AMELIORATIVE POTENTIAL OF *Entandrophragma utile* STEM BARK ON SOME LIVER ENZYMES IN CCl_4 INTOXICATED RATS

BY

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCES (B.sc) IN BIOCHEMISTRY

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CERTIFICATION

This is to certify that the project report titled 'Ameliorative potential of *Entandrophragma utile* on some liver enzymes in CCl_4 intoxicated rats' has been carried out by ITEBALUMHE ELVIS AFIMHIA (LSC2103761) under the supervision of Dr. K.O. Orumwesodia and approved in partial fulfilment of the requirement for the award of Bachelor of Science degree in the Department of Biochemistry of the University of Benin, Benin City, Edo State.

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We the undersigned attest and declare that the dissertation of ITEBALUMHE ELVIS AFIMHIA titled "Ameliorative potential of *Entandrophragma utile* on some liver enzymes in CCl_4 intoxicated rats" has successfully passed the plagiarism test and does not violate any copyright regulations.

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DEDICATION

This report is dedicated to the Almighty God through whom all things are possible and to my lovely family for their care, unconditional love, and support.

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ABSTRACT

The liver is a vital organ responsible for detoxification, metabolism, and synthesis of essential biomolecules, but its exposure to toxicants such as carbon tetrachloride (CCl_4) often results in severe hepatic damage. This study investigated the ameliorative potential of *Entandrophragma utile* stem bark extracts on selected liver enzymes in CCl_4-intoxicated Wistar rats. Fresh stem bark of E. utile was collected, air-dried, and extracted using methanol, after which the crude extract was fractionated into ethyl acetate and ethanol residue fractions. Thirty male Wistar rats were divided into six groups of five animals each: normal control, CCl_4 control, silymarin-treated, and groups treated with crude, ethyl acetate, and ethanol residue fractions of E. utile. The extracts were administered orally for 28 days, while CCl_4 was given intraperitoneally in the final week to induce hepatotoxicity. Blood samples were analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) activities using standard colorimetric methods. Results revealed that CCl_4 intoxication caused significant elevation in all liver enzyme markers compared with the control, indicating hepatic injury, whereas treatment with E. utile extracts markedly reduced these enzyme levels in a manner comparable to the standard drug, silymarin. The ethyl acetate fraction exhibited the most pronounced hepatoprotective effect, suggesting the presence of potent antioxidant constituents capable of mitigating oxidative stress and restoring normal liver function. In conclusion, Entandrophragma utile possesses significant hepatoprotective properties against CCl_4-induced liver damage, validating its traditional use in managing hepatic disorders and indicating its promise as a natural source of therapeutic agents.

CHAPTER ONE

1.0 Introduction and Literature Review

1.1 Introduction

The liver, one of the largest and most metabolically active organs in the human body, plays a crucial role in maintaining homeostasis through its involvement in metabolism, detoxification, and synthesis of vital biomolecules. Globally, liver diseases represent a major public health challenge, accounting for nearly two million deaths annually, with the majority arising from cirrhosis, hepatocellular carcinoma (HCC), and viral hepatitis (Asrani et al., 2019; Moon et al., 2020). Epidemiological data indicate that liver disease prevalence is particularly high in regions of Africa and Asia, where environmental and occupational exposures to toxicants are widespread (Schuppan and Afdhal, 2008; Mokdad et al., 2014). Various xenobiotics such as industrial solvents, aflatoxins, alcohol, and certain pharmaceuticals can lead to oxidative stress, lipid peroxidation, and hepatocellular damage, culminating in acute or chronic liver injury (Abdel-Misih and Bloomston, 2010; Das and Vasudevan, 2019). These toxicant-induced hepatic injuries not only disrupt enzymatic and mitochondrial functions but also initiate inflammatory cascades that contribute to fibrosis and carcinogenesis. Hence, the burden of toxicant-related liver diseases underscores the necessity for discovering natural hepatoprotective agents that can mitigate or reverse such damage through antioxidant and anti-inflammatory mechanisms.

