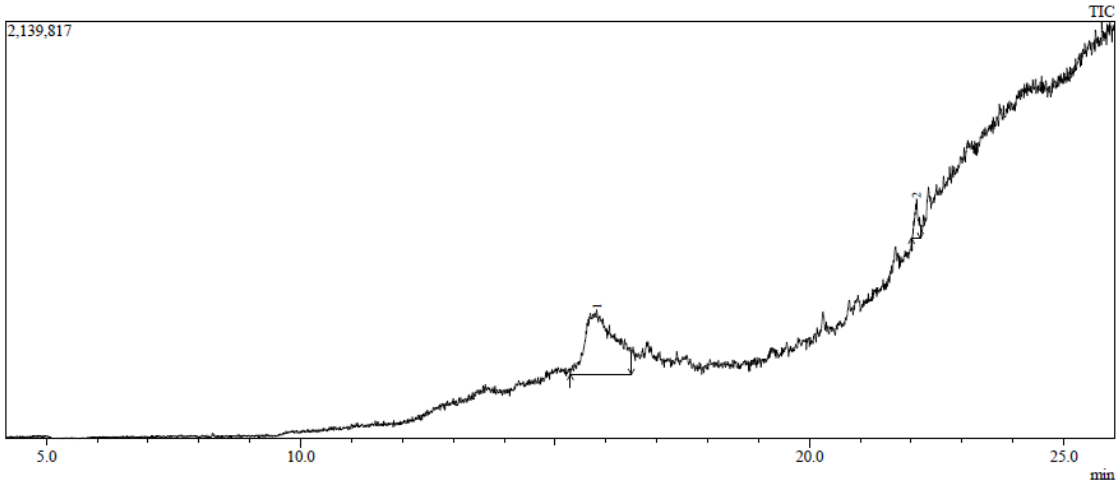


Figure 3: Chromatogram of the GC-MS analysis of activated charcoal of *Terminalia catappa* showing two peaks

Each peak stands for a different compound. Each peak's covered area refers to the ion intensities at a given mass to charge m/z values, which represent the compound's relative abundance (concentration). The peak area increases with increasing ion intensities, which in turn leads to a higher area percentage. Table 2 displays the identified substance and its area percentage value for each peak. The chromatogram illustrates that the GC-MS equipment required a total of 25 minutes for every injection of analysis.

3.1 PHYTOCHEMICALS IDENTIFIED 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.

Table 2: Functional compounds (phytochemicals) detected in activated charcoal of *Terminalia catappa*

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
		14049575	100	533905			

Phytochemicals identified in activated charcoal of *terminalia catappa* by gc-ms **s**hows 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 4: GC-MS analysis of biochemical compounds found in *Terminalia catappa*

