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EFFECTS OF *Adansonia digitata* AND *Corchorus olitorius* LEAVES CONCOCTION ON SELECTED PARAMETERS ASSOCIATED WITH FRIED OIL-INDUCED HEPATIC DAMAGE IN RATS

BY

OLADAPO, OYINKANSOLA PRECIOUS

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BEING A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF ILORIN, ILORIN, KWARA STATE, NIGERIA.

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NOVEMBER, 2022

CERTIFICATION

This is to certify that the project work titled “EFFECTS OF Adansonia digitata AND Corchorus olitorius LEAVES CONCOCTION ON SELECTED PARAMETERS ASSOCIATED WITH FRIED OIL-INDUCED HEPATIC DAMAGE IN RATS” is an original work carried out by OLADAPO OYINKANSOLA PRECIOUS, with matric number 17/55EH140, under my supervision.

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Mr. O.K. Bello
Project Supervisor

Date

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Prof. J.O. Adebayo
Head of Department

Date

.....
External Examiner .....
Date

DEDICATION

This project work is dedicated to my family for their immense support, love, patience and guidance during the period of this project work and at every phase of my life. I'm forever grateful to you all.

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Foremost, I give all glory to God Almighty, the giver of all things great and beautiful, for His unfailing love and gift of life, wisdom and provision at every phase of life.

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ABSTRACT

This study investigated the effects of aqueous leaves concoctions of *Adansonia digitata* and *Corchorus olitorius* on the serum proteins, antioxidants and the liver enzymes of rats administered with fried oil.

A total of thirty-six (36) Wistar rats were distributed into 6 groups of 6 rats each, such that, all groups were fed on rat chow and distilled water. All other groups were administered with fried oil; groups 3, 4, and 5 were administered with aqueous leaves concoctions of *A. digitata*, *C. olitorius*, and the combination of both respectively. Group 6 was placed on Vitamin C.

The secondary metabolites constituents of *A. digitata* and *C. olitorius* revealed the presence of saponins, alkaloids, flavonoids, tannins, terpenoids, steroids and polyphenols. The administration of fried oil significantly ($p<0.05$) altered cellular parameters associated with oxidative stress as shown in the control group (group 2). There was significant ($P<0.05$) increase in the activities of Superoxide dimutase (SOD), Catalase (CAT), Glutathione-S-transferase (GST), Glutathione peroxidase (GPx), Glutathione (GSH) and albumin on all the groups administered with the leaves concoctions when compared with control group (group 2) and the most effective is the combined concoction group (group 5). There was significant ($P<0.05$) decrease in total protein, bilirubin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) in all the groups administered with the leaves concoctions when compared with control group (group 2) and the most effective is the combined concoction group (group 5). Hence, we can therefore conclude that the leaves of *A. digitata* and *C. olitorius* might be useful as nutraceuticals.

Keywords: *Adansonia digitata*, *Corchorus olitorius*, antioxidants, free radicals, oxidative stress, hepatic damage

CHAPTER ONE

1.0 Introduction

The liver is the largest internal organ providing essential metabolic, exocrine and endocrine functions. These include production of bile, metabolism of dietary compounds, detoxification, regulation of glucose levels through glycogen storage and control of blood homeostasis by secretion of clotting factors and serum proteins such as albumin. Hepatocytes are the principal cell type in the liver accounting for approximately 70% of the mass of the adult organ. Hepatocytes, along with biliary epithelial cells (BECs; also known as cholangyocytes) are derived from the embryonic endoderm (Zorn, 2008).

The liver has an essential role in nutrient metabolism including the control and maintenance of the blood glucose level; in detoxification and excretion of hydrophobic metabolites and xenobiotics; in the synthesis of most plasma proteins; and in digestion through synthesis, biliary secretion, and conservation of bile acids that are essential both for digestion and intestinal absorption of fats and other lipids including fat soluble vitamins. The clinical manifestations of hepatic disease are directly attributable to alterations in the metabolic, excretory, synthetic, and digestive functions of the liver. The liver has great reserve, and signs of hepatic failure often do not develop until 70% or more of functional capacity is lost. Importantly, even when a major fraction of the hepatocellular mass has been lost following acute injury, recovery is possible because of the unique regenerative capacity of the liver (Tennant and Center, 2017).

1.1 Liver Disease

Liver diseases can be inherited or caused by a variety of factors that damage the liver. In fact, there are many types of liver diseases that can be caused by a virus, damage from drugs or chemicals, obesity, diabetes or an attack from own immune system, when the condition is left untreated, it can become life threatening and can permanently damage the liver or the bile duct. This damage leads to malignancy and cause liver cancer (Krishnan, 2019).

1.1.1 Acute Liver Failure

Acute liver failure occurs when liver rapidly loses its ability to function. More commonly, liver failure develops slowly over the course of years but acute liver failure develops in a matter of days. Acute liver failure can cause many complications, including excessive bleeding and increasing pressure in the brain. Another term for acute liver failure is fulminant hepatic failure. Acute liver failure is a medical emergency that requires hospitalization. Some causes of acute liver failure can be reversed with treatment. But in other situations, a liver transplant may be the only cure for acute liver failure (Larson *et al.*, 2005).

1.1.2 Chronic Liver Disease

Chronic liver disease (CLD) is a progressive deterioration of liver functions for more than six months, which includes synthesis of clotting factors, other proteins, detoxification of

harmful products of metabolism, and excretion of bile. CLD is a continuous process of inflammation, destruction, and regeneration of liver parenchyma, which leads to fibrosis and cirrhosis. The spectrum of etiologies is broad for chronic liver disease, which includes toxins, alcohol abuse for a prolonged time, infection, autoimmune diseases, genetic and metabolic disorders (Sharma and Nagalli, 2021).

Cirrhosis is a final stage of chronic liver disease that results in disruption of liver architecture, the formation of widespread nodules, vascular reorganization, neoangiogenesis, and deposition of an extracellular matrix. The underlying mechanism of fibrosis and cirrhosis at a cellular level is the recruitment of stellate cells and fibroblasts, resulting in fibrosis, while parenchymal regeneration relies on hepatic stem cells. Chronic liver disease is an extremely common clinical condition, and the focus is done on the common etiologies, clinical manifestations, and management (Sharma and Nagalli, 2021).

According to Sharma and Nagalli (2021), there are several etiologies of chronic liver disease:

Alcoholic Liver Disease

Alcoholic liver disease is a spectrum of disease which includes alcoholic fatty liver with or without hepatitis, alcohol hepatitis (reversible because of acute ingestion) to cirrhosis (irreversible). Patients with severe alcohol use disorder mostly develop chronic liver disease; this is the most frequent cause of CLD.

Non-alcoholic Fatty Liver Disease (NAFLD/NASH)

NAFLD has an association with metabolic syndrome (obesity, hyperlipidemia, and diabetes mellitus). Some of these patients develop non-alcoholic steatohepatitis, which leads to fibrosis of the liver. All the risk factors of metabolic syndrome can aggravate the disease process.

Viral Hepatitis

Chronic hepatitis B, C, and D infections are the most common causes of chronic liver disease in East Asia and Sub-Saharan Africa. There are various genotypes of hepatitis C. In Europe and North America, genotype 1a and 1b are more prevalent, while in Southeast Asia, genotype 3 is more common. A molecular epidemiological study revealed a high prevalence of HCV genotype 4, subtype 4a among Egyptian patients living in Sharkia governorate, Egypt. Chronic hepatitis C, if not treated, may lead to hepatocellular carcinoma.

Genetic Causes of Chronic Liver Disease

- **Alpha-1 antitrypsin deficiency**: This is the most common genetic cause of CLD among children.

- **Hereditary hemochromatosis**: It is an autosomal recessive disorder of iron absorption. Here due to a mutation involving the HFE gene that regulates the iron

absorption from the intestine, excessive iron is absorbed from the gastrointestinal tract. As a result, there is a pathological increase in total body iron (such as ferritin and hemosiderin). This process leads to the generation of hydroxyl free radicals, which in turn causes organ fibrosis.

- **Wilson disease**: Autosomal recessive disorder leading to copper accumulation

Autoimmune Causes of Chronic Liver Diseases

Autoimmune hepatitis is a rare disease in which there is the destruction of liver parenchyma by autoantibodies. Most of the patients who present with this disease have already developed cirrhosis. Females are more commonly affected than males.

- **Primary biliary cirrhosis (PBC)**: This is an autoimmune and progressive disease of the liver, which is the destruction of intrahepatic biliary channels and portal inflammation and scarring. It leads to cholestatic jaundice and fibrosis of liver parenchyma. PBC is more common in middle-aged women. Alkaline phosphatase levels increase in PBC.

- **Primary Sclerosing Cholangitis (PSC)**: commonly associated with ulcerative colitis. This condition is characterized by a decrease in the size of intrahepatic and extrahepatic bile ducts due to inflammation and fibrosis.

- **Autoimmune hepatitis (AIH)**: This is a form of chronic inflammatory hepatitis, more common in women than men, and is characterized by elevated autoantibodies such as antinuclear antibodies, anti-smooth muscle antibodies, and hypergammaglobulinemia.

Other causes of chronic liver disease include drugs such as amiodarone, isoniazid, methotrexate, phenytoin, nitrofurantoin, vascular causes such as Budd-Chiari syndrome and Idiopathic or cryptogenic causes which accounts for about 15%.

1.1.3 Acute-On-Chronic Liver Failure

The definition of acute-on-chronic liver failure (ACLF) remains contested. In Europe and North America, the term is generally applied according to the European Association for the Study of the Liver-Chronic Liver Failure (EASL-CLIF) Consortium guidelines, which defines this condition as a syndrome that develops in patients with cirrhosis and is characterized by acute decompensation, organ failure and high short-term mortality. Onethird of patients who are hospitalized for acute decompensation present with ACLF at admission or develop the syndrome during hospitalization (Arroyo *et al.,* 2016).

ACLF frequently occurs in a closed temporal relationship to a precipitating event, such as bacterial infection or acute alcoholic, drug-induced or viral hepatitis. However, no precipitating event can be identified in approximately 40% of patients. The mechanisms of ACLF involve systemic inflammation due to infections, acute liver damage and, in cases

without precipitating events, probably intestinal translocation of bacteria or bacterial products (Arroyo *et al.,* 2016).

ACLF is graded into three stages (ACLF grades 1–3) on the basis of the number of organ failures, with higher grades associated with increased mortality. Liver and renal failures are the most common organ failures, followed by coagulation, brain, circulatory and respiratory failure. The 28-day mortality rate associated with ACLF is 30%. Depending on the grade, ACLF can be reversed using standard therapy in only 16–51% of patients, leaving a considerable proportion of patients with ACLF that remains steady or progresses. Liver transplantation in selected patients with ACLF grade 2 and ACLF grade 3 increases the 6- month survival from 10% to 80% (Arroyo *et al.,* 2016).

1.1.4 Test for Liver Diseases

A number of liver function test are available to test the proper function of the liver, (serum proteins, serum albumin, bilirubin (direct and indirect), ALT, AST, GGT, ALP, PT and PTT). Imaging tests such as transient elastography, ultrasound and magnetic resonance imaging can be used to examine the liver tissue and bile ducts. Liver biopsy can be performed to examine liver tissue to distinguish between various conditions; tests such as elastography may reduce the need for biopsy in some situations (Tapper and Lok, 2017).

Liver injury is defined as a rise in either ALT level more than three times of the upper limit of normal (ULN), alkaline phospatase (ALP) level more than twice ULN, or total bilirubin

level more than twice ULN when associated with increased ALT or ALP. Liver damage is further characterized into hepatocellular (predominantly initial alanine transferase elevation) and cholestatic (initial alkaline phosphatase rise) types (Mumoli *et al.*, 2006).

However, they are not mutually exclusive and mixed types of injuries are often encountered.The liver synthesizes, concentrates, and secretes bile acids and excretes other toxicants, such as bilirubin. Drug induced injury to hepatocytes and bile duct cells can lead to cholestasis. Cholestasis, in turn, causes intrahepatic accumulation of toxic bile acids and excretion products, which promotes further hepatic injury (Jaeschke *et al.*, 2002).

Marker enzymes are widely used to assess liver damage; hence, we determined the levels of enzymes such as ALP, AST and ALT. Necrosis or membrane damage releases the enzyme into circulation; hence, it can be measured in the serum. High levels of AST indicate liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury; AST catalyzes the conversion of alanine to pyruvate and glutamate and is released in a similar manner.Therefore, ALT is more specifific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Choudhary and Devi, 2014).

Serum ALP, bilirubin and total protein levels, on the other hand, are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in the presence of increasing biliary pressure (Muriel and Garcipiana, 1992). Increase in the level of these

serum enzymes ALP, acid phosphatase (ACP), AST, ALT, lactate dehydrogenase (LDH) and gamma-glutamyl transferase (γ-GT) was also observed. Lipid peroxidation leads to generation of free radicals (such as peroxyl, alkoxyl and aldehyde) that cause cell damage and leads to the release of marker enzymes (Choudhary and Devi, 2014).

The disruption of the ordered lipid-bilayer of the membrane structure is probably due to the presence of reactive oxygen species produced due to oxidative stress, leading to the escape of detectable quantity of these enzymes out of the cell into the extracellular fluid. The reactive oxygen species might have oxidized the polyunsaturated fatty acids which make up the lipid bilayer resulting in its disruption. The elevated levels of serum globulin also suggest adverse effect of reactive oxygen species on the secretory ability of the liver, and hence it also affects the normal functioning of the organ. Increased bilirubin production, enhanced hepatic conjugation and biliary excretion of the pigment present in aspartame-treated animals may be a result of decreased uptake, conjugation or increased bilirubin production (Choudhary and Devi, 2014).