Medicinal plants have long served as a valuable source of bioactive compounds for treating liver disorders, particularly in traditional medicine systems such as Ayurveda and African ethnobotany. Among these, *Entandrophragma utile* (Dawe and Sprague), commonly known as Sipo, Utile, or African mahogany, has attracted increasing scientific attention for its potential therapeutic benefits. Belonging to the family Meliaceae, E. utile is a large deciduous hardwood tree native to tropical West and Central Africa, predominantly found in countries such as Nigeria, Cameroon, Ghana, and the Democratic Republic of Congo (Orwa et al., 2009; Adedeji et al., 2022). The tree can reach up to 45 meters in height, characterized by a straight bole, reddish-brown bark, and pinnate leaves, with its wood highly valued for its durability and fine grain. Phytochemical investigations have revealed the presence of limonoids, terpenoids, flavonoids, and phenolic compounds, many of which exhibit significant antioxidant, anti-inflammatory, and hepatoprotective activities (Adedeji et al., 2022; Owolabi et al., 2021). Recent studies have explored E. utile extracts for their ability to attenuate liver damage induced

by carbon tetrachloride and paracetamol in animal models, suggesting its potential as a natural hepatoprotective agent (Owolabi et al., 2021; Adedeji et al., 2022). Consequently, the current study aims to further elucidate the hepatoprotective properties and mechanistic actions of Entandrophragma utile, contributing to the growing evidence supporting medicinal plants as effective alternatives for managing toxicant-induced liver injury. The reason for this study is to evaluate the effective potential of *Entandrophragma util*e stem bark as an effective hepatoprotective agents and This study is aimed at determining the ameliorate potential of *Entandrophragma utile* on some liver enzymes in CCl_4 intoxicated rats.

1.1.1 Justification of the Study

The liver is a vital organ responsible for numerous critical functions, including metabolism, detoxification, protein synthesis, and bile production.

Consequently, liver damage, arising from various etiologies such as chemical exposure (e.g., carbon tetrachloride-CCl_4), viral infections, alcohol abuse, and drug-induced toxicity, poses a significant global health burden (Min Li *et al.,* 2023). Liver diseases, ranging from steatosis to fibrosis, cirrhosis and hepatocellular carcinoma, contribute substantially to morbidity and mortality worldwide (Lu *et al.,* 2023). Current conventional treatments often have limitations, including side effects, high costs, and sometimes limited efficacy, particularly in advanced stages of liver damage.

Over the years, medicinal plants have been used by man for the prevention, management and treatment of several diseases. Medicinal plants contain secondary metabolites which can be quite effective when synthetic drugs fail. The presence of Phytochemical constituents in medicinal plants are very efficient in treatment of diseases and they play an important role in healing (Garg *et al.,* 2022). This study was conducted to evaluate the ameliorative potential of *Entandrophragma utile* on some liver enzyme in CCl_4 intoxicated rats.

1.1.2 Aim of the Study

This study is aimed at determining the ameliorate potential of Entandrophragma utile on some liver enzymes in CCl_4 intoxicated rats.

1.1.3 Specific Objective of Study

The specific objectives of this study are to:

- This study will demonstrate whether or not the various extracts of *Entandrophragma utile* stem bark is an effective treatment for liver damage.
- To determine the effect of *Entandrophragma utile* on tetrachloromethane (CCl4) intoxicated wistar rats
- To determine the levels of some liver enzymes on CCl4 intoxicated rats.
- Determining the effect of *Entandrophragma utile* on plasma levels of ALT, AST, ALP, GGT of CCl_4 intoxicated rats.
- Determine the effect of *Entandrophragma utile* on the architecture of the liver of CCl_4 intoxicated rats.

1.2 Literature Review

1.2.1 *Entandrophragma utile*

The taxonomic classification of Entandrophragma utile is as follows:

- **Kingdom:** Plantae

  subkingdom: Tracheobionta
- **Superdivision: Spermatophyta**
- **Division: Magnoliophyta**
- **Class: Magnaliopsida**
- **Subclass: Rosidae**
- **Order:** Sapindales
- **Family:** Maeliaceae
- **Genus:** Entandrophragma
- **Species: Entandrophragma utile**

  Features of E. utile

It is a tall tree, reaching heights of approximately 60 meters, and is commonly found throughout forest regions of west and central Africa. It is characterized by its extensive branch structure, short, reddish-brown hairs clustered at the ends of its branchlets, pinnate leaves and bark that is about 4 cm thick. Its seed pods typically open while still on the tree. This species thrives primarily in moist semi-deciduous forests. The tree features hairy flower clusters (tomentellous inflorescence) with white flowers and distinctive buttress roots (Adeniran *et al.,* 2023). Belonging to the family Meliaceae, E. utile is a large deciduous hardwood tree native to tropical West and Central Africa, predominantly found in countries such as Nigeria, Cameroon, Ghana, and the Democratic Republic of Congo (Orwa et al., 2009; Adedeji et al., 2022). it is commonly known as Sipo, Utile, or African mahogany.