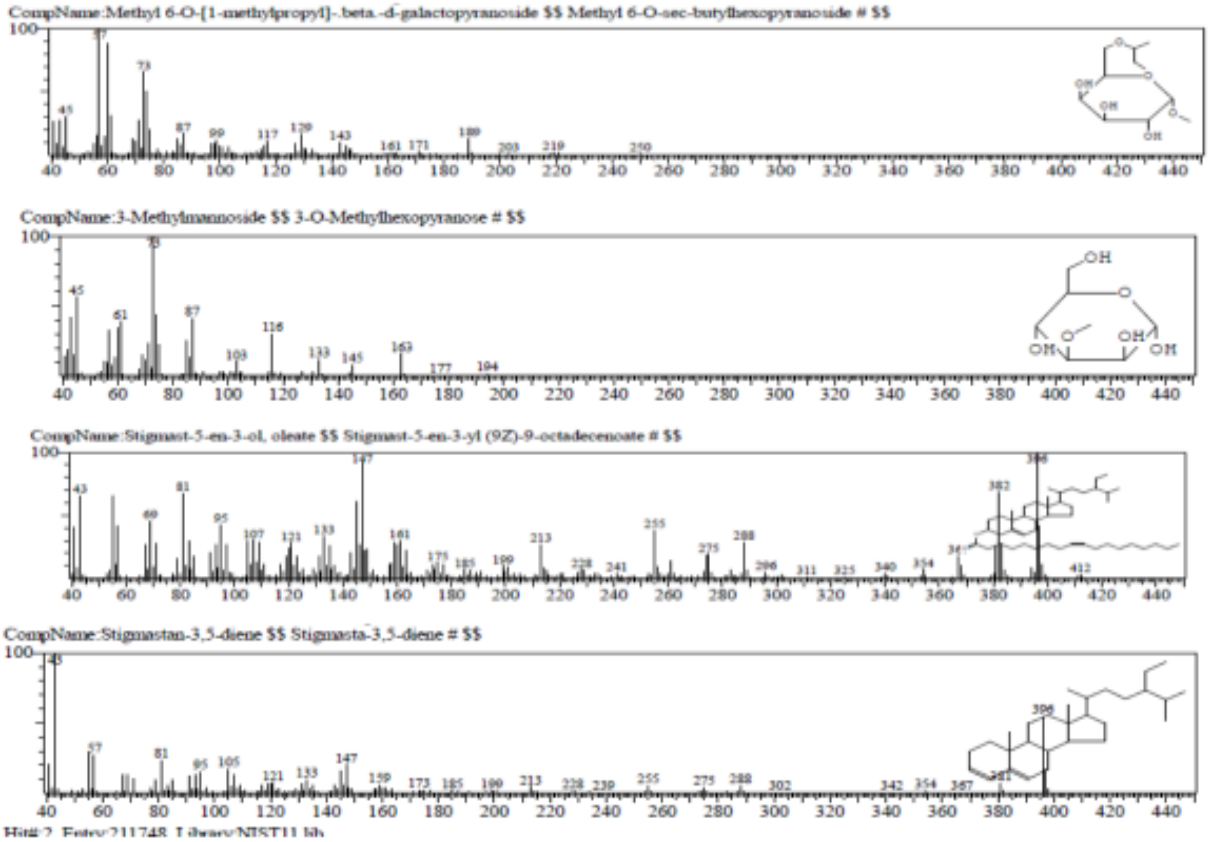
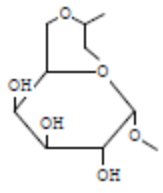
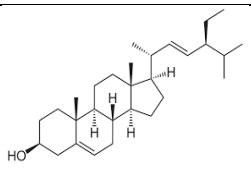


Table 3: Biological activity of activated charcoal of *Terminalia catappa*

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

Stigmastan 3,5-diene		$C_{47}H_{82}O_2$ 396	Anti-diabetic potential. Anti-osteoarthritis, anticancer, anti-inflammatory effect.	6.76

3.2 RESULT OF ASSAY CARRIED OUT ON SERUM OF EXPERIMENTAL ANIMALS

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.

Table 4: Table showing the result gotten from the various assays carried out

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)*10^2	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)*10^2	2.318 ± 0.1090	3.098 ± 0.079*	2.546 ± 0.044*	2.829 ± 0.015*
HDL (mmol/L)*10^2	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

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 LIVER FUCTION TEST

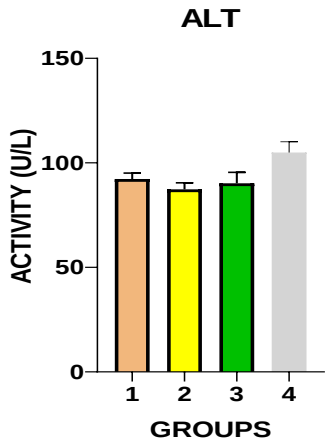


Figure 5: Statistical analysis showing the level of ALT in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal and 10mg Atorvastatin Tablets (group 3 and 4), fed with animal lard, and induced with 5% cholesterol showed an increase when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM values of ALT level.

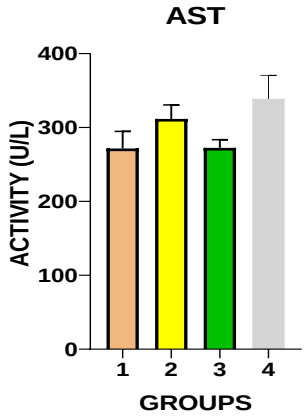


Figure 6: Statistical analysis showing the level of ALT in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal (group 3), fed with animal lard, and induced with 5% cholesterol showed a decrease when compared to animals not treated but induced with high

level of cholesterol in group 2, and animals treated with 10mg Atorvastatin Tablets (group 4), fed with animal lard, and induced with 5% cholesterol showed an increase when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM values of ALT level.