1.2 Fats

Fat is the most rigorous foundation of food energy. There are nine calories in every gram of fats (Noakes, 2003). There are various groups of compounds of this type of diet that are basically soluble in organic solvents and insoluble in water. Fat is defined chemically as triglycerides; trimesters of glycerol with several fatty acids (Brouwer *et al*., 2010). On the basis of their structure and composition, fats may be both solid and liquid at room

temperature. Several terms are frequently used to refer to fats; some are oils, fats and lipid, in which oils such as vegetable oil, canola oil, olive oil etc. are liquids at normal room temperature, and are frequently used to refer to fats. On the other hand, fats is typically used to refer to fats that are solids at typical room temperature (e.g., lard, butter, shortening etc.) (Banik and Hossain, 2014).

Fats are the principal energy supplies of the body, which form a vital source of energy during hunger or other labour works. They play important roles in the absorption of fats soluble vitamins A, D, E and K. They also control cholesterol level, and so act as important regulating compound. They help to maintain healthy skin and hair and acts like "cushion" and heat regulator to protect the heart, liver and other vital organs (Banik and Hossain, 2014).

Lipid is another familiar term which is generally used to refer to both liquid and solid fats, besides other linked substances, generally in a medical and biochemical context (Banik and Hossain, 2014). Lipids are a chemical family that includes cholesterol, phospholipids and fats and oils and make up a major part of the average human diet (Abdel-Razek, 2017). They are important constituents of many foods and cell membrane. As a result of this, lipids play specific roles in membrane signaling (Maduelosi *et al.*, 2019).

There are abundant diverse kinds of fats, but each is a distinction on the identical chemical arrangement. More specifically, the entire fats are sources of fatty acids and glycerol (Banik and Hossain, 2014).

The main sources of fats are food items such as meat, egg yolk, milk, nuts, butter, ghee, cheese, different types of vegetables, etc. (Banik and Hossain, 2014).

1.2.1 Classification of Fats

According to Banik and Hossain (2014), fats can be classified in the following ways:

a) **Saturated fats:** They have no double bond in their hydrocarbon chains and are solids at room temperature. Butter, ghee etc. are familiar instances of saturated fats. High intake of saturated fats help to raise blood cholesterol level (especially LDL cholesterol) that can lead to coronary disorders and high blood pressure (Ravnskov, 1998). The people who spend a sedentary daily life and old people have to reduce the **i**ngestion of saturated fats. On the other hand, the energy requirement is high for the people who are involved in heavy labour; they need more fat intake from their daily requirement.

b) **Unsaturated fats:** They have one or more double bonds and 'kink' in the hydrocarbon chain and are liquid at room temperature. Oils are unsaturated fats. Unsaturated fats may be monounsaturated fats, polyunsaturated fats (more than one double bond). High intake of these types of fats help to lower LDL (bad) cholesterol and help to raise HDL (good) cholesterol. Olives, Olive oil, sunflower oil, peanut oils are unsaturated fats.

Depending on the dietary necessities, fats are of two kinds:

a) **Essential fats:** Essential fats are categorized as those types of fats that are not manufactured by the body such as linoleic acid, linolenic acid, arachidonic acid etc. and must be included in the diet.

b) **Non-essential fats:** Those types of fats are synthesized by means of the body and are not indispensable in the diet. An example of non-essential fat is glycine.

All types of fats are not similar. Some are as essential for maintaining good health (good fat) and others are dangerous for the body (bad fat). The bad fats are responsible for increasing blood cholesterol levels or have other harmful effects on cardiovascular health (Food Standard Board, 2007).

1.2.2 Healthy Fats

Unsaturated fats are categorized as good fats. These types of fats are found chiefly in plant foods or in fish that eat tiny flora. Further unsaturated fats are categorized into two groups; monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). The two polyunsaturated fatty acids crucial for physical condition are omega-3 and omega-6. Omega-3 and omega-6 fatty acids are very essential because they play various roles in the body but they cannot be synthesized by the body and are required to come from food. The beneficial effects of omega-3 fatty acids are brain and eye development of growing fetus during pregnancy and for maintaining and promoting health all over life (Hooper, 2004). Omega-6 fatty acids also take part in the development of brain and heart function and also

in normal growth and development (Harris *et al.,* 2009). Both mono-and polyunsaturated fats may help to lower LDL (bad) cholesterol and decrease risk of heart disease. Olive, canola and peanut oils are high in monounsaturated fats, and safflower, sunflower, and corn oils contain more polyunsaturated fats (Banik and Hossain, 2014).

Omega-3 fatty acids

The common food sources of omega-3 fatty acids are fatty fish such as salmon, herring, sardines, trout and flaxseed, walnuts as well as canola oil. The American Heart Association (AHA) has suggested that healthy adults eat at least two servings of fish per week to boost omega-3 fatty acid intake (Lichtestein *et al.*, 2006). It has numerous potential health promoting benefits, such as:

- It decreases inflammation in case of heart disease, inflammatory bowel disease and rheumatoid arthritis.
- It helps to prevent blood from clotting and sticking to artery walls.
- It reduces risk for blocked blood vessels and heart attacks.
- It prevents hardening of the arteries.
- It lowers risk of sudden death and abnormal heart rates.
- It reduces triglyceride levels.
- It lowers blood pressure.

Omega-6 fatty acids

The common sources of omega-6 fatty acids are vegetable oils such as corn oil, safflower oil, sesame oil, soybean oil, sunflower oil; soft (liquid or tub) margarine, preferably one that is trans-fat free; walnuts; sunflower seeds, pumpkin seeds, sesame seeds; soy "nuts" (roasted soy beans), soy nut butter and tofu etc. (Banik and Hossain, 2014).

Omega-6 fatty acids also play key roles in health promoting benefits, for instance:

- Neutral or reduced levels of inflammatory mediators.
- Replacing saturated and trans fats with omega-6 fatty acids linked with decreasing risk of heart disease.
- Development of insulin resistance and decrease in the occurrence of diabetes
- Inferior blood pressure.
- Inferior cholesterol levels.

Monounsaturated fats

The available food sources of monounsaturated fats are vegetable oils: olive oil, canola oil, peanut oil; nuts: almonds, cashews, peanuts, pecans, pistachios; avocado; peanut butter and almond butter. The main functions of this is to lower LDL cholesterol, while increasing HDL (good) cholesterol (Banik and Hossain, 2014).

1.2.3 Unhealthy Fats

Fats that produce unwanted effects in the body are known as bad fats such as saturated fats and Trans fats (Uauy *et al.*, 2009; Jakobsen, 2008). Meats with high-fat and dairy foods such as cheese, whole milk etc. are the main foundation of saturated fatty acids. The enlisted food items are the most important resource of unhealthful fats and have to be avoided or eaten scarcely (Banik and Hossain, 2014).

Saturated fats

The familiar food sources of saturated fats are fatty cuts of beef, pork and lamb; poultry skin, chicken wings, dark meat chicken; high fat dairy products: cheese, butter, whole milk, 2% reduced fat milk, cream, cream cheese, sour cream, ice cream; tropical oils: coconut oil, palm oil, palm kernel oil, cocoa butter; lard and suet etc. (Banik and Hossain, 2014).

Trans fatty acids (Trans fats)

Trans fats (occasionally called Trans fatty acids) are produced during the progression of hydrogenation of vegetable oils (Bakker *et al.*, 1997; Mozaffarin *et al.*, 2006). Hydrogenation is a method used by food manufacturers to add hydrogen to vegetable oils to create a solid fat. Trans fat is the worst kind of fat, even worse than saturated fat. Trans fats are also known as partially hydrogenated oil. Trans fats may demoralize the "good" blood cholesterol (HDL cholesterol) level when eaten in large quantities, and are called hidden killers of the body (Banik and Hossain, 2014).

There are two sources of Trans fat; natural and man-made. The common sources of Trans fat are vegetable shortening and stick margarines; refrigerated dough products; fried foods: doughnuts, French fries, other deep fried fast food items; commercially ready foods containing partially hydrogenated oils: crackers, cookies, cakes, pastries, microwave popcorn and other snack foods. High intake of saturated fats and Trans fats can raise the level of bad cholesterol (LDL cholesterol) in the body. These fats may also make the lining of blood vessels (the endothelium) less flexible (Banik and Hossain, 2014).

1.3 Oxidative Stress and Lipid Peroxidation

All living aerobic multicellular organisms require molecular oxygen (O_2) to survive rather than oxygen, which is susceptible to radical formation due to its electronic structure. Reactive oxygen species (ROS) are small molecules derived from oxygen molecules including free oxygen radicals, such as superoxide ($O_2^{\cdot-}$), hydroxyl ($OH^{\cdot}$), peroxyl ($RO_2^{\cdot}$), and alkoxyl ($RO^{\cdot}$) as well as hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2), which are non-radicals. These nonradicals are either oxidizing agents or easily converted into radicals. Nitrogen-containing oxidants, such as nitric oxide (NO) peroxynitrite($ONOO^-$), nitrogen dioxide (NO_2) are called reactive nitrogen species (RNS) (Klebanoff, 1980; Bedard and Krause, 2007).

ROS generation is generally a cascade of reactions which starts with the production of superoxide. Superoxide rapidly dismutates to hydrogen peroxide spontaneously, particularly at low pH or is catalyzed by SOD. The other generations include the reaction

of superoxide with NO to form peroxynitrite, the peroxidase-catalyzed formation of hypochlorous acid from hydrogen peroxide, and the iron-catalyzed Fenton reaction leading to the generation of hydroxyl radical (Klebanoff, 1980; Thannickal and Fanburg, 2000).

The generation of ROS can occur as a product of biochemical reactions, in mitochondria, peroxisomes, cytochrome P450, and other cellular components (Balaban *et al.*, 2005; Gonzalez, 2005). ROS are generated mainly by the mitochondrial ETC. Almost all cells, and tissues continuously convert a small proportion of molecular oxygen to ROS in ETC. ROS produced by other pathways, including the respiratory burst taking place in activated phagocytes, are ionizing the damaging effect of the radiation on components of cell membranes, and by-products of several cellular enzymes (NADPH oxidases, xanthine oxidase, nitric oxide synthase) (Alfadda and Sallam, 2012).

The formation of ROS is a natural consequence of aerobic metabolism and is integral for cell signaling and maintaining tissue oxygen homeostasis. When oxygen homeostasis is not maintained, there is an increase in oxidative stress in the cellular environment. Superoxide, hydrogen peroxide and hydroxyl radicals are normal metabolic by-products which are generated continuously by the mitochondria in growing cells. Microsomal cytochrome P450 enzymes, flavoprotein oxidases and peroxisomal enzymes are other significant intracellular sources of ROS (Seifried *et al.*, 2007).

Reactive species or free radicals that include reactive oxygen and nitrogen species collectively and are called reactive oxygen nitrogen species (RONS). They are released from macrophages, neutrophils and dendritic cells in response to an inflammatory stimulus. RONS are highly reactive due to the presence of unpaired valence shell electrons or non-static bonds, and their proper regulation is vital for an efficient immune response and for limiting tissue damage (Salman and Ashraf, 2013).

ROS play the key roles in both health and disease. ROS also have an important role in several physiologic processes such as normal vascular cell functioning and maintaining vascular diameter regulation. ROS carry out this function by mounting effective immune response, acting as possible signaling molecules and regulating glucose uptake by skeletal muscle (Salman and Ashraf, 2013). They have a role in response to growth factor stimulation and control of inflammatory responses. They participate in the regulation of differentiation, proliferation, growth, apoptosis, cytoskeletal regulation, migration, and contraction. ROS also have an important role in host defense because ROS generation deficiencies reduce the killing ability of leukocytes (Brieger *et al.*, 2012).

ROS contributes to a wide range of pathologies and many of the implicated diseases which lead to death, such as chronic inflammation and autoimmune diseases (diabetes, rheumatoid arthritis, lupus), sensory impairment (ocular disease, hearing loss), cardiovascular diseases (atherosclerosis, hypertension, ischemia/reperfusion injury), cancer (breast, renal, lung), fibrotic disease (pulmonary and liver fibrosis, diabetic

nephropathy), obesity, insulin resistance, neurological disorders (Parkinson's, Alzheimer's, ALS, schizophrenia), and infectious diseases (septic shock, influenza, hepatitis, HIV) (Brieger *et al.*, 2012; Salman and Ashraf, 2013).

During times of environmental stress (e.g., UV radiation, heat exposure and ionizing radiation), their levels could increase dramatically. At high concentrations, ROS reacts readily with biomolecules such as lipids, proteins, carbohydrates, and nucleic acids. This may result in significant damage to cell structures, and cumulates into a situation known as oxidative stress (Conner and Grisham, 1996; Brieger *et al.*, 2012).

Oxidative stress is a condition when the balance between the production of oxidants and their removal by antioxidants gets disturbed leading to increased production and accumulation of oxidants in the body. Aging, chronic inflammatory diseases, smoking, diabetes neurodegenerative diseases, cancer, etc., lead to generation of oxidative stress (Salman and Ashraf, 2013). Lipid peroxidation is the process under which oxidants such as free radicals or non radical species attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids (PUFAs) that involve hydrogen absraction from a carbon, with oxygen insertion resulting in lipid peroxyl radicals and hydroperoxides (Yin *et al.*, 2011).