Phytochemical Composition and Traditional Uses

Research has identified several medically active compounds in the stem bark of *Entandrophragma utile.* These include tetranortriterpenoids like utilins, lactone entandrophragma, methyl angolensate, heptane triterpenoids called entilins, and ergosterol derivatives (Ngouana *et al*., 2024). Traditionally, a maceration of the bark has been used as a tonic and stimulant (Adesanwo *et al.,* 2022). It has also been found to have anti-sickling (Adejumo *et al.,* 2011), anti-inflammatory (Adeola *et al.,* 2023), anti-oxidant (Usman *et al.,* 2018), pain relief (Kola Mustapha *et al*., 2023), anti-malarial activities (Ngonuana *et al.,* 2024).

Physical uses of Entandrophragma utile

The physical uses of Entandrophragma utile (Sipo Mahogany) are diverse, primarily revolving around its valuable timber. Its wood is extensively utilized in construction, highly prized for crafting furniture and cabinetry, employed for durable flooring, and often used in boat construction due to its resistance and strength (Dainou *et al.,* 2021).

Plate 1(A): *Entandrophragma utile stem*

Plate 1(B): *Entandrophragma utile*

Mechanism of Carbon tetrachloride (CCl_4)

Carbon tetrachloride (CCl_4) causes liver damage through its conversion into highly reactive, short-lived free radicals such as trichloromethyl (CCl_3) and trichloromethyl peroxy ($CCl_3OO\cdot$) by the enzyme cytochrome P450 in the liver a process known as reductive dehalogenation. These reactive intermediates damage liver cells in two main ways. First, they can directly attach to essential cellular components, disrupting their normal functions. Second, they initiate lipid peroxidation, a process in which the free radicals react with oxygen and attack unsaturated fatty acids in cell membranes, particularly unsaturated phospholipids. This lipid destruction compromises membrane integrity and leads to cellular injury. Experimental studies have confirmed the presence of these free radicals during CCl_4 metabolism. The resulting cell damage can occur either through covalent binding of the reactive intermediates to cellular molecules or through increased lipid peroxidation caused by their interaction with oxygen. The breakdown products of this lipid degradation, mainly reactive aldehydes, spread throughout the cell, causing further harm such as increased membrane permeability an indicator of impending cell death (Cheeseman et al., 1985).

The extent of carbon tetrachloride (CCl_4) toxicity is influenced by the tissue's oxygen partial pressure. At low loxygen levels, the metabolism of CCl_4 primarily produces trichloromethyl ($CCl_3\cdot$) and dichloromethyl ($CHCl_2\cdot$) radicals, leading to the covalent binding of these reactive inermediates to cellular components (Ontario *et al.,* 2022). This process mainly disrupts lipid metabolism causing increased lipid synthesis and reduced lipid export from hepatocytes which ultimately results in steatosis, or fatty liver (journal of toxicology and Environmental Health, 2020). Conversely, at high oxygen partial pressures. CCl_4 metabolism shifts toward the formation of trichloromethyl peroxy ($CCl_3OO\cdot$) radicals, which promote lipid peroxidation. This oxidative damage drives the progression of liver injury from steatosis to apoptosis (Mrwad *et al.,* 2025)

In the present communication we extend the observations on CCl4-induced liver damage and report on experiments where hepatocytes were exposed to CCl4, and the ensuing toxicity was modified with a variety of substances targeted at different steps of the CCl4 attack. These substances included a cytochrome P450 inducer, change in oxygen pressure, chemicals that induced lipid peroxidation without covalent binding, anti-oxidants, and a radical scavenger, several typical parameters of CCL4 toxicity were monitored, among them lipoprotein release and composition covalent binding of activated metabolites to subcellular fractions, and

aldehyde formation from lipid peroxidation. The results contribute to a better understanding of the mechanism of CCl4-induced steatosis and hepatotoxicity.