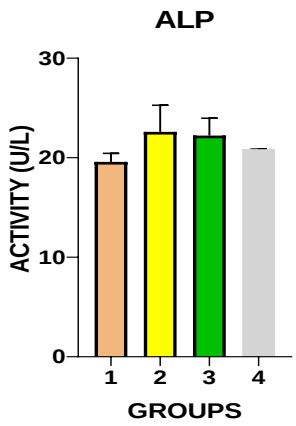


Figure 7: Statistical analysis showing the level of ALP in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal and 10mg Atorvastatin Tablets (group 3 and 4), fed with animal lard, and induced with 5% cholesterol showed a decrease when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM values of ALT level.

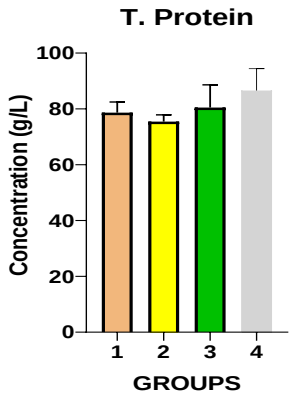


Figure 8: Statistical analysis showing the level of Total protein in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal and 10mg Atorvastatin Tablets (group 3 and 4), fed with animal lard, and induced with 5% cholesterol showed an increase in total protein levels when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM. Values of total protein level.

3.2.2 KIDNEY FUCTION TEST

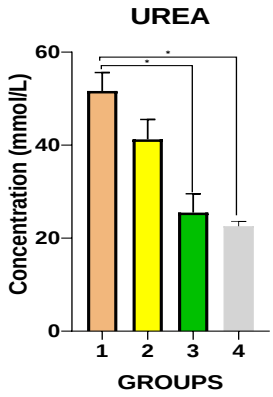


Figure 9: Statistical analysis showing the level of urea in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal and 10mg Atorvastatin Tablets (group 3 and 4), fed with animal lard, and induced with 5% cholesterol showed significant ($p<0.05$) decrease in urea levels when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM. Values of urea level.

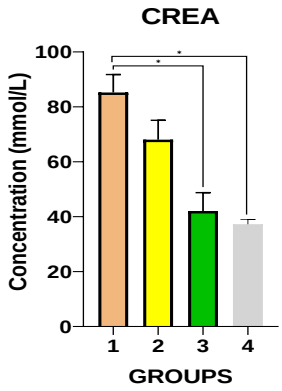


Figure 10: Statistical analysis showing the level of creatinine in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal and 10mg Atorvastatin Tablets (group 3 and 4), fed with animal lard, and induced with 5% cholesterol showed significant ($p<0.05$) decrease in creatinine levels when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM. Values of creatinine level.

3.2.3 TEST FOR ELECTROLYTE ACTIVITY

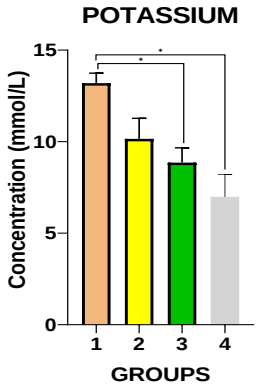


Figure 11: Statistical analysis showing the level of potassium in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal and 10mg Atorvastatin Tablets (group 3 and 4), fed with animal lard, and induced with 5% cholesterol showed significant ($p<0.05$) decrease in potassium levels when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM. Values of potassium level.

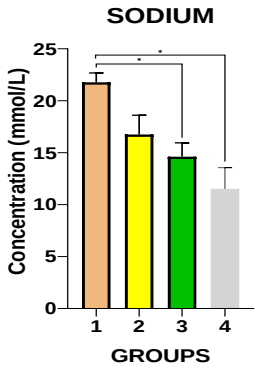


Figure 12: Statistical analysis showing the level of sodium in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal and 10mg Atorvastatin Tablets (group 3 and 4), fed with animal lard, and induced with 5% cholesterol showed significant ($p<0.05$) decrease in sodium levels when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM. Values of sodium level.