1.4 Role of Oxidative Stress in Liver Diseases

Due to their special chemical characteristics, ROS/RNS can initiate lipid peroxidation, cause DNA strand breaks, and indiscriminately oxidize virtually all molecules in biological membranes and tissues, resulting in injury. Although, since the body is able to remove ROS/RNS to a certain degree, these reactive species are not necessarily a threat to the body under physiological conditions (Brieger *et al.*, 2012; Salman and Ashraf, 2013). As a matter of fact, ROS are required at certain level in the body to perform its important physiological functions. The generation of ROS is a natural part of aerobic life, which is responsible for the manifestation of cellular functions including signal transduction pathways, defense against invading microorganisms and gene expression to the promotion of growth or death (Bedard and Krause, 2007).

Oxidative/nitrosative stress represents the bodies' imbalance in the production and the elimination of reactive oxygen and nitrogen species as well as decreased production of antioxidants. In terms of oxidative stress, in specific physiological conditions, it is actually useful. For example, it could strengthen biological defense mechanisms during appropriate physical exercise and ischemia, and induce apoptosis to prepare the birth canal for delivery. However, this is confined to particular situations, and in most other cases, large levels of ROS and oxidative stress will induce cell death through necrotic and/or apoptotic mechanisms, leading to cellular and tissue injury (Klebanoff, 1980; Salman and Ashraf, 2013).

Liver is a major organ attacked by ROS (Conner and Grisham, 1996). Parenchymal cells are primary cells subjected to oxidative stress induced injury in the liver. The mitochondrion, microsomes and peroxisomes in parenchymal cells can produce ROS, regulating on PPARα, which is mainly related to the liver fatty acid oxidation gene expression. Moreover, Kupffer cells, hepatic stellate cells and endothelial cells are potentially more exposed or sensitive to oxidative stress-related molecules. A variety of cytokines like TNF-α can be produced in Kupffer cells induced by oxidative stress, which might increase inflammation and apoptosis. With regard to hepatic stellate cells, the proliferation and collagen synthesis of hepatic stellate cells is triggered by lipid peroxidation caused by oxidative stress (Agarwal *et al.,* 2006; Perrone *et al.,* 2010).

When the ROS is excessive, the homeostasis will be disturbed, resulting in oxidative stress, which plays a critical role in liver diseases and other chronic and degenerative disorders (Halliwell, 2007).

The oxidative stress not only triggers hepatic damage by inducing irretrievable alteration of lipids, proteins and DNA contents and more importantly, modulating pathways that control normal biological functions. Since these pathways regulate genes transcription, protein expression, cell apoptosis, and hepatic stellate cell activation; oxidative stress is regarded as one of the pathological mechanisms that results in initiation and progression of various liver diseases, such as chronic viral hepatitis, alcoholic liver diseases and nonalcoholic steatohepatitis (Drew and Leeuwenburgh, 2002; Bolisetty and James, 2013).

It has also been suggested that there are complicated cross-talks among pathological factors, inflammation, free radicals and immune responses (Boveris, 1976; Bolisetty and James, 2013).

Moreover, systemic oxidative stress arising during liver disease can also cause damage to extra-hepatic organs, such as brain impairment and kidney failure (Lee *et al.*, 2004). It was suggested systemic oxidative stress might be a significant "first hit", acting synergistically with ammonia to induce brain edema in chronic liver failure (Liochev and Fridovich, 2002). With regard to kidney failure, systemic oxidative stress is considered to play a critical role in the pathophysiology of several kidney diseases (Foote *et al.*, 1985: Barbusıńskı, 2009). The general mechanism scheme of oxidative stress induced by various factors on liver disease is concluded in Figure 1.

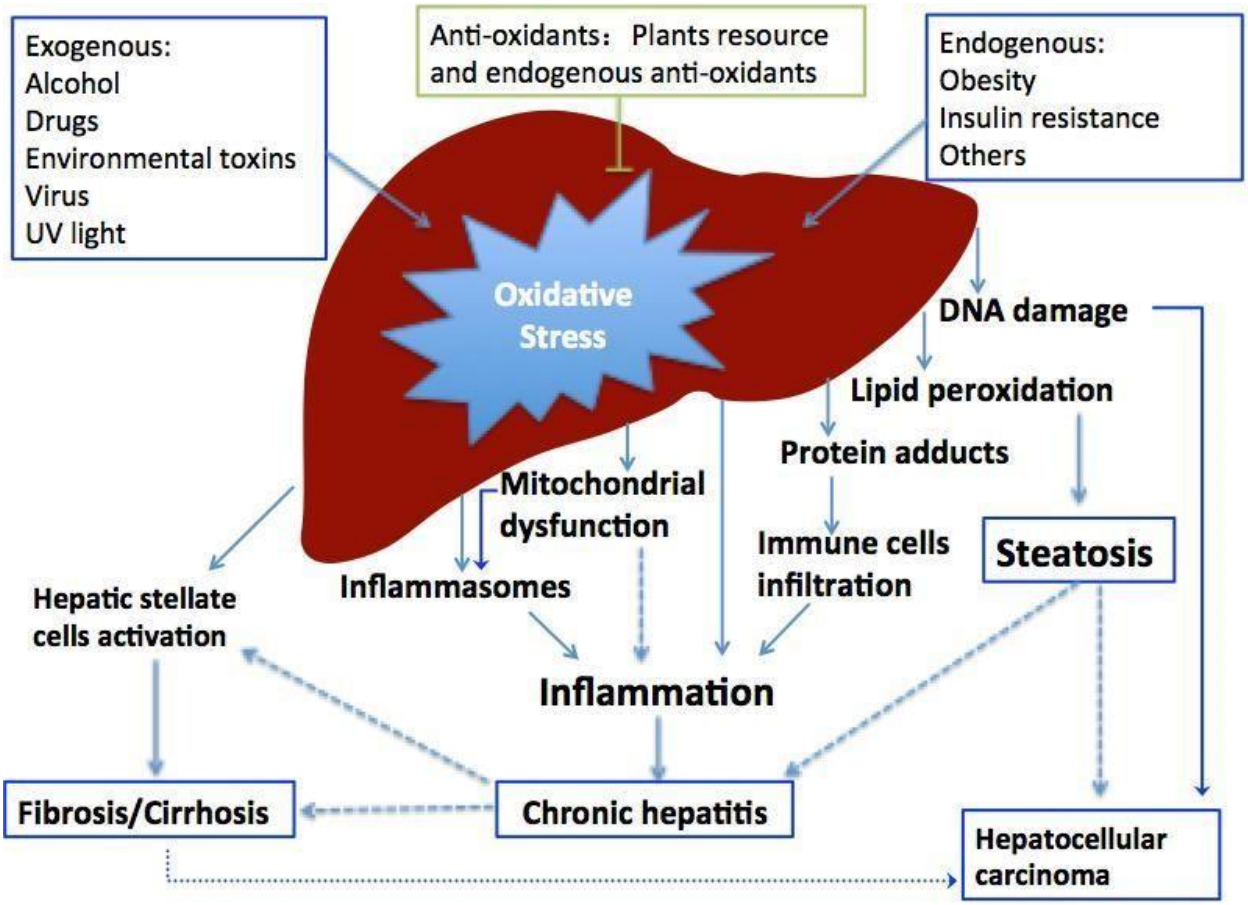

Figure 1: The general mechanism scheme of oxidative stress induced by various factors on liver diseases Source: Li *et al.* (2015)

1.5 Effects of Fried Oil on Hepatic Function

Cooking oils (edible oils) are vegetable oils which are extensively used for cooking. These edible oils are rich in triglycerides, sterol, tocoferol, carotenes and pigments (Shastry *et al.*, 2011).

Edible oils are sources of high energy supply to the body. Each gram of oil or fat supplies 9kcal of energy which is double the quantity of energy provided by protein and carbohydrate (Saha *et al.,* 2005). According to Nutritional Science News of November (1996), the consumption of diet rich in fish oil do not only lower blood cholesterol, triglycerides and lowdensity lipoprotein (LDL, which is the bad cholesterol), but also raises levels of beneficial high-density lipoprotein (HDL, which is the good cholesterol) (Frank and Painter, 1996).

The reuse of vegetable oils for food preparation has become a very common practice in both food outlets and homes. Several changes in flavour and taste are observed in the food prepared with reused vegetable oils. According to an earlier research carried out, the average number of times a batch of vegetable cooking oils is used before being discarded ranged from three to six times (Mensah and Obeng, 2013). This is because of its high thermal stability. During the frying process, various chemical reactions occur such as thermal oxidation, hydrolysis and polymerization due to the exposure of the oil to high temperature in the presence of air and moisture (Moreira *et al*., 1999; Gupta, 2005).

Fat associated liver conditions may be related to humans consuming foods heated at high temperatures in degraded and reused unstable fats. Consumption of these deep fried foods and the highly oxidized lipids is an emerging public health issue in human populations where the quality of frying fat is neither monitored nor regulated (Wafula *et al.*, 2021).

Frankel *et al.* (1984) observed that when saturated fats are excessively heated under high temperatures for a prolonged time, they undergo decomposition. Chow and Gupta (1994) were able to demonstrate that cooking fats start breaking down producing free fatty acids, diacylglycerols, and monoaclyglycerols that break down further to produce hydro peroxides and finally transformed into polymers that are of high molecular weight (Wafula *et al.,* 2021).

The repeated heating of oil at high temperature (180^{o}C and above) also results in thermal oxidation of the oil which causes the configuration of the fatty acids to change from the cis isomer to the trans isomer (Kamsiah *et al.*, 2011). This configuration change causes the polyunsaturated fatty acid to acquire undesirable properties associated with saturated fatty acids such as the correlation with increased serum cholesterol levels and higher lower density lipoprotein (LDL) cholesterol in particular (Wolfrum and Spenner, 2014). Oxidation of oil results in formation of various products, some of which are toxic and these oxidized fats in deep fried foods have been demonstrated to play a highly significant role in the pathogenesis of various diseases (Wafula *et al.*, 2021). Ingestion of these decomposition products or unstable oxidized lipids causes several alterations in the lipid and fatty acid metabolism that might be of great physiological relevance as suggested by Crnjar *et al.* (1981).

For example, radical species that are generated in the course of oxidation process damage some biomolecules (membrane lipid, protein and DNA) which manifest as cell

dysfunction, ageing, cardiovascular and neurodegenerative disease (Ayodeji *et al.,* 2015). The destruction of enzymes such as alanine transaminase, aspartate transaminase, lactate dehydrogenase etc. through continuous oxidation of oils also contribute to liver, heart and kidney dysfunction (Goldberg and Kirsch, 1996).

Wafula *et al.* (2021) in their study of the effects of reused high-fat diet on male rat livers observed that 20% reused high fat diet had caused liver steatosis in the study animals and it was suggested that reused high fat diet is the main cause of accelerated progression of steatosis to non alcoholic fat liver disease and finally liver cirrhosis. Further implications of these changes were the failure of the liver to synthesis and secrete major plasma proteins, which will consequently affect other body systems (Wafula *et al*., 2021).

Shastry *et al.* (2011) evaluated the effect of reused edible oils on the vital organs of rats using both sunflower and palm oil. Increase in biochemical parameters such as SGPT (serum glutamate–pyruvate transaminase), SGOT (serum glutamate-oxaloacetate transaminase), and ALP (alkaline phoshphatase) were increased in reused oils fed groups and the histopathological study showed the change in size of liver, heart, kidney and testes cells in reused oil groups. It was then concluded that reused sunflower oil and palm oil, can be toxic and can cause considerable damage to the vital organs of the experimental animals (Shastry *et al.,* 2011).

In another study, Maduelosi *et al.* (2019) investigated the effects of vegetable oil used for frying akara balls on lipid profile and liver enzymes of albino rats. The results revealed high level of CHOL and LDL, and low levels of HDL, TRIG and VLDL in all the test groups over the control with the levels increasing/decreasing with frying time. The liver enzymes (ALT, ALP and AST) levels in the test groups were also found to be high in test groups, suggesting hepatic injury as it was deduced to be as a result of liver damage, liver cell destruction and alteration in the permeability of the membrane. The study then suggested that consumption of vegetable oil that has been repeatedly used for frying has harmful effects in the body (Maduelosi *et al.,* 2019) .

1.6 Aims and Objectives of the Study

The main aim of the research work is to determine;

- the effect of aqueous concoctions of *Adansonia digitata* and *Cochorus olitorius* on fried oil induced hepatic damage in rats.
- to ascertain whether the aqueous concoctions of *Adansonia digitata* and *Cochorus olitorius* were potent enough to normalize the abnormal physiological conditions of the induced rats.
- to determine which of the two plants can be considered a better alternative to synthetic drugs.

CHAPTER TWO

2.0 Literature Review

2.1 *Adansonia digitata*

African baobab is a very long-lived tree with multipurpose uses. It is said that some trees are over 1000 years old. Earlier attempts to describe African Baobab on the basis of fruit difference are not accepted till now as they are not grown agronomically or domesticatedly (Chevalier, 1906). It is commonly found in the thorn woodlands of African savannahs, which tend to be at low altitudes with 4-10 dry months per year. It tends to grow as solitary individuals, though it can be found in small groups depending on the soil type. It is not found in areas where sand is deep. It is sensitive to water logging and frost. All locations where the tree is found are arid or semi-arid (Salim *et al.,* 2012).

The baobab tree and its related species belong to the Family of Malvaceae and the genus *Adansonia*. The tribe, which is pantropical, includes Bombax and Ceiba producing fruit fibres used as kapok. The family includes about 30 genera, 6 tribes and about 250 species (Baum, 1995). A number of these species are used locally for leaves, wood, fruits, seeds or gum. The African baobab (*A. digitata*) occurs naturally in most countries of Sahara as a scattered tree in the savannah, and is also present in human habitation. In the past, some ethnic groups in Mali such as the Dogon, Kagolo and Bambara used to take seedlings from the wild to plant them around their villages (Sidibé *et al.,* 1996). The tree has been

introduced in many countries used as an ornamental plant. It is also known as the dead-rat tree (from the appearance of the fruits), monkey-bread tree (the dry fruit as food for monkeys), upside-down tree (the bare branches looked like roots) and cream of tartar tree (the acidic taste of the fruits) (Sidibé and Williams, 2002).