Oxidative Stress

Reactive oxygen species (ROS), including superoxide anion ($O_2^{-}\cdot$) and hydrogen peroxide (H_2O_2), arise primarily from the incomplete reduction of molecular oxygen during mitochondrial oxidative phosphorylation. In addition to mitochondrial respiration, various enzymatic systems—such as NADPH oxidases, xanthine oxidase, and cytochrome P450 monooxygenases—also contribute to cellular ROS generation (Murphy, 2019).

Hydrogen peroxide, though relatively stable, can participate in Fenton and Haber–Weiss reactions, where it oxidizes ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), generating highly reactive hydroxyl radicals (·OH) (Winterbourn, 2020). Ferric iron can then be reduced back to ferrous iron by superoxide, creating a redox cycle that continuously produces hydroxyl radicals in the presence of oxygen and H_2O_2. This iron-catalyzed process amplifies oxidative stress, leading to the oxidation of proteins, lipids, and nucleic acids (Sies and Jones, 2020).

Superoxide radicals can also interact with nitric oxide ($NO\cdot$) to form peroxynitrite ($ONOO^{-}$), a potent oxidant capable of nitrating tyrosine residues and damaging mitochondrial enzymes (Pacher et al., 2007; Radi, 2018). Such oxidative and nitrosative stress leads to structural and functional impairment of biomolecules—protein oxidation disrupts enzymatic activity, DNA oxidation can induce mutagenesis, and lipid peroxidation compromises membrane integrity.

Lipid peroxidation of polyunsaturated fatty acids (PUFAs), in particular, plays a crucial role in triggering ferroptosis, a regulated, iron-dependent form of cell death characterized by the accumulation of lipid peroxides (Stockwell et al., 2017; Jiang et al., 2021). Thus, iron not only catalyzes ROS generation but also acts as a central mediator of oxidative damage and ferroptotic signaling pathways.

Reactive oxygen species (ROS) owe their reactivity to their ability to either extract an electron from, or donate their unpaired electron to, nearby molecules, thereby attaining a more thermodynamically stable state (Loriente, 2020). In contrast, molecules such as hydrogen peroxide (H_2O_2) or peroxynitrite ($ONOO^{-}$) are non-radical oxidants that, while more stable, can still exert significant oxidative effects under certain conditions (Sies and Jones, 2020).

Although ROS were once regarded solely as harmful by-products of metabolism, it is now well-established that at low or controlled concentrations, they act as critical secondary

messengers in redox signaling. These signaling roles influence gene expression, immune defense, host-pathogen interactions, and vascular homeostasis, including nitric oxide (NO·)-mediated vasodilation (Holmström and Finkel, 2019; Forman et al., 2023).

However, excessive accumulation of ROS disturbs the redox equilibrium between oxidants and antioxidants, leading to oxidative stress—a condition characterized by cellular and molecular damage (Jones and Sies, 2015). To refine this concept, recent literature distinguishes between oxidative eustress, which refers to homeostatic redox signaling necessary for normal physiology, and oxidative distress, denoting pathological ROS overproduction and oxidative damage (Sies et al., 2023).

A similar duality applies to reactive nitrogen species (RNS), such as nitric oxide and peroxynitrite. At physiological levels, RNS act as signaling molecules modulating vasodilation and immune responses; in excess, they provoke nitrosative stress, disrupting protein function and cellular redox homeostasis (Radi, 2018; Forrester et al., 2018).

Thus, both ROS and RNS exemplify the fine balance of redox biology: essential for signaling at moderate levels, yet deleterious when their production overwhelms cellular antioxidant defenses.

Antioxidant Defense Mechanisms

Antioxidants are molecules that inhibit or neutralize oxidative damage by scavenging reactive species, chelating transition metals, or enhancing the activity of enzymatic defense systems. They maintain cellular redox equilibrium and protect biomolecules such as lipids, proteins, and nucleic acids from oxidative and nitrosative stress (Sies et al., 2023)

Classifications

1. Endogenous Antioxidants

Endogenous antioxidants comprise enzymatic and non-enzymatic systems that form the first line of defense against oxidative stress.

A. Enzymatic Antioxidants

These are catalytically active enzymes that convert reactive species into less harmful molecules:

Superoxide Dismutases (SODs)

Convert superoxide radicals ($O_2^{-}\cdot$) into hydrogen peroxide (H_2O_2) and oxygen. There are cytosolic (Cu/Zn-SOD), mitochondrial (Mn-SOD), and extracellular (EC-SOD) isoforms (Zelko et al., 2021).