3.2.4 TEST FOR ANTIOXIDANT ACTIVITY

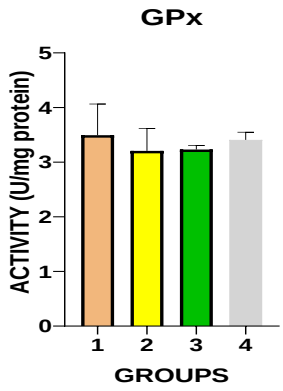


Figure 13: Statistical analysis showing the level of Glutathione Peroxidase (GPx) in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal (group 3), fed with animal lard, and induced with 5% cholesterol showed a slight increase in glutathione peroxidase activity when compared to animals not treated but induced with high level of cholesterol in group 2. Animals treated with 10mg Atorvastatin Tablets (group 4), fed with animal lard, and induced with 5% cholesterol showed an increase in glutathione peroxidase activity when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM. Values of glutathione peroxidase activity.

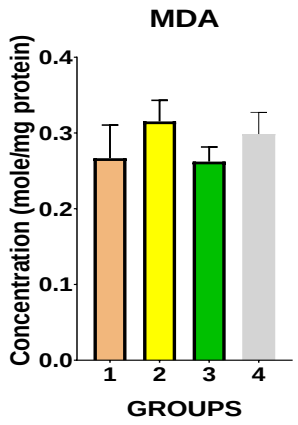


Figure 14: Statistical analysis showing the level of Malondialdehyde (MDA) in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal (group 3), fed with animal lard, and induced with 5% cholesterol showed a decrease in malondialdehyde level when compared to animals not treated but induced with high level of cholesterol in group 2. Animals treated with 10mg Atorvastatin Tablets (group 4), fed with animal lard, and induced with 5% cholesterol showed a slight decrease in malondialdehyde level when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM. Values of malondialdehyde level.

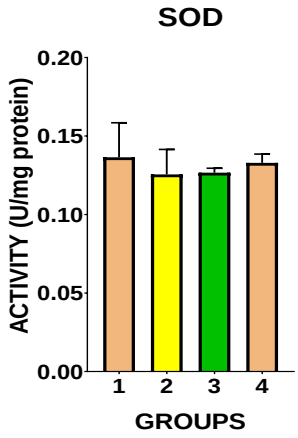


Figure 15: Statistical analysis showing the activity of Sodium dismutase (SOD) in group 1, 2, 3, and 4.

The activity of Superoxide dismutase in animals treated with 200mg activated charcoal (group 3), fed with animal lard, and induced with 5% cholesterol is almost the same as the activity of superoxide dismutase in animals not treated but induced with high level of cholesterol in group 2. Animals treated with 10mg Atorvastatin Tablets (group 4), fed with animal lard, and induced with 5% cholesterol showed a slight increase in Superoxide dismutase activity when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM. Values of sodium dismutase activity.

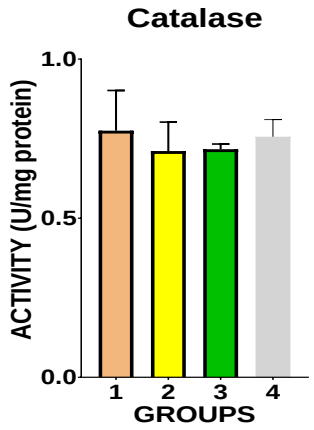


Figure 16: Statistical analysis showing the activity of Catalase in group 1, 2, 3, and 4.

The activity of catalase in animals treated with 200mg activated charcoal (group 3), fed with animal lard, and induced with 5% cholesterol is almost the same as the activity of catalase in animals not treated but induced with high level of cholesterol in group 2. Animals treated with 10mg Atorvastatin Tablets (group 4), fed with animal lard, and induced with 5% cholesterol showed a slight increase in catalase activity when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM. Values of catalase activity.