The binomial name of *A. digitata* was given by Linnaeus, the generic name honouring Michel Adanson who had been to Senegal in the eighteenth century to describe baobab (Adanson, 1771).

Although *A. digitata* is mostly regarded as a fruit-bearing forest tree, it is a multipurpose and widely-used species with medicinal properties, numerous food uses of various plant parts, and bark fibers that are used for a variety of purposes. Centuries ago the products were traded: it was well known in Cairo markets in the sixteenth century (Sidibé and Williams, 2002). The various parts of the plant (leaves, bark and seeds) are used as a panacea, that is, to treat almost any disease and specific documented uses include the treatment of malaria, tuberculosis, fever, microbial infections, diarrhoea, anaemia, dysentery, toothache, etc. (Watt and Breyer-Brandwijk, 1962).

The seeds, leaves, roots, flowers, fruit pulp and bark of baobab are edible. Baobab leaves are used in the preparation of soup. Seeds are used as a thickening agent in soups, but they can be fermented and used as a flavouring agent or roasted and eaten as snack (Kabore *et al.*, 2011). The flora from Tikamgarh District in Bundelkhand Region has immense

pharmaceutical and commercial potential (Verma, 2014). India has about more than 45 000 plants species and among them several thousand are claimed to possess medicinal properties (Taur and Patil, 2011).

The fruit consists of pulp and large seeds embedded in the dry acidic pulp and shell. The fruit pulp has very high vitamin C content, almost ten times that of oranges. It contains sugars but no starch and is rich in pectin. It can be dissolved in water or milk. The liquid is then used as a drink and sauce for food, a fermenting agent in local brewing, or as a substitute for cream of tartar in baking. In India, it is reported that baobab pulp is used externally with buttermilk for the relief of diarrhea and dysentery, while the young leaves are crushed and used to treat painful swellings (Sidibé and Williams, 2002).

The leaves are used to make soup, and the pulp is used to make beverage and for food preparation (Yazzie *et al.,* 1994). During the rainy season when the baobab leaves are tender, people harvest a fresh batch of leaves. During the last month of the rainy season, leaves are harvested in great abundance and are dried for domestic use and for marketing during the dry season. The leaves are typically sun-dried and either stored as whole leaved or pounded and sieved into a fine powder (Gebauer *et al.,* 2002). Young leaves are widely used, cooked as spinach, and frequently dried, often powdered and used for sauces over porridges, thick gruels of grains, or boiled rice (Sidibé and Williams, 2002). The leaves and fruit pulp are used as febrifuge as well as an immune stimulant (El-Rawy *et al.,* 1997).

In recent years, due to industry seeks natural alternatives, demand for seed oils as ingredients for food, cosmetics and biofuel has been greatly increased. The seed is a good source of phosphorus, calcium and magnesium. Seed oils are important sources of nutritional oils, industrial and pharmaceutical importance (Nzikou *et al.,* 2012).

In some countries in West Africa, the leaves, fruit pulp and seeds are the main ingredients in sauces, porridges and beverages (Chadare *et al.,* 2009). Recently, baobab has been referred to as a "superfruit" based on its nutritional profile (e.g. vitamin, fatty acid, mineral) (Gruenwald, 2009). The major interest in baobab products is as a result of its ascorbic acid and dietary fiber content. The level of vitamin C contained in fruit pulp is high and can range from 2.8 to 3 g/kg. (Vertuani *et al.,* 2002). It was noted that baobab fruit pulp has very high vitamin C content (280–300 mg/100 g), which is seven to ten times more than oranges (51 mg/100 g) (Manfredini *et al.,* 2002).

One study demonstrated that the consumption of 40 g of baobab pulp provided 100% of the recommended daily intake of vitamin C in pregnant women (19–30 years) (Chadare *et al.*, 2009). The ascorbic acid content was evaluated in the fruit of *A. digitata* (Diop *et al.,* 1988); and it was found to contain 337 mg/100 g of ascorbic acid (Eromosele *et al.,* 1991). Sidibe and Williams recommended that baobab leaves should be stored as whole leaves rather than ground leaf powder in order to preserve the high vitamin content (Sidibé and Williams, 2002).

Abioye *et al.* (2020) in a research to determine the effects of drying methods on the nutritional and quality attributes of baobab leaves revealed that shade drying was found most suitable among the drying methods because it was able to retain most of the nutrients with significant difference in proximate, ascorbic acid and mineral contents from other drying methods.

2.1.1 Phytochemistry of Baobab Fruit Tree (*Adansonia digitata* L.)

Phytochemical investigation revealed the presence of flavonoids, phytosterols, amino acids, fatty acids, vitamins and minerals. Baobab (*Adansonia digitata* L.) leaves are being rich in secondary metabolites such as phenolic acids, flavonoids, particularly quercetin, kaempferol glycosides and procyanidins (Malabadi *et al.*, 2021).

A phytochemical study of a Nigerian baobab fruit pulp confirmed to the isolation of hydroxycinnamic acid glycosides, iridoid glycosides, and phenylethanoid glycosides. Further, procyanidin B2, gallic acid, and epicatechin has been identified in a Malawi baobab fruit pulp. Malian baobab fruit pulp and leaves were also the rich source of procyanidins and flavonol glycosides, with different in a qualitative composition compared to those of other African countries, indicating that growing region has affected their chemical profile (Zahra'u *et al.*, 2014; Venter *et al.*, 2017).

Stem bark and leaves contain a glycoside adansonin, scopoletin, tannin and friedeline. Seeds contain steroid, terpenoid, alkaloids and saponin. Roots have fatty oil containing

oleic, palmatic, stearic, linoleic and linolinic acid. Fruit pulp contains mucilage, gum, glucose, and tartrate. Baobab also contains fat, potassium, carbohydrate, fibre, protein, vitamin-C, calcium, iron, magnesium, phosphorous and thiamine (Gebauer *et al.,* 2016; Braca *et al.*, 2018; Sokeng *et al.,* 2019).

The ascorbic acid content was evaluated in the fruit of *Adansonia digitata* and it was found to contain 337 mg/100 g of ascorbic acid. The fruit pulp have a very high vitamin C, calcium, phosphorus, carbohydrates, fibers, potassium, proteins and lipids content, which can be used in seasoning as an appetizer and also make juices. Seeds contain an appreciable quantities of phosphorus, magnesium, zinc, sodium, iron, manganese, whereas they have high levels of lysine, thiamine, calcium and iron (Zahra'u *et al.*, 2014).

The alkaloid 'adansonin' in the bark is thought to be the active principle for the treatment of malaria and other fevers, as a substitute for quinine. Saponins present in the leaves of African baobab exhibited a good antibacterial, anti-inflammatory, and immune-boosting properties. The flavonoid is considered to have good antioxidant abilities, antiviral, anticancer, anti-inflammatory, and anti-allergic properties with good therapeutic agents. Total phenols possessed anti-carcinogenic and antimutagenic effects by acting as protective agents of DNA against free radicals (Adesanya *et al.*, 1998; Zahra'u *et al.*, 2014; Sundarambal *et al.*, 2015; Gebauer *et al.*, 2016; Venter *et al.,* 2017; Braca *et al*., 2018; Zagga *et al.*, 2018).

Adansonia digitata L. seeds contain trypsin inhibitors, tannins, cyanogenic glycosides, oxalate, phytic acid, and alkaloids. The trypsin inhibitor has been implicated to cause adverse effects on the utilization and availability of protein. The African baobab seed also contains about 13 mg/100 g of phytic acid and 23.5 mg/ 100 g catechin. These antinutritional factors can be decreased by varying processing factors and methods consequently increasing the bioavailability of essential nutrients. These methods include thermal processing, soaking, sprouting, boiling, fermentation, dehulling, germination or a combination of two (2) or more. The accceptability and optimal utilization of baobab parts as a nutrient source is limited by the presence of anti-nutrients such as protease inhibitors, tannins and phytates but the processing techniques may reduce or destroy the anti-nutrient profile (Adesanya *et al.*, 1998; Zahra'u *et al.*, 2014; Sundarambal *et al.*, 2015; Gebauer *et al.*, 2016; Venter *et al.,* 2017; Braca *et al*., 2018; Zagga *et al.*, 2018).

2.1.2 Hepato-protective Activity of *Adansonia digitata*

The hepatoprotective activity of an aqueous extract of the fruit pulp was evaluated in vivo against chemical-induced toxicity with CCl_4 in rats. The results clearly showed that the water extract exhibited significant hepatoprotective activity. The liver protective ability of *A. digitata* extract was 76, 77, and 87% for alanine transferase, aspartate transferase and alkaline phosphatase activity, respectively when the plant extract was administered at the start of CCl_4 toxicity. The results suggest that consumption of *A. digitata* fruit may play an

important part in resistance to liver damage in areas where baobab is consumed (Al-Qarawi *et al.*, 2003).

Although the mechanism of action of liver protection could not be established, this protective activity could be due to triterpenoids, β-sitosterol, β-amyrin palmitate, and/or α-amyrin and ursolic acid present in the fruit (Al-Qarawi *et al.*, 2003). The acute toxicity of baobab fruit pulp extract was tested in vivo on rats and the results showed that the LD50 was 8000 mg/kg following parenteral administration suggesting low toxicity (Ramadan *et al.*, 1994).

In another research by Sa'id *et al.* (2020) evaluating the photochemical composition and hepato-protective potential of aqueous fruit pulp extract of *Adansonia digitata* against CCl_4 induced liver damage in rats revealed the presence of certain phytochemical constituents in *A. digitata* like tannins, saponins, flavonoids, alkaloids, terpenes and glycosides indicates its hepatoprotective effect as some of the constituents have antioxidant activity. Also, there was significant decrease in serum AST, ALT, ALP, TBIL, bilirubin, total protein (TP) and albumin (ALB) relative to the CCl_4 treated group suggesting lessened hepatic damage by the administration of *A. digitata* fruit pulp extracts indicated recovery from CCl_4 hepatic damage and suggested its healing potentials as well (Sa'id *et al.,* 2020).

The effects of baobab seed oil on drug metabolising enzymes (cyclopropenoid fatty acids) were evaluated in vivo in rats fed either with baobab seed oil (1.27% cyclopropenoid fatty acids) or heated baobab seed oil (0.046% cyclopropenoid fatty acids in the diet). The rats

fed baobab oil showed retarded growth when compared to other groups of animals. Furthermore, the relative liver weights were markedly increased whereas cytochrome P-450 content and NADPH cytochrome C reductase and NADH cytochrome C reductase activities were decreased (Andrianaivo-Rafehivola *et al.,* 1995).

2.2 *Corchorus olitorius*

Corchorus olitorius L. commonly known as molokhia in Middle-Eastern countries is an annual herbaceous plant with slender stem belonging to Tillaceae family. Although molokhia is believed to be native to Africa and Asia, it is cultivated in a number of countries, including Australia, South America, and some parts of Europe for food and industrial use (Kundu *et al.,* 2012). Molokhia is used as green leafy vegetable in Egypt, Sudan, India, Bangladesh, Philippines and Malaysia, Africa, Japan, South America, the Caribbean, and Cyprus (Tulio *et al.,* 2002). It is commonly known as Jew's mallow, bush okra, long- fruited jute, tossa jute, and jute (Islam, 2013).

There is no consensus on the origins of *C. olitorius* because it has been cultivated and used in Africa and Asia for centuries and also occurs in the wild in both continents. Kundu *et al.*, 2012). Currently, since it is used as a leafy vegetable, it occurs in all of tropical Africa including Benin, Nigeria, Ivory Coast, Cameroon, Sudan, Kenya, Uganda, and Zimbabwe. It is a popular soup vegetable in Caribbean, Cyprus, Brazil, India,

Bangladesh, Sri Lanka, China, Japan, Philippines, Malaysia, Egypt, and the Middle Eastern countries. Apart from food use, it is grown as a commercial crop for jute production in India, Bangladesh and China (Holm, 1979).

Molokhia is valued as a nutritious leafy vegetable due to its high vitamin, mineral, and phenolic content (Giro and Ferrante, 2016). The leaves also contain high amounts of mucilaginous polysaccharides with gives viscous consistency and widely consumed as soup in Middle Eastern countries. Dried leaves are used in herbal tea, while seeds are used as flavoring agent (Ohtani *et al*., 1995). Apart from its food uses, it is valued as an herbal remedy in fevers, enteritis, dysentery, chronic cystitis, aches, and pains (Zakaria *et al.*, 2006). Different parts of *C. olitorius* are reported to exhibit a range of biological effects antimicrobial, antidiabetic, aantihistaminic cardioprotective, hepatoprotective, nephroprotective, anticonvulsant, antiestrogenic, and antimalarial effects (Khan *et al.,* 2006).

The leaves are rich sources of Vitamin A (β- carotene), C, E, B1, B2, folic acid, and minerals such as iron and calcium (Helaly *et al.,* 2016). Among soluble polysaccharides abundantly present in molokhia leaves, an acidic polysaccharide rich in uronic acid rhamnose, glucose, galcturonic acid, and glucuronic acid has been isolated (Ohtani *et al.,* 1995). It is noteworthy that, molokhia leaves retain key nutrients even after cooking (Abdalla *et al.,* 2016).

The seeds contain alkaloids, flavonoids, tannins, cardiac glycosides, steroids, saponins, and anthraquinones (Maxwell *et al.,* 2014). The roots reportedly contain triterpenes including corosin and sterols including sitosterol and stigmasterol (Manzoor-I-Khuda and Habermehl, 1979). It is noteworthy that molokhia is also known to contain antinutritional factors including phytates, hydrocyanic acid, oxalic acid, and tannins (Helaly *et al.,* 2016).