Reaction: $2\ O_2^{-}\cdot + 2\ H^+ \rightarrow H_2O_2 + O_2$

Catalase (CAT)

Found mainly in peroxisomes, catalase decomposes hydrogen peroxide into water and oxygen, thus preventing hydroxyl radical formation through Fenton chemistry (Glorieux and Calderon, 2017).

Reaction: $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

Glutathione Peroxidases (GPx)

Selenium-dependent enzymes that reduce hydrogen peroxide and lipid peroxides using reduced glutathione (GSH) as a cofactor. GPx4, in particular, prevents lipid peroxidation and ferroptosis (Ursini and Maiorino, 2020).

Peroxiredoxins (Prx)

A family of thiol-specific peroxidases that detoxify peroxides and participate in redox signaling (Rhee, 2020).

Thioredoxin (Trx) and Glutaredoxin (Grx) Systems

Maintain thiol redox status of proteins and work in concert with NADPH to restore oxidized enzymes (Zhou et al., 2023).

B. Non-Enzymatic Endogenous Antioxidants

Glutathione (GSH) – The most abundant cellular thiol, GSH directly scavenges radicals and serves as a substrate for GPx and GST enzymes. A decline in GSH levels is a hallmark of oxidative distress (Forman et al., 2023).

Uric Acid – An effective scavenger of hydroxyl and peroxynitrite radicals in plasma.

Bilirubin – Formed from heme catabolism; acts as a potent peroxyl radical scavenger.

Coenzyme Q10 (Ubiquinol) – Functions in mitochondrial electron transport and lipid antioxidant defense.

Lipoic Acid – Regenerates other antioxidants (vitamin C, E, and GSH) and chelates metal ions.

2. Exogenous Antioxidants

These are dietary or supplemental compounds that bolster the body's defense system and provide protection against chronic diseases associated with oxidative stress.

A. Vitamin Antioxidants

Vitamin C (Ascorbic Acid) – A water-soluble antioxidant that donates electrons to neutralize ROS and regenerates vitamin E. It also chelates iron to reduce Fenton chemistry (Carr and Maggini, 2017).

Vitamin E (Tocopherols and Tocotrienols) – Lipid-soluble antioxidants that protect polyunsaturated fatty acids in membranes from peroxidation (Traber and Atkinson, 2019).

Vitamin A and Carotenoids – Quench singlet oxygen and inhibit lipid peroxidation; β-carotene and lycopene are key examples.

B. Polyphenols and Flavonoids

Plant-derived secondary metabolites such as quercetin, resveratrol, and curcumin exhibit strong free radical–scavenging and anti-inflammatory properties. They modulate transcription factors like Nrf2 and NF-κB, promoting antioxidant gene expression (Pandey & Rizvi, 2022).

The Impact of Oxidative Stress on Liver Cells

The liver is continuously exposed to different toxic and reactive metabolites including Reactive Oxygen Species (ROS) and Reactive Nitrogen Specie (RNS). A shift in the redox balance toward oxidative stress can be considered an initial step in the pathogenesis of liver diseases, (Li, *et al.,* 2015). This process is affected by comorbidities such as diabetes/insulin resistance and by various exogenous factors such as alcohol abuse, viral infection, drug overdose, high-caloric diet, and exposure to environmental toxins, UV light or heavy metals. A surge in reactive oxygen species and reactive nitrogen species levels is important in the onset of inflammatory reactions, fibrosis, necrosis, apoptosis or malignant transformation, (Li *et al.,*2015).

Hepatocytes are important sites of ROS production, especially in mitochondria, and are also sensitive to ROS-mediated injury. Each hepatocyte contains 1000 to 2000 mitochondria occupying about 20% of the cell volume. ROS-mediated damage of lipids and particularly, PUFAs, can alter cell membrane fluidity and permeability. Mitochondrial lipid and oxidative stress. Mitochondrial dysfunction in hepatocytes has been linked to the development and progression of chronic liver disorders. For instance, patients with non-alcoholic steatohepatitis (NASH) exhibit hepatic oxidative stress due to impaired mitochondrial respiratory capacity and proton leakage (M. K. Arumugam *et al.,* 2023). Oxidative stress promotes an influx of calcium into cells and redistribution of cellular calcium from the endoplasmic reticulum (ER) to the cytosol, mitochondria and nuclei, which in turn may trigger apoptotic and necrotic death (Görlach *et al*., 2021). This response increases mitochondrial permeability transition and facilitates the release of pro-apoptotic factors such as cytochrome c, and the activation of calcium-dependent endonucleases, proteases and lipases, contributing to the death of hepatocytes and other liver cell types. In addition, oxidative stress can affect the secretory functions of hepatocytes by disrupting the formation of bile flow, leading to cholestasis (Basiglio *et al.,* 2021).