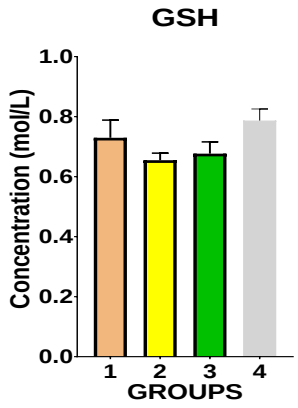


Figure 17: Statistical analysis showing the level of Glutathione (GSH) in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal (group 3), fed with animal lard, and induced with 5% cholesterol showed a slight increase in glutathione level when compared to animals not treated but induced with high level of cholesterol in group 2. Animals treated with 10mg Atorvastatin Tablets (group 4), fed with animal lard, and induced with 5% cholesterol showed an increase in glutathione level when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM. Values of glutathione level.

3.2.5 LIPID PROFILE TEST

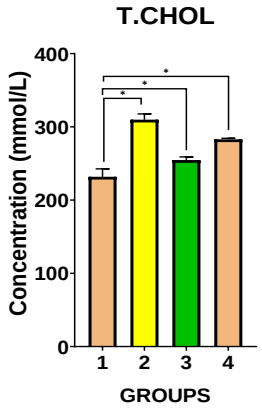


Figure 18: Statistical analysis showing the level of Total cholesterol in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal and 10mg Atorvastatin Tablets (group 3 and 4), fed with animal lard, and induced with 5% cholesterol showed significant ($p<0.05$) decrease in cholesterol levels when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM. Values of total cholesterol level.

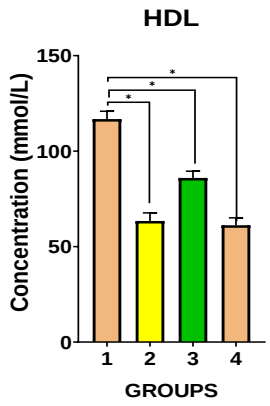


Figure 19: Statistical analysis showing the level of High-density lipoprotein cholesterol (HDL-C) in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal (group 3), fed with animal lard, and induced with 5% cholesterol showed significant ($p<0.05$) increase in HDL-C levels when compared to animals not treated but induced with high level of cholesterol in group 2. Animals treated with 10mg Atorvastatin Tablets (group 4), fed with animal lard, and induced with 5% cholesterol showed a slight decrease in the level of HDL-C. Each column represents the mean ± SEM. Values of high-density lipoprotein cholesterol level.

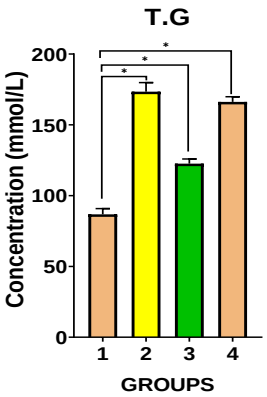


Figure 20: Statistical analysis showing the level of Triglyceride (TG) in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal and 10mg Atorvastatin Tablets (group 3 and 4), fed with animal lard, and induced with 5% cholesterol showed significant ($p<0.05$) decrease in triglyceride levels when compared to animals not treated but induced with high level of cholesterol in group 2. Each column represents the mean ± SEM. Values of triglyceride level.

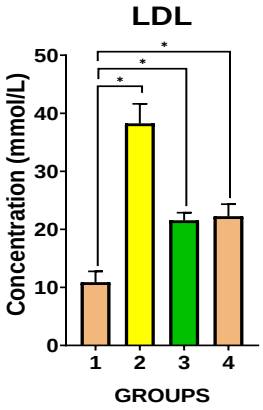


Figure 21: Statistical analysis showing the level of Low-density lipoprotein cholesterol (LDL-C) in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal and 10mg Atorvastatin Tablets (group 3 and 4), fed with animal lard, and induced with 5% cholesterol showed significant ($p<0.05$) decrease in LDL-C levels when compared to animals not treated but induced with high level of cholesterol in

group 2. Each column represents the mean ± SEM. Values of low-density lipoprotein cholesterol level.