Molokhia leaves contains high amounts of mucilaginous polysaccharides which yield viscous soup when cooked and usually used as an accompaniment for main dishes (Adebo *et al.,* 2018). In Middle Eastern countries, the leaves are cut into small pieces and boiled in water with salt and pepper to make soup. Molokhia soup is very popular in the Middle east. In Mediterranean regions, young green leaves and shoots are used to add flavour and viscous texture to soups and stews. Seeds are used for flavouring. Tender leaves and shoots are also eaten raw as salad vegetable in Egypt and India (Ali and Nasir, 1999). Dried leaves are used in the preparation of herbal tea. The leaves are used to prepare a stew called "ewedu" in Nigeria, while in Philippines, the leaves along with bamboo shoots are consumed as a leafy vegetable (Ahmed, 2021).

In Nigeria, sticky sauce comparable to okra is prepared and eaten as an accompaniment for starchy dumplings made from cassava, yam or millet. Since, Molokhia is an annual herb dried leaf powder is used to make this sauce during off season. Sauce is also prepared from powdered and dried immature fruits (bush okra). In East Africa, it is cooked with cowpeas, pumpkin, cocoyam leaves, sweet potato, milk, butter, and meat flavored with pepper and

lemon (Abdul and Kaushik, 2015). Recently, molokhia leaves are also used for the development of Sushi wrap as a promising viable substitute for Nori (Castro *et al.,* 2018).

The leaf extract of the plant is also employed in folklore medicine in the treatment of gonorrhea, pain, fever, and tumors. Its leaves and roots are eaten as herbal medicine in South east Asia (Afolayan and Ndlovu, 2008). In some parts of Nigeria, its leaves decoctions are used for treating iron deficiency, folic acid deficiency, as well as treatment of anemia. Leaves are used in ascites, pains, cystitis, piles, dysentery, dysuria, pectoral pain, tumors, gonorrhea, and female infertility (Fasinmirin and Olufayo, 2009). The leaves are particularly used as an herbal medicine in typhoid and malarial fevers (Adebo *et al.,* 2018).

Leaves are also used as blood purifier and leaf twigs are used cardiac problems while, leaf infusion is taken as a tonic and appetite enhancer. The leaves are also used in the treatment of constipation in Tanzania (Fasinmirin and Olufayo, 2009). In Benin leaves are used as tonic, diuretic, emollient, as blood purifier, in heart disease and infantile malnutrition (Fagbohoun and Ibrahim, 2011). Root scrapings are used to treat toothache, while decoction of the roots is used as a tonic to increase strength. In Nigeria, seeds are used as purgative and febrifuge (Calleja, 2010).

2.2.1 Phytochemistry of *Corchorus olitorius*

Different parts of *C. olitorius* have been reported to exhibit a wide range of biological activities in vitro and in vivo which are attributed to its phytochemical composition (Ahmed, 2021).

The leaves are reported to contain triterpenes, sterols and fatty acid, phenolics, ionones, oxydase, chlorogenic acid, glycosides, saponins, tannins, and flavones in addition to carbohydrates, protein, fat, fiber, ash, acidic polysaccharides, lignin, and other mucilaginous polysaccharides (Azuma *et al.,* 1999).

Jute leaf is a unique plant part which is a rich source of many chemical compounds and plays an important role in the National and international market (Islam, 2013). Jute leaves are reported to contains as many as 17 active nutrients compounds including protein, fat, carbohydrate, fiber, ash, calcium, potassium, iron, sodium, phosphorous, beta-carotene, thiamine, riboflavin, niacin, ascorbic acid etc. (Calleja, 2010; Islam, 2010).

Leaves contain oxydase and chlorogenic acid. The folic acid content is substantially higher than that of other vegetables, ca 800 micrograins per 100g (ca 75% moisture) or ca 3200 micrograms on a zero moisture basis (Chen and Saad, 1981). This green, leafy vegetable is rich in beta-carotene for good eyesight, iron for healthy red blood cells, calcium for strong bones and teeth, and vitamin C for smooth, clear skin, strong immune cells, and fast wound-healing. Vitamins A, C and E present in Saluyot “sponge-up” free radicals, scooping them up before they can commit cellular sabotage (Chen and Saad, 1981).

Some other compounds that can be found in other parts of the plants e.g. the seeds and flowers include Chorchoroside A-E, Corosin, Corosolic acid, Olotoriside, Oxocorocin, Hexadecanoic acid, Hexanoic acid, methyl ester, Hexanone, Hexenyl benzoic acid, Hydrocarbons, Isoamyl butyrate, Limonene, Methyl tiglate, Nerolidol, Nonadecane, Octadecanoic acid, Phenyl ethyl tiglate, Sabinene, Terpenes, etc. (Ahmed, 2021).

2.2.2 Hepato-protective Activity of *Corchorus olitorius*

Although various extracts of molokhia leaves have shown antioxidant activity both in vitro and in vivo, studies on hepatoprotective effects in various animal models report conflicting findings. Some of the studies report strong hepatoprotective effect, while some studies report adverse effects in hepatotoxicity models (Ahmed, 2021).

Ethanol extract of the leaves were evaluated for protective effect against CCl_4 induced hepatotoxicity in Wistar rats at dosage levels of 500, 750, and 100 mg/kg. Oral administration of the extract for 15 days showed dose-dependent reduction in serum transaminases (ALT and AST), alkaline phosphatase, and serum albumin toward control levels. Furthermore, a significant decrease in serum albumin, platelet, and white blood cell count, but no significant differences were observed with respect to serum bilirubin, hemoglobin, and packed cell volume. On the contrary, total protein concentration was found to be elevated in extract-treated groups (Ujah, 2014).

Pretreatment with aqueous leaf extract at a dose 50 and 100 mg/kg of showed significant restoration of key hepatic and renal biomarkers in sodium arsenite-induced toxicity in rats. The extract not only attenuated the effects of sodium arsenite as evidenced by increased levels of catalase, SOD, GSH reductase GSH-S-transferase, and GSH peroxidase but also reduced fragmentation of DNA in liver and kidney tissues. The biochemical findings were substantiated by histological studies (Das *et al.,* 2010).

Similar findings were reported by Haridy *et al.* (2020) wherein, aqueous extract at the dose of 500 and 1000 mg/kg exhibited significant hepatoprotection against CCl_4-induced hepatotoxicity in rats. The extract restored elevated levels of alanine transaminase, AST, alkaline phosphatase and malondialdehyde to normal levels. The activity of GSH peroxidase which was increased on administration of CCl4 also restored to normal levels with extract treatment. The hepatoprotective effect was dose dependent and 1000 mg/kg dose showed higher effect mediated through strong antioxidant activity (Haridy *et al.,* 2020).

The hepatoprotective activity of molokhia leaf supplemented diet (100 mg/g) was evaluated in streptozotocin induced diabetic rats. The results indicated as significant increase in the activities of hepatic δ-aminolevulinic acid dehydratase (δ-ALAD), catalase, SOD and decreased serum transaminases (AST and ALT). The study concluded that restoration of hepatic δ-ALAD activity, strengthen antioxidant defense systems and modulating hepatic

function biomarkers could be possible factors responsible for the hepatoprotective effects of molokhia leaves in diabetes (Saliu *et al.,* 2019).

Azeez et al. (2019) reported hepatoprotective activity of molokhia leaves grown with synthesized biogenic AgNPs restored hydrogen peroxide induced reduction in catalase concentrations and elevated malondialdehyde levels toward normal levels in the liver. It was concluded that molokhia leaves possess significant antioxidant and hepatoprotective effects. Ethanol extracts of molokhia leaves at 200 mg/kg dose significantly reduced the levels of serum transaminases, alkaline phosphatase, bilirubin, urea, and creatinine levels in thioacetamide induced hepatotoxicity in experimental rats. Histopathology revealed that the extracts restored tissue architecture of both liver and kidney tissues (Sule *et al.,* 2017).

In another study, hepatoprotective effects ethanol extract of molokhia leaves at three dosage levels, namely, 50, 100, and 200 mg/kg was evaluated in normal rats. Oral feeding of the extract for 28 days significantly reduced the levels of ALT, aspartate aminotransferase, and alkaline phosphatase. The extract also reduced total cholesterol levels at 50 and 100 mg/kg dose and resulted in a dose dependent increase in HDL levels. Since the extract at 200 mg/kg increased cholesterol levels, it was opined that the extract offers hepatoprotection with possible tendency to increase total cholesterol levels (Omeje *et al.,* 2016). However, controlled experiments are required to arrive at meaningful conclusion in this regard because molokhia leaf supplemented diet resulted in a significant hepatotoxic effect in CCl_4 induced hepatotoxicity in rats. Supplementation of molokhia

leaves in the at 5% and 10% levels did not result in significant improvements in hepatic function biomarkers and also did not enhance antioxidant defense systems. However, histological study revealed a significant damage to the liver tissue in molokhia supplemented CCl_4 treated rats indicating potentiation of hepatotoxic effects of CCl_4(Iweala and Okedoyin, 2014) .

2.3 Phytochemicals in the Treatment of Liver Diseases

Plants are the major source of medications in folk medicinal systems, nutraceuticals, pharmaceutical intermediates, modern medicines, food supplements, and also act by adding chemical moieties in synthetic medicines (Dewick, 1996). Phytochemicals are categories under several classes such as flavonoids, phenol, carotenoids, alkaloids, tocopherols, terpenoids, polyphenols, saponins, phytosterols, and organosulfur compounds. Phytochemicals within plants generally provide protection against pests, pathogens, predators, and the external environment (Cowan, 1999). There are more than 7500 different polyphenolic compounds are identified in plant kingdom which are medicinally important. Grains, Cereals, fruits, legumes, nuts, vegetables and spices are rich in polyphenols (Opara and Rockway, 2006).

Bioactive compounds are a natural source of antioxidants and antiinflammatory biomolecules, which are actively used in amelioration of hepatic injury. Generally they are profusely present in plants such as phenol, flavonoids, alkaloids, polyphenols, terpenoids, glycosides, and saponins. Several in vivo, in vitro, and ex-vivo studies have suggested that

bioactive components possess immunomodulatory, antioxidant, antimicrobial, antimutagenic, hypoglycemic, hypolipidemic, anti-inflammatory, gastroprotective, anticancer, and chemopreventive activities (Kumar and Pandey, 2013).

One of the major problems in interpretation of studies attempting to show a benefit of antioxidant therapy of liver disease is the inability to know the exact mechanisms of action of specific compounds labeled "antioxidants". Many plant derived compounds demonstrate in vitro and even in vivo antioxidant capacity but these effects may not be responsible for their biological activity. Of the various available antioxidants, strong evidence exists that several agents mediate their action primarily based on changes in
ROS and redox state of the cell. These agents include vitamins E and C, N-acetylcysteine (NAC), mitoquinone (mitoq), and polyenyl phosphatidylcholine (PPC). Other compounds, such as Silymaryin, S-adenosyl methionine, and betaine have additional prominent nonantioxidant effects that may be responsible for their clinical effects (Singal *et al.,* 2011).

The major cell antioxidants include glutathione (GSH), tocopherol (vitamin E) and vitamin C. (WHO, 2019). Vitamin E serves as an antioxidant by complexing with unpaired electrons thus stabilizing these free radical compounds and preventing lipid peroxidation (Zingg, 2007). Some effects observed include a decrease in production of tumor necrosis factor (TNF) in alcoholic hepatitis (Hill *et al.,* 1999), and prevention of hepatic stellate cell (HSC) activation in chronic hepatitis C. Alpha tocopherol accounts for almost 90% of the total vitamin E in the human tissues (Houglum *et al.,* 1997).

Vitamin C or ascorbic acid serves as an electron donor and thus can terminate free radical chain reactions. Similar to vitamin E, however, its ability to serve as an electron donor makes it possible for it to actually generate free radicals when at high concentrations in the presence of metal ions (Abudu *et al.,* 2004). NAC acts by increasing hepatic GSH levels, and serving as a free thiol itself. It is widely used for the treatment of acetaminophen overdose (Zafarullah *et al.,* 2003). As the only member of this group that is itself a reduced thiol, it has unique potential to augment GSH levels and drive protein thiol redox reactions to the reduced form (Singal *et al.*, 2011).

However, the available data regarding the benefits of antioxidant treatment in patients with chronic liver diseases is insufficient. A differentiation between antioxidants regarding their liver-protective effects is not possible until now, as they have different chemical structures and different anti-oxidizing potency. The results of many studies have suggested that antioxidants may be used as adjuvants in numerous diseases, especially in those where an increase of oxidative stress is involved, including chronic liver diseases (Gheorghe *et al.,* 2019).

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

3.1.1 Plant Materials and Authentication

Leaves of *Adansonia digitata* and *Corchorous olitorius* were obtained from Ipata Market, Ilorin, Kwara state, Nigeria. They were authenticated at the Department of Plant Biology, University of Ilorin and the Voucher Numbers were obtained as UICH/OO1/951 and UICH/002/154 respectively. They were then deposited at the herbarium. The leaves of *Adansonia digitata* and *Corchorous olitorius* were removed from their stalks, washed thoroughly with clean water and air-dried at room temperature (28^{o}C) for two weeks. The leaves were pulverized with electric blender (Steelman K207, H.M and co Ltd, China) to powdered form, sealed and stored in polythene bags in a cool, dry place pending the period of use.