While ROS and lipid peroxidation products impair hepatocellular function and via ability, they also promote the differentiation and activation of hepatic stellate cells to myofibroblasts, leading to the secretion and accumulation of collagen and other extracellular matrix components within the liver (Cichoz-Lach, H., and Michalak, A. (2021). Therefore, chronic activation of hepatic stellate cells in response to oxidative stress favors the development of liver fibrosis, which may progress to cirrhosis and hepatocellular carcinoma (Ramos-Tovar and Muriel, 2020; Liang *et al.,* 2016).

Liver Enzymes

The liver is a vital organ that plays a crucial role in maintaining overall health and wellbeing. One of the key functions of the liver is to produce enzymes that help to break down and process nutrients, toxins, and waste products. These liver enzymes catalyse chemical reactions necessary for various bodily functions. Some of these key functions of living enzymes include detoxification, metabolism, energy production, and protein synthesis (Mokdad *et al*., 2020). Detoxification involves the breakdown and elimination of toxins, such as drugs, alcohol and environmental pollutants. Metabolism involves the breakdown and processing of nutrients, such as carbohydrates, proteins, and fats. Energy production involves the breakdown and

processing of energy-rich molecules, such as lactate and glucose. Protein synthesis involves the processing of amino acids into distinct proteins.

Liver enzymes are proteins produced by the liver that are crucial indicators of liver health and function. When liver cells are damaged or stressed, these enzymes are released into the bloodstream, where the levels can be measured to assess liver function.

Types of Liver enzymes

There are several types of liver enzymes, each with specific functions and characteristics. Some of the most common liver enzymes that are used for liver function tests include:

1. Aminotransferases (Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT)): they are indicators of liver cell damage. They play a role in gluconeogenesis by transferring amino groups from aspartic acid or alanine to ketoglutaric acid, resulting in the production of oxaloacetic acid and pyruvic acid, respectively. AST exists as both cytosolic and mitochondrial isoenzymes and is found in the liver, heart muscle, skeletal muscle, kidneys, brain, pancreas, lungs, white blood cells, and red blood cells (Kim *et al*., 2020). It is less sensitive and specific to the liver compared to ALT, and elevated AST levels can result from non-living related causes. In newborns and infants, AST levels are about twice as high as in adults, but these levels decrease to adult values by around six months of age (Oh *et al.,* 2017).

   ALT is a cytosolic enzyme highly concentrated in the liver, it has a half-life of about 47 ± 10 hours (Agrawal *et at.,* 2019). In most liver diseases where the activity of both enzymes originates mainly from the hepatocyte cytosol, ALT levels are generally higher than AST (Pandeya *et al*., 2021). The release of these enzymes into the bloodstream is triggered by hepatocellular damage, not necessarily by cell death (Lala *et al.,* 2023).

2. Alkaline Phosphatase (ALP): Alkaline phosphatase belongs to a group of zinc metalloenzymes that are highly concentrated in the microvilli of the canaliculi and also present in various other tissues, including bone, intestines, and placenta (Iluz-Freundich *et al.,* 2020). They facilitate the hydrolysis of organic phosphate esters located in the extracellular space.

3. Gamma-glutamyl transferase (GGT): GGT is a glycoprotein enzyme found on the membranes of cells with high secretory or absorptive activity. Its function is to transfer

a gamma-glutamyl group from peptides to other amino acids. GGT is also found in several other parts of the body, including the kidney, pancreas, intestine, prostate, testicles, spleen, heart, and brain (Whitfield, 2022). However, compared to alkaline phosphatase, GGT is more specific for diagnosing biliary diseases because it is not present in bone (Koenig and Seneff, 2015).