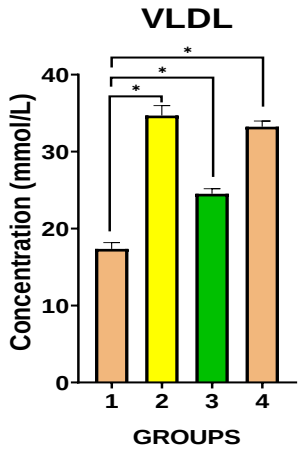


Figure 22: Statistical analysis showing the level of very low-density lipoprotein cholesterol (VLDL-C) in group 1, 2, 3, and 4.

Animals treated with 200mg activated charcoal (group 3), fed with animal lard, and induced with 5% cholesterol showed significant ($p<0.05$) decrease in VLDL-C levels when compared to animals not treated but induced with high level of cholesterol in group 2. Animals treated with 10mg Atorvastatin Tablets (group 4), fed with animal lard, and induced with 5% cholesterol showed a slight decrease in the level of VLDL-C. Each column represents the mean ± SEM. Values of very low-density lipoprotein cholesterol level.

CHAPTER FOUR

4.1 DISCUSSION

The research that has been given offers significant insights into how various treatments or experimental setups affect antioxidant defense pathways and oxidative stress indicators. When there is an imbalance between the body's generation of reactive oxygen species (ROS) and antioxidant activity, it can lead to oxidative stress. It is characterized as an excess of oxidants over antioxidants, which can cause molecular damage and/or disturbance of redox signaling and regulation. Over time, the idea of oxidative stress has changed to take into account new insights into the function of redox signaling. (Sies, 2020).

ROS are produced as metabolic byproducts of biological systems and have physiological functions, including cell signaling. Examples of ROS include superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. But as ROS production rises, they may begin to negatively impact crucial cellular components including proteins, lipids, and nucleic acids (Pizzino *et al.*, 2017).

Superoxide dismutase (SOD) is an enzyme that catalyzes the breakdown of superoxide into oxygen and hydrogen peroxide, which is a vital part of the antioxidant defense mechanism of cells. Superoxide is a negatively charged free radical that is created when oxygen receives one electron. It has a limited half-life and has trouble passing through cell membranes (Wang *et al.*, 2018). SODs are found in a wide range of organisms, including microbes, plants, and animals. They are crucial for preserving the body's redox balance (Zheng *et al.,* 2023).

The induction of cholesterol reduced the superoxide dismutase (SOD) activity but rat in group 3 and 4 treated with activated charcoal and atorvastatin drug respectively, showed increase SOD activity but not significant.

Catalase is an enzyme that is essential to cells' defense against antioxidants because it catalyzes the two-step reaction mechanism that converts hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2) (Nandi *et al.,* 2019).

Reactive oxygen species (ROS) must be balanced in the body, and catalase is necessary for this since too much hydrogen peroxide can cause oxidative stress and damage to tissues and cells. The enzyme is especially significant when it comes to diseases linked to oxidative stress, like inflammatory disorders, cardiovascular diseases, and neurodegenerative diseases (Rasheed, 2024).

The induction of cholesterol reduced the catalase activity but rat in group 3 and 4 treated with activated charcoal and atorvastatin drug respectively, showed increase catalase activity but not significant.

It can therefore be inferred that activated charcoal of *Terminalia catappa* has an antioxidant activity to reduces oxidative stress caused by reacting oxygen species (ROS).

CONCLUSION

The activities of key antioxidant enzymes, including glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase, as well as the levels of the antioxidant glutathione (GSH), were slightly increased in the group treated with activated charcoal of *Terminalia catappa*. These findings suggest a compensatory upregulation of the antioxidant defense system in response to the increased oxidative stress burden caused by increased level of cholesterol.

Importantly, the total protein levels, high-density lipoprotein cholesterol increased significantly. The level of aspartate amino transferase was reduced compared to the untreated group 2 which have a high level of AST. The level of triglyceride, very low-density lipoprotein cholesterol, low-density lipoprotein cholesterol, malondialdehyde were also reduced compare to the untreated group 2. Indicating that the observed changes were specific to oxidative stress pathways, increased protein level, increase in level of good cholesterol, decrease in bad cholesterol, and reduced lipid peroxidation.

Data from this study shows that activated charcoal contains phytochemical compounds and can reduce hypercholesterolemia.

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