3.1.2 Animals

A total of thirty-six (36) male Wistar rats with an average weight of 100g were purchased from the Animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were distributed into six groups, of six rats each, housed in clean and well ventilated cages and maintained on pelletized rat feed (Vital Feed, Grand Cereals, Jos, Nigeria) and distilled water for one week.

3.1.3 Fried Oil

Used Vegetable oil was purchased from Oke-odo junction, Tanke, Ilorin, Kwara State.

3.1.4 Drugs, Chemicals and Assay Kits

Vitamin C used in this study was a product of Monobind International, Lake Forest, USA. Assay kits for ALT, ALP and AST, albumin, bilirubin, SOD, CAT, GST, GPx and GSH

kits were products of Randox Laboratories Ltd. Company, Antrim, UK. All chemicals were prepared in all-glass distilled water.

3.2 Methods

3.2.1 Administration of Fried Oil

A known volume (1ml) of fried oil was orally administered to each rats in groups 2 to 6 for a period of one week. All groups (1 to 6) were maintained on the pelletized feed and distilled water during this period.

3.2.2 Administration of Vitamin C, *Adansonia digitata* and *Corchorus olitorius* Leaves

- *Adansonia digitata* leaves concoction was prepared by the addition of 240mg of each pulverized leaves into 2000ml of distilled water and stored in the refrigerator a day before use.
- *Corchorus olitorius* leaves concoction was prepared by the addition of 240mg of each pulverized leaves into 2000ml of distilled water and stored in the refrigerator a day before use.
- A mixture of *Adansonia digitata* and *Corchorus olitorius* leaves concoction was prepared by adding 120mg of each in 2000ml of water then mixed together and stored in the refrigerator a day before use.

- Vitamin C was prepared first by grinding Vitamin C tablets into powder using mortar and pestle, then 240mg/kg body weight was dissolved in 2000ml of water and stored in the refrigerator a day before use.

The prepared concoctions were then administered to the rats.

Group 1 (positive control) and group 2 (negative control) were not administered any of the concoctions prepared. Group 3 were administered 4ml of *Adansonia digitata* concoction, Group 4 were administered 4ml of *Corchorus olitorius* concoction, Group 5 were administered 4ml of a mixture of *Adansonia digitata* and *Corchorus olitorius* concoction while Group 6 were administered 4ml of Vitamin C (synthetic drug). These procedures were repeated for one week and all groups were maintained on pelletized feed and clean water during this period.

Table 1: Animal Grouping and Administrations

Groups	**Pelletized feed and water**	**Fried oil**	**Treatment (Administration of *Adansonia digitata,* *Corchorus olitorius* and Vitamin C)**
1	Maintained	Not administered	Not treated
2	Maintained	Administered	Not treated
3	Maintained	Administered	*Adansonia digitata* concoction
4	Maintained	Administered	*Corchorus olitorius* concoction

5	Maintained	Administered	*Adansonia digitata* and *Corchorus olitorius* concoction
6	Maintained	Administered	Vitamin C solution

3.2.3 Preparation of Potassium Chloride (KCl) Solution

1M KCl solution was prepared by dissolving 25g of KCl salt crystals in 100ml of distilled water.

3.2.4 Preparation of Phosphate Buffer Solution (pH=7.4)

A known weight (7.30g/dm^3) of Monosodium Dihydrogen Phosphate (NaH_2PO_4) and 14.30g/dm^3 of Dipotassium Hydrogen Phosphate (K_2HPO_4) were each dissolved in 1000ml of distilled water and both solutions were mixed together. The pH of the buffer was determined using a pH meter and it read 6.97. 10% NaOH solution was then added adjust buffer to pH of 7.4.

3.2.5 Animal Sacrifice

After administration, the rats were anaesthetized using diethyl ether soaked in cotton wool. The soaked cotton wool was housed in a closed glass container and the rats were placed in the container to lose consciousness. The blood of each rats were collected by ocular puncture into separate and appropriately labeled heparinized and non-heparinized bottles for haematological and biochemical serum analysis. The animals were further dissected using sterilized dissecting tools to collect the livers which were rinsed in 1.15%

KCl solution and housed in universal tubes before being stored in the refrigerator.

3.2.6 Homogenization

A known weight (1g) of each liver was homogenized in homogenizing buffer, in the ratio 4:1 (4ml of buffer for every 1g of liver). The homogenates were then collected in plain tubes, labelled appropriately and stored in a refrigerator until required for further analysis.

3.2.7 Preparation of Serum

The blood samples collected in non-heparinized tubes as well as the homogenized liver were cold-centrifuged at 4000rpm for 10 minutes so as to separate the plasma from the serum and the supernatants were pipetted and collected, labeled appropriately and stored in a refrigerator for subsequent biochemical analysis.

3.3 Biochemical Assays

3.3.1 Secondary Metabolite Screening of *Adansonia digitata* and *Corchorus olitorius*

Qualitative and quantitative secondary metabolites screening of *A. digitata* and C. *olitorius* leaves were determined by adopting the methods described by Harborne (1993); Sofowora (1993) and Ajayi *et al.* (2010). All determinations were done in triplicates.

3.3.1.1 Saponins

Exactly 1g of each powdered sample were separately boiled with 10ml of distilled water in water bath for 10 mins. The mixture was filtered while hot and allowed to cool.

Exactly 2ml of filtrate was diluted to 10ml with distilled water and shaken vigorously for 2 mins.

3.3.1.2 Terpenoids

Exactly 5ml of each extract was mixed with 2ml of chloroform. Concentrated H_2S0_4 (3ml) was then added to form a layer. A reddish-brown precipitate was formed, which indicated the presence of terpenoids.

3.3.1.3 Flavonoids

A known weight (1g) of powdered sample of *A. digitata* and *C. olitorius* leaves were separately boiled in 20ml of water and then filtered. Exactly 5ml of dilute ammonia solution was added to a portion of the filtrate, followed by the addition of concentrated H_2SO_4. A yellow coloration indicated the presence of flavonoids.

3.3.1.4 Tannins

A known weight (1g) of each powdered sample of *A. digitata* and *C. olitorius* were separately boiled in 20ml distilled water for five minutes in a water bath and was then filtered while hot. Exactly 1ml of cooled filtrate was diluted with 5ml distilled water and a few drops of 10% ferric chloride was added. The reaction mixture showed the formation of a brownish green colouration, which revealed the presence of tannins.

3.3.1.5 Alkaloids

A known weight (lg) of each powdered sample of *A. digitata* and *C. olitorius* were separately boiled with water and acidified with 5ml of 1% HCI on a steam bath. The solutions obtained were filtered after which 2ml of the filtrate was treated with few drops

of Mayer's reagent(potassium mercuric iodide). The formation of a creamywhite

precipitate indicated the presence of alkaloids in the extracts.

3.3.1.6 Cardiac Glycosides

Exactly 5ml of each, of the extracts of *A. digitata* and *C. olitorius* were treated with 2ml of glacial acetic acid, containing one drop of ferric chloride solution. After which, 1ml of concentrated sulphuric acid was added to the mixture. There was no brown-ring precipitate observed at the interface, which indicated that deoxy sugar characteristics of cardenolides was not present.

3.3.1.7 Polyphenol

Exactly 0.5g of the powdered leaf of each of *A. digitata* and *C. olitorius* were boiled in 10ml distilled water for 5mins and filtered while hot. Then 1ml of ferric chloride solution was added to each solution separate. There was formation of blue-black colouration, which revealed, the presence of polyphenol.

3.3.1.8 Steroids

Exactly 0.2g each of the powdered *A. digitata* and *C. olitorius* leaves were dissolved in 2ml chloroform. After which 0.2ml of concentrated H_2S0_4 were carefully added to form a reddish-brown colour at the interface, which indicated the presence of steroid.

3.3.1.9 Phlobatannins

Exactly 2.5 ml of aqueous extract of *A. digitata* and *C. olitorius* leaves was boiled with 1% aqueous hydrochloric acid, there was no deposition of a red precipitate, which is an indication for the absence of phlobatannins.

3.3.1.10 Anthraquinones

Exactly 0.5g of the powdered leaf of *A. digitata* and *C. olitorius* were dissolved in 5 ml of chloroform separately. The resulting mixture was shaken for 5 minutes, after which it was filtered. The filtrates were thoroughly mixed with equal volume of 10% ammonia solution. The absence of a bright pink colour in the aqueous layer indicates the absence of anthraquinones.

3.3.2 Quantitative Constituents of *Adansonia digitata* and *Corchorus olitorius* Leaves

3.3.2.1 Polyphenol Content

The amount of polyphenol in the methanol extract was determined by using Folin-Ciocalteu reagent. A known volume (2.5ml) Of 10% Folin-Ciocalteu reagent and 2 ml of Na_2C0_3 solution (2%) was added to 1ml each of the leaf extracts of *A. digitata* and *C. olitorius* leaves. The resulting mixture was incubated for 15 minutes at 28ºC. The absorbance of the sample was read at 765nm. Gallic acid was used as standard (1mg/ml). All the tests were performed in triplicates. The results were determined from the standard calibration curve and were expressed as gallic acid equivalent (mg/g of extracted compound) (Siddhuraju and Becker, 2003).

3.3.2.2 Total Flavonoid Content

Aluminium chloride colorimetric method was used to determine the flavonoid content. 1ml each of *Adansonia digitata* and *Corchorus olitorius* leaves was mixed with 3ml of methanol, 0.2 ml (10%) of Aluminium chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water at 27ºC for 30 minutes. The absorbance was read at 420nm.

Quercetin was used as standard (1mg/ml). All the tests were performed in triplicates. Flavonoid content was determined from the standard calibration curve and was expressed as quercetin equivalent (mg/g of extracted compound) (Zhishen *et al.*, 1999).

3.3.2.3 Alkaloid content

A known weight (5g) of the sample or *Adasonia digitata* and *Corchorus olitorius* was weighed into a 250ml beaker containing 200 ml of 10% acetic acid, ethanol was added and covered for 4 hours. It was filtered using filter Number 4 filter paper and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated 5ml ammonium hydroxide was added dropwise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1993).

3.3.2.4 Saponin Content

The method used was according to Obadoni and Ochuko (2001). Exactly 20g each of the powdered form of *Adansonia digitata* and *Corchorus olitorius* was put into a conical flask and 100ml of 20% aqueous ethanol was added. The mixture was heated over a hot water bath for 4h with continuous stirring at about 55ºC. The mixtures were filtered and residue

re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90ºC. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. About 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in water bath. After evaporation the samples were made to dry in the oven to a constant weight and the saponin content was calculated as percentage.

3.3.2.5 Determination of Tannins Content

Dried plant of *Adansonia digitata* and *Corchorus olitirius* (0.5g) was extracted with 300ml of diethyl ether for 20 hours at 28ºC. The residues were boiled for 2 hours with 100ml of distilled water, and then allowed to cool before being filtered. The extract was adjusted to a volume of 100ml in a volumetric flask. The content of tannins in the extract was determined colourimetrically using Folin-Denis's reagent, and by measuring absorbance of the blue complex at 760nm, using tannic acid solution as a standard solution (Boham and Kocipai, 1994).

3.3.3 Toxicity Study

The procedure for the determination of liver functional indices, haematologieal parameter, antioxidant parameters and serum lipid profile were carried out as stated below.

3.3.3.1 Determination of Liver Function Indices

3.3.3.1.1 Serum Bilirubin

Principle:

Bilirubin was determined by adopting the procedure described by Sherlock (1951). This principle was based on the calorimetric method described by Sherlock (1951).

Procedure:

(a)Total bilirubin

A known volume (0.2ml) of reagent A (Sulphanilic), a drop of reagent B (Caffeine) and 1ml of reagent C (NaOH) was pipetted into a test tube. A known volume (0.2ml) of the diluted serum was pipetted into the test tube. Exactly 0.2 ml of reagent A, a drop of reagent B and 0.1 ml of reagent C was pipetted into another test tube and 0.2 ml of distilled water was added to serve as the blank. The mixture was incubated at 37^{o}C for 30 minutes. A known volume (1ml) of reagent D was added to all the test tubes. The mixtures were incubated further at 25^{o}C for 30 minutes. Absorbance was read at 578 nm.

Calculation:

Total bilirubin (µmol/L) = 185 x A_{TB} (578nm)

3.3.3.1.2 Serum Albumin

The procedure described by Doumas *et al.* (1971) was used for the determination of albumin in the serum of the animals.

Principle:

The measurement of serum albumin is based on its quantitative binding to indicator 3, 3', 5, 5'- tetrabromo-cresol sulphonapthalein (bromocresol green BCG). The

albumin-BCG complex absorbs maximally at 578 nm, the absorbance being directly proportional to albumin concentration in the sample.

Procedure:

A known volume (0.01ml) of the diluted serum was pipetted into a test tube and 3ml of reagent B (BCG reagent) was added. Also, 3ml of reagent B was added to 0.01ml of distilled water in another test tube which constituted the blank. 3ml of reagent B was added to 0.01ml of reagent A (Albumin standard) in another test tube which served as standard. The mixture will be incubated at 37^{o}C for 10 minutes. Absorbance was read spectrophotometrically at 630 nm.

Calculation: Albumin concentration (mg/mL) = $\frac{A_{sample} \times \text{Concentration of Standard}}{A_{standard}}$

Concentration of standard = 46.73 mg/mL

3.3.3.1.3 Protein Concentration

The protein concentration in the tissues and serum of animals was assayed, using Biuret reagent as described by Gornall *et al.* (1949).

Principle:

Cupric ions form a purple-coloured complex with compounds containing repeated amide linkages (CONH-) in basic medium. The purple colour is due to the coordination between the cupric ions and the unshared electron pair of peptide nitrogen and the oxygen of water.