Liver function tests (Cholestatic Pattern)

Cholestatic pattern of liver function test is essential for observing and diagnosing the disproportionate increased levels of alkaline phosphatase and gamma-glutamyl transferase compared to alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Lala, *et al.,* 2023). This pattern typically indicates impaired bile flow, either due to obstruction of the bile ducts or dysfunction of the hepatocytes in producing bile both resulting in increased bilirubin concentrated in the blood or serum.

Alkaline Phosphatase (ALP) is an enzyme found in the liver, bile ducts, and bone. Elevated levels of ALP are often associated with bile duct obstruction, as the enzyme is released into the bloodstream when bile flow is impeded. However, ALP can also be elevated in bone diseases, so its interpretation must be considered in the clinical context.

Gamma-glutamyl transferase (GGT) is another enzyme that, when elevated along with ALP, strongly suggests a biliary origin of the obstruction (Li and Zhang, 2025). GGT is present in bone, making it a more specific marker for biliary disease compared to ALP.

Bilirubin is a breakdown product of haemoglobin, and its levels are typically elevated in cholestasis due to impaired bile excretion. The disproportionate elevation of bilirubin compared to AST and ALT indicates that the primary issue is with bile flow rather than liver cell damage.

The ratio of ALT to ALP is useful for distinguishing the type of liver damage by hepatotoxins. The ratio of ALT to ALP is equal or greater than five (5) during hepatocellular damage, while that of cholestatic damage, the ratio of ALT to ALP is two (2) or less than. During mixed liver damage, the ratio of ALT to ALP falls between two and five (Kozielewicz *et al*., 2025).

Clinical significance of the Cholestatic pattern

The cholestatic pattern can be observed in a variety of liver diseases, including:

- Primary Sclerosing Cholangitis (PSC): PSC is another chronic liver disease characterized by inflammation and scarring of the bile ducts. It often presents with a cholestatic pattern on liver function tests.
- drug-Induced Liver damage: Certain medications can cause cholestasis as a side effect. This drug-induced liver damage may present with a cholestatic pattern.
- Viral Hepatitis: some types of viral hepatitis, particularly those affecting the bile ducts, can cause a cholestatic pattern.
- Biliary tract obstruction: Obstruction of the bile ducts, whether caused by gallstones, tumors, or strictures, can lead to a significant rise in ALP and GGT.
- Primary Biliary Cholangitis (PBC): This autoimmune disease targets the small bile ducts, causing inflammation and ultimately leading to their destruction. The resulting cholestasis manifests as elevated ALP and GGT.

For diagnosing hepatocellular damage involving animals, ALT and AST (sometimes combined with total bilirubin) are the primary indicators. ALT is considered a more precise and sensitive marker for hepatocellular damage than AST, (Lala *et al.,* 2023).

CHAPTER TWO

2.0 Materials and Method

2.1 Materials

Vis Spectrophotometer (Searchtech Instrument, England)

Centrifuge 80-2 ((Life Assistance Scientific, U.K.)

HH-S Water bath (Searchtech Instruments, England)

Beakers (Pyrex, England)

Test tubes (plastic and glass) (Pyrex, England)

Test tube racks (Pyrex, England)

Analytical weighing balance (S. Mettler, England)

Spatula (Pyrex, England)

Rat cage

Rat chow

Micropipette (Pyrex, England)

Measuring cylinder (Pyrex, England)

Filter paper

Feeding plate

Cuvettes (Searchtech Instrument, England)

Cotton wool (Pyrex, England)

Refrigerator

Stirring rod (Pyrex, England)

pH meter (Pyrex, England)

Micropipette tips (Pyrex, England)

Mortar and pestles

Latex gloves (Pyrex, England)

Syringes (Pyrex, England)

Gavage

Face mask

Plain bottle (Pyrex, England)

Dissecting kit (Pyrex, England)

2.1.1 Reagents and Chemicals

Alkaline phosphatase test kits (Pyrex, England)

Gamma-glutamyl transferase test kits (Pyrex, England)

Aspartate Aminotransferase test kits (Pyrex, England)

Alanine Aminotransferase kits (Pyrex, England)

Formalin (Pyrex, England)

Tri-sodium citrate Dihydrate (Pyrex, England)

Tetrachloromethane (Pyrex, England)

Sodium chloride (Pyrex, England)

Potassium Dihydrogen phosphate (Pyrex, England)

Chloroform (Pyrex, England)

TrichloroAcetic Acid (Pyrex, England)

EDTA DiSodium Phosphate (Pyrex, England)

Hexane (