Procedure:

A known volume (1ml) of the appropriately diluted supernatant was pipetted into the test tube and 4ml of Biuret reagent was added. This were thoroughly mixed and left undisturbed for 30 minutes at 27ºC. Exactly 4ml of Biuret reagent was added to 1 ml of distilled water in another test tube which constituted the blank. The absorbance was read against the blank at 540 nm.

Protein concentrations of the samples were calculated from a calibration curve obtained using Bovine Serum Albumin (BSA). For the calibration curve, different concentrations of BSA (110mg/ml) were prepared into ten test tubes. Each test tube was made up to 1ml with appropriate volume of distilled water. 4.0ml of Biuret reagent was added to each test tube. The mixture was left undisturbed at 27ºC for 30 minutes, after which absorbance was read at 540 nm against the blank. A calibration curve of absorbance against BSA concentration was plotted.

3.3.3.2 Determination of Enzyme Concentrations

3.3.3.2.1 Alkaline Phosphatase Activity

The procedure described by Wright *et al.* (1972b) was adopted for the determination of alkaline phosphatase activity.

Principle:

The amount of phosphate ester cleaved within a given time is a measure of the phosphatase enzyme. P-nitrophenyl phosphate was hydrolyzed to para-nitrophenol and phosphoric acid at pH of 10.1. The P-nitrophenol conferred a yellowish colour on reaction mixture and its intensity was read spectrophotometrically at 400 nm.

Procedure:

A known volume (0.2 ml) of the diluted supernatant was pipetted into the test tube, 2.2 ml of

Carbonate buffer (O.1M) and 0.1ml of $MgS0_4.7H_20$ (O.1M) was added. The samples were incubated for 30 minutes at 37°C Then, 0.5ml of the substrate (p-nitrophenyl phosphate 10 mM) was added. The mixtures were thoroughly mixed and incubated for 30 minutes at 37°C.

The reaction was stopped by adding 2.0 ml of IN NaOH. Blank was obtained by following the same procedure, only that 0.2ml was used in lieu of 0.2ml of diluted homogenates. The absorbance was read spectrophotometrically at 400 nm. Enzyme activity was calculated using the following expression:

Enzyme activity (nM/min/ml) = $\Delta \frac{AB/min \times 1000 \times TV \times F}{9.9 \times SV \times L}$

Where:

ΔAB/min = Change in absorbance of reaction mixture per minute

TV = Total volume of the reaction mixture.

F = Total dilution factor

SV = Volume of enzyme source

L = Light path length (1cm)

9.9 = Extinction co-efficient of 1µm of p-nitrophenol in an alkaline solution of 1ml and 1cm path length.

1000 = The factor introduced to enable the enzyme activity to be expressed in nM/min/mg protein. The specific activity for alkaline phosphatase will be calculated from the expression:

Specific

enzyme activity (nM/min/mg protein)

$$= \frac{\text{Enzyme activity}}{\text{Protein concentration}}$$

3.3.3.2.2 Aspartate Aminotransferase

The procedure described by Reitman and Frankel (1957) was used to assay for the activity of aspartate aminotransferase.

Principle:

The enzyme catalyses the reversible reaction involving a-oxoglutarate and L-aspartate to form Lglutamate and oxaloacetate. The activities of aspartate aminotransferase were determined by monitoring the concentration of oxaloacetate hydrazine formed with 2,4dinitrophenylhydrazine as illustrated by the equation below:

$$\text{a-oxoglutarate} + \text{L-aspartate} \xleftrightarrow{\text{AST}} \text{L-glutamate} + \text{oxaloacetate}$$

Procedure:

A known volume (0.1ml) of diluted sample was pipetted into a test tube, 0.5 ml of reagent A was added. Also, 0.5 ml of reagent A was added to 0.1 ml of distilled water in another test tube to constitute the blank. The mixture was incubated at 37^{o}C for 30 minutes. Then, a known volume (0.5 ml) of reagent B was added into all the test tubes. The mixtures were mixed and left undisturbed for 20 minutes at 25^{o}C. The reaction was stopped by adding 0.5 ml of 0.4N NaOH. The solution was mixed and the absorbance was read against blank after

Specific
5 minutes at 468 nm. The enzyme activity was obtained from the calibration curve of aspartate amino transferase.

$$\text{activities of aspartate aminotransferase (U/L protein)} = \frac{\text{Enzyme activity}}{\text{Protein concentration}}$$

3.3.3.2.3 Alanine Aminotransferase

The procedure described by Reitman and Frankel (1957) was used for the determination of the activity of alanine aminotransferase.

Principle:

The enzyme catalyses the reversible reaction involving a-oxoglutarate and L-alanine to form

L-glutamate and pyruvate. The activity of alanine aminotransferase activity was determined by monitoring the concentration of pyruvate hydrazine formed with 2,4dinitrophenyl hydrazine as illustrated in the equation below.

$$\text{a-oxoglutarate + L-alanine} \xleftrightarrow{\text{ALT}} \text{L-glutamate + Pyruvate}$$

Procedure:

A known volume (0.1 ml) of diluted sample was pipetted into a test tube, 0.5ml of reagent A was added. Also, 0.5 ml of reagent A was added to 0.1ml of distilled water in another test tube to constitute the blank. The mixture was incubated at 37^{o}C for 30 minutes. Then, a known volume (0.5ml) of reagent B was added into all the test tubes. The mixtures were mixed and left undisturbed for 20 minutes at 25^{o}C. The reaction was stopped by adding 0.5

Specific
ml of 0.4N NaOH. The solution was mixed and absorbance was read against blank after 5 minutes at 546 nm. The enzyme activity was obtained from the calibration curve.

$$\text{activities of aspartate aminotransferase (U/L protein)} = \frac{\text{Enzyme activity}}{\text{Protein concentration}}$$

3.3.3.2.4 Superoxide Dismutase

The level of SOD activity will be determined by the method of Mistra and Fridovich (1972).

Principle:

A known volume (1ml) of samples were diluted to 9ml of distilled water to make 1 in 10 dilutions. An aliquot of 0.2ml of the diluted sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The absorbance at 480nm was monitored every 30 seconds for 150 seconds.

$$\text{Increase in absorbance per minute} = \frac{A_3 - A_o}{2.5}$$

A_o= absorbance after 0 seconds, A_3 = absorbance after 150 seconds

Specific

% SOD inhibition = increase in absorbance for substrate x 100 Increase in absorbance of blank 1 1 unit of SOD activity will be given as the amount of SOD necessary to cause 50%

inhibition of the oxidation of adrenaline.

3.3.3.2.5 Catalase

Catalase activity was determined according to the method of Sinha (1971).

Principle:

This method was based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide with the formation of perchromic acid as an unstable intermediate. The chromic acetate then produced is measured colorimetrically at 570 - 610nm. Since dichromate has no absorbency in this region, the presence of the compound in the assay mixture does not interfere at all with the colorimetric determination of chromic acetate. The catalase preparation is allowed to split hydrogen peroxide for different periods of time. This reaction is stopped at a particular time by the addition of dichromate/acetic acid mixture and the remaining hydrogen peroxide is determined by measuring chromic acetate colorimetrically after heating the reaction mixture.

Procedure:

Prior to use, the enzymes source was diluted in 0.05M phosphate buffer (pH 7.0) to obtain a rate of 0.03-0.07 $\Delta A/\min$. The assay mixture was thereafter constituted by adding 1.0cm^3 of distilled water. The mixture was incubated in spectrophotometer for 5 minutes to achieve temperature equilibration. This was then followed by the addition of 0.1cm^3 of the enzyme source (appropriately diluted). Decrease in absorbance was monitored at 240 nm for 2 minutes immediately after the addition of the appropriately diluted enzymes source.

Change in absorbance was calculated from the initial 45 seconds. Catalase activity was obtained from the following expression:

Catalase activity (Units/mg) = $\frac{\Delta A_{240}/\text{min} \times 100}{43.6 \times \text{mg protein/ml reaction mixture}}$

3.3.3.2.6 Glutathione-S-Transferase

Glutathione-S-transferase (GST) activity will be determined according to Habig *et al.* (1974).

The principle was based on the fact that all known glutathione-S-transferase demonstrate a relatively high activity with 1-chloro-2, 4,-dinitrobenzene as the second substrate. Consequently, the conventional assay for glutathione-S-transferase activity utilizes 1chloro-2, 4,-dinitrobenzene as substrate. When this substance was conjugated with reduced glutathione, its absorption maximum shifts to a longer wavelength. The absorption increases at the new wavelength of 340nm provided a direct measurement of the enzymatic reaction.

Procedure:

The medium for the estimation was prepared as shown in Table 2 below and the reaction was allowed to run for 60 seconds each time before the absorbance was read against the blank at 340nm. The temperature was maintained at 31^{o}C. The absorbance was measured.

Table 2: Reaction Estimation of GST

Reagents	Amount

- Phosphate buffer	500ul - 0.5ml
- NaN_3	100ul - 0.1ml
- GSH	200ul - 0.2ml
- H_2O_2	100ul - 0.1ml
- Sample	500ul - 0.5ml

3.3.3.2.7 Glutathione Peroxidase

Glutathione peroxidase (GPx) activity was determined according to the method of Rotruck *et al.* (1973)

Principle:

Glutathione peroxidase activity was observed by the concentration of the reduced glutathione left during the reaction measured by the absorbance at 412nm (GSH).

Table 3: Reaction Estimation of GPx

Reagents	Amount
- Phosphate buffer	500ul - 0.5ml
- NaN_3	100ul - 0.1ml
- GSH	200ul - 0.2ml
- H_2O_2	100ul - 0.1ml
- Sample	500ul - 0.5ml
- Distilled water	600ul - 0.6ml

The whole mixture was incubated at 37ºC for 3 minutes after which 0.5ml of TCA was added and centrifuged at 3000 rpm for 5 minutes. A known volume (1ml) each of the supernatant, 2ml of K_2HPO_4 and 1ml DNTB were added in a test tube and the absorbance was read at 412nm against the blank. Glutathione peroxide activity was observed by plotting the standard curve and the concentration of the remaining reduced glutathione (GSH) was extrapolated from the curve.

GSH consumed = 245.84 - GSH remaining

$$\text{Glutathione peroxide activity} = \frac{\text{GSH consumed}}{\text{Protein}} \text{ mg}$$

3.3.3 Statistical Analysis

Data were expressed as mean ± SEM of 6 determinations except otherwise stated. All results were statistically analysed using Duncan Multiple Range Test and complemented with Student's t-test, Statistical Package for Social Sciences, version 21 (SPSS Inc., Chicago, IL, USA) were used for statistical analyses. Statistical significance was set at 95% confidence interval (Mahajan, 1997).

CHAPTER FOUR

4.0 Results

4.1 Secondary Metabolite Screening of *Adasonia digitata* and *Corchorous olitorius* Leaves

The quantitative screening of the secondary metabolites of *Adasonia digitata* and *Corchorous olitorius* leaves revealed the presence of saponins, alkaloids, polyphenols, tannins, steroids, flavonoids and terpenoids as shown in Table 4. Anthraquinones, cardiac glycosides and phlobatannins were absent in both leaves. Quantitative screening of the concoction leaves of *Adasonia digitata* and *Corchorous olitorius* revealed that tannins (198.98 mg/dL) were the most abundant in *Adasonia digitata* whereas polyphenols (203.14 mg/dL) occurred the most in *Corchorous olitorius* as shown in Table 4. It is notable that the abundance of polyphenols (147.9 mg/dL in *Adansonia digitata* and 203.14mg/dL in *Corchorus olitorius*) contributes greatly to their pharmacological properties.

Table

4: Secondary Metabolite Constituents of *Adasonia digitata* and *Corchorous olitorius* Leaves

Metabolites	***Adasonia digitata*** **(mg/dL)**	***Corchorous olitorius*** **(mg/dL)**
Alkaloids	78.76±0.43	72.36±2.05
Anthraquinones	ND	ND
Cardiac glycosides	ND	ND
Flavanoids	13.42±1.03	104.81±0.18
Polyphenols	147.90±0.68	203.14±0.32
Phlobatannins	ND	ND
Saponins	16.59±1.85	22.17±0.24
Tannins	198.98±0.14	127.40±0.16
Terpenoids	42.45±0.45	21.25±1.08
Steroids	30.45±0.51	12.59±1.85

Values are expressed as Means ± SEM; n=10 at ($p<0.05$).

ND: None Detected.

4.2 Toxicity Screening

4.2.1 Determination of Liver Function Indices

Quantitative screening of Total protein, Albumin and Bilirubin content in the serum revealed an elevated level of Total protein (98.12±0.18) and Bilirubin (86.14±0.22) in Group 2 (negative control) as compared to Group 1 (Normal) with Total protein and Bilirubin levels of 41.14±0.11 and 41.14±0.39 mg/dL respectively. Albumin content in Group 2 (15.14±0.26) decreases in comparison to Group 1 (68.14±0.21). Administration of *Adasonia digitata*, *Corchorus olitorius,* and Vitamin C lowered Total Protein and Bilirubin levels and increased Albumin levels for Groups 3 to 6 as shown in Table 5 with the concoction of *Adasonia digitata* and *Corchorus olitorius* showing the best pharmacological effect.

5:

Selected Toxicity Parameters of Fried Oil Administered Rats

Groups	**Total Protein**	**Albumin**	**Bilirubin**
		(mg/dL)	
Normal	41.14±0.11	68.14±0.21	41.14±0.39
Negative	98.12±0.18	15.14±0.26	86.14±0.22
FO - AD	59.61±0.21	38.12±0.64	53.16±0.27
FO - CO	54.12±0.18	40.16±0.81	55.12±0.34

Table Effects of *Adasonia digitata* and *Corchorus olitorius* Leaves Concoction on

FO – AD/CO	40.81±0.15	41.14±0.81	32.64±0.37
FO- Vit C	54.24±0.11	46.21±0.71	40.14±0.28

Values are expressed as Means ± SEM; n=6 at ($p<0.05$).

Normal - Group 1 maintained only on pelletized rat feed and distilled water.

Negative - Group 2 administered fried oil with no treatment.

FO – AD - Group 3 administered fried oil then treated with *Adansonia digitata.*

FO – CO - Group 4 administered fried oil then treated with *Corchorus olitorius.*

FO – AD/CO - Group 5 administered fried oil then treated with *Adansonia digitata* and *Corchorus olitorius.*

FO - Vit C- Group 6 administered fried oil then treated with Vitamin C.

4.2.2 Toxicity Enzymes Concentrations

Quantitative screening of Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) content in serum revealed an elevated level of ALP (5.32±0.51), AST (12.46±0.12) and ALT (9.46±0.21) in Group 2 as compared to Group 1 with ALP, AST and ALP levels of 2.11±0.64, 6.12±0.41 and 4.12±0.03 IU/L respectively. Administration of *Adasonia digitata*, *Corchorus olitorius,* and Vitamin C significantly lowered ($p<0.05$) ALP, AST and ALP levels for Groups 3 to 6 with the

concoction of both *Adasonia digitata* and *Corchorus olitorius* having the most ameliorating effect as shown in Table 6.

Table 6: Effects of *Adasonia digitata* and *Corchorus olitorius* Leaves Concoction on Selected Serum Toxicity Enzymes on Fried Oil Administered Rats

Groups	ALT	AST	ALP
	(IU/L)		
Normal	4.12±0.03	6.12±0.41	2.11±0.64
Negative	9.46±0.21	12.46±0.12	5.32±0.51
FO - AD	6.82±0.23	8.91±0.16	4.18±0.42
FO - CO	6.71±0.23	8.61±0.23	4.00±0.41
FO – AD/CO	3.84±0.11	6.41±0.41	2.19±0.46
FO – Vit C	7.01±0.21	7.61±0.22	3.99±0.51

Values are expressed as Means ± SEM; n=6 at ($p<0.05$).

ALP = Alkaline Phosphatase; AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase.

FO = Fried Oil; AD = *Adansonia digitata*; CO = *Corchorus olitorius*; AD/CO = *Adansonia digitata* + *Corchorus olitorius*; Vit C = Vitamin C.

4.2.3 Antioxidant Screening

Quantitative screening of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione-STransferase (GST), Glutathione Peroxidase (GPx), Glutathione (GSH) revealed an reduced level of SOD (2.98±0.21), CAT (9.14±0.66) GST (21.12±0.71) GPx (19.81±0.04) and GSH (12.41±0.04) in the negative control group in comparison to Group 1 with SOD, CAT, GST, GPx, and GSH levels of 9.18±0.16, 21.41±0.78, 56.12±0.81, 46.15±0.06 and 36.14±0.06 nM/min/mg respectively. Administration of *Adasonia digitata*, *Corchorus olitorius,* and Vitamin C increased SOD, CAT, GST, GPX, and GSH levels for Groups 3 to 6 with the concoction of *Adasonia digitata* and *Corchorus olitorius* having the most restorative effect as shown in Table 7.

Table 7: Effects of *Adasonia digitata* and *Corchorus olitorius* Leaves Concoction on Antioxidant Capacity of Fried Oil Administered Rats

Groups	SOD	CAT	GST	GPx	GSH
		(nM/min/mg protein)			
Normal	9.18±0.16[a]	21.41±0.78[a]	56.12±0.81[a]	46.15±0.06[a]	36.14±0.06[a]
Negative	2.98±0.21[b]	9.14±0.66[b]	21.12±0.71[b]	19.81±0.04[b]	12.41±0.04[b]
FO - AD	4.48±0.19[c]	14.48±0.41[c]	36.12±0.64[c]	36.64±0.06[c]	31.12±0.07[c]
FO - CO	5.01±0.20[d]	15.11±0.52[d]	35.12±0.55[c]	35.64±0.07[c]	34.14±0.08[c]
FO – AD/CO	12.41±0.17[e]	20.84±0.44[a]	54.48±0.48[a]	52.14±0.02[d]	45.12±0.03[d]
FO – Vit C	5.03±0.18[d]	15.44±0.51[d]	38.94±0.58[d]	41.16±0.03[a]	28.14±0.07[e]

Values are expressed as Means ± SEM; n=6 at ($p<0.05$); Values with different superscripts are significantly different from each other.

SOD = Superoxide Dismutase; CAT = Catalase; GST = Glutathione-S-Transferase; GPx = Glutathione Peroxidase; GSH = Glutathione.

FO = Fried Oil; AD = *Adansonia digitata*; CO = *Corchorus olitorius*; AD/CO = *Adansonia digitata* + *Corchorus olitorius*; Vit C = Vitamin C.

CHAPTER FIVE

5.0 DISCUSSION

The liver is responsible for several functions, including primary detoxification of various metabolites, synthesizing proteins, and producing digestive enzymes. The liver also plays a significant role in metabolism, regulation of red blood cells (RBCs), glucose synthesis and storage. Evaluation of the liver based on these functions can help to determine the area and extent of hepatic injury (Iluz-Freundlich *et al.,* 2020).

Several studies have revealed secondary metabolites as the major factors responsible for the pharmacological properties of medicinal plants (Kumar and Pandey, 2013; Bello *et al.,* 2017). The pharmacological activities of these secondary metabolites contribute greatly to their therapeutic and phytochemical properties (Bello *et al.,* 2017).

Plants' most critical secondary metabolites and bioactive chemicals are flavonoids and phenolic acids (Velu *et al.,* 2018). Their abundant presence in this study may suggest their roles as natural antioxidants due to their free superoxide radicals scavenging properties. The presence of polyphenols may suggest its pharmacological effects as phenolics have also been reported to enhance bile secretion and lower blood cholesterol and lipid levels (Saxena *et al.,* 2013). Flavonoids also protect cell membranes from lipid peroxidation-induced damage (Bhatti *et al.,* 2022).

In this study, the greater abundance of some of these secondary metabolites in either *Adansonia digitata* or *Corchorus olitorius* leaves concoction in comparison with the other suggests that the consumption of both *Adansonia digitata* and *Corchorus olitorius* leaves concoction is more likely to provide better pharmacological and therapeutic effects.

The liver synthesizes, concentrates, and secretes bile acids and excretes other toxicants, such as bilirubin. Injury to hepatocytes and bile duct cells can lead to cholestasis which in turn, causes intrahepatic accumulation of toxic bile acids and excretion products, which promotes further hepatic injury (Jaeschke *et al.,* 2002).

A significant elevation ($P \leq 0.05$) in bilirubin level is observed in the negative control group, a contrast to the decreased albumin level shown in table 5. This may be an indicator of hepatic dysfunction as hypoalbuminemia has been reported as an indicator of several liver diseases (Chen *et al.*, 2021). Since maintenance of plasma albumin concentrations can be achieved with only 10% of normal hepatocyte mass, a fall in serum albumin reflects decreased synthesis of albumin by the hepatic parenchymal cells (Chen *et al.,* 2021).

A study which investigated the serum biochemical responses under oxidative stress of aspartame in wistar albino rats revealed that increased bilirubin production and enhanced hepatic conjugation in aspartame-treated animals may have been as a result of decreased uptake, conjugation or increased bilirubin production (Choudhary and Devi, 2014).

In this study, the decrease in the level of albumin may have resulted in the inability of albumin to bind to bilirubin, which may have led to the elevated level of unconjugated bilirubin in the plasma, resulting in hepatic jaundice.

However, a significant increase ($p<0.05$) in albumin level and decrease in bilirubin and total protein level was observed in the groups treated with *Adansonia digitata and Corchorus olitorius* leaves concoctions. This is in accordance with the study of Said *et al.* (2020) which investigated the hepatoprotective potential of aqueous fruit pulp extract of *Adansonia digitata* against CCl_4 induced liver damage in rats. The study suggested that the presence of secondary metabolites in the fruit pulp of *Adansonia digitata* enhanced the healing potential of *Adansonia digitata* and showed excellent protection on liver architecture (Said *et al.,* 2020).

Therefore, the greatest ameliorating effect observed in the group administered a concoction of both *Adansonia digitata and Corchorus olitorius* may be as a result of the combination of the secondary metabolites present in *Adansonia digitata* and *Corchorus olitorius.*

AST and ALT participate in gluconeogenesis by catalyzing the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to produce oxaloacetic acid and pyruvic acid, respectively. ALT is a cytosolic enzyme found in the liver that helps convert proteins into energy for the liver cells. AST is present as cytosolic and mitochondrial isoenzymes and is found in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs,

leucocytes, and red cells. It is not as sensitive or specific for the liver as ALT. ALP is an enzyme found in the liver and bone and is important for breaking down proteins. In the case of hepatocellular injury, over abundance of these enzymes is observed. This triggers the release of these enzymes into the bloodstream for circulation (Prati *et al.,* 2002).

Maduelosi *et al.* (2019) investigated the effects of vegetable oil reused for frying on the liver of albino rats. The study deduced that high level of liver enzymes (ALT, ALP and AST) in the test groups was as a result of liver damage, liver cell destruction and alteration in the permeability of the membrane due to the harmful effects of the consumption of vegetable oil that had been repeatedly used for frying (Maduelosi *et al.,* 2019).

In this study, a significant increase ($p<0.50$) in the level of ALT, AST and ALP in the negative control group therefore suggests hepatic damage, disease such as a blocked bile duct, certain bone diseases, or cellular leakage. The efflux of these liver enzymes into the bloodsream indicates cell membrane damage caused by oxidative stress (Tamas *et al.*, 2002; Ghouri *et al.*, 2010).

A study that investigated the protective effects of methanolic leaf extracts of *Monanthotaxis caffra* against aflatoxin B1-induced hepatotoxicity in rats concluded that pre-treatment of rats with extracts of *Monanthotaxis caffra* normalized the serum level of the liver enzymes on exposure to toxin and this was attributed to the presence of secondary metabolites present in *Monanthotaxis caffra* (Makhuvele *et al.,* 2022).

The significant decrease ($P \leq 0.05$) in the level of liver enzymes after treatment with *Adansonia digitata* and *Corchorus olitorius* further implicates the hepatoprotective properties of the secondary metabolites present in the plants.

The liver plays a central role in maintaining inter-organ homeostasis of GSH by exporting nearly all of the synthesized GSH into plasma and bile (Ookhtens and Kaplowitz, 1998).

This study revealed a significant decrease ($P \leq 0.05$) in the antioxiant enzymes levels in the negative control group. Consequently, an alteration of the hepatic ability to synthesize or export GSH as shown in decreased GSH level (table 7) may have an impact on systemic GSH homeostasis (Lu, 2013).

The decrease in SOD, GPx and CAT levels may play a role in oxidative injury and ultimately in the pathogenesis of chronic liver disease as these antioxiant enzymes have been reported to catalyze decomposition of ROS (Czuczejko *et al.,* 2003; Novo *et al.,* 2006). This is in line with the study of Salem *et al.* (2005) which suggested that deficient antioxidant defence mechanisms may lead to excess oxygen free radical formation that promotes pathological processes in the liver.

Bello *et al.* (2017) investigated the effects of methanolic extracts of *Cochorous olitorous* and *Adansonia digitata* leaves against irradiation-induced Atherosclerosis in male wistar rats. The study implied that the combination of the extract from *Adansonia digitata* and *Corchorus olitorius* showed better pharmacological activities and induced better

antioxidant enzymes, lessening the effect of free radicals induced by radiation (Bello *et al.*, 2017).

In this study, the ability of *Corchorus Olitorous* and *Adansonia digitata* to induce better antioxidant enzymes which have been implicated for their free radical scavenging properties is revealed by the increase in the antioxiant enzyme levels as observed in the test groups treated with *Adansonia digitata* and *Corchorus olitorius*, especially in the group administered a concoction of both *Adansonia digitata* and *Corchorus olitorius*. This may therefore be characterized to the antioxiant properties of the secondary metabolites present in the leaves of *Adansonia digitata* and *Corchorus olitorius*.

5.1 CONCLUSION

This study concludes that the consumption of oil that has been repeatedly used for frying has several deteriorating effect on the liver as evidenced in the predisposition of the test groups to various types of hepatic damage and diseases.

The administration of *Adansonia digitata* and *Corchorus olitorius* leaves concoctions increased the efficacy of antioxidant enzymes which reduced the risk of oxidative stress. It also normalized the level of the liver enzymes and proteins, suggesting that the concoctions were able to repair hepatic damage that had been induced by fried oil. This confirms the hepatoprotective properties of *Adansonia digitata* and *Corchorus olitorius*.

Therefore, a combination of both *Adansonia digitata* and *Corchorus olitorius* leaves concoctions can be used as therapy against free radical-induced diseases. This is because it possesses better pharmacological effects than the orthodox medicine as well as better than each individual plant.

5.2 RECOMMENDATIONS

On the basis of the results obtained in this study, it is recommended that the consumption of oil that has been repeatedly used for frying should be avoided as it has proven not to be hepatic friendly.

In the case where consumption of repeatedly used fried oil is inevitable, there is need for protection. Frequent consumption of a combination of both *Adansonia digitata* and *Corchorus olitorius* is therefore advised due to their proven antioxidant, hepatoprotective and pharmacological properties.

Further studies should also be carried out to further assess the damages caused by the consumption of repeatedly used oil on other tissues of the body and the protective effects of *Adansonia digitata, Corchorus olitorius* and other phytochemicals in the body, and if present, their toxic effects should also be investigated.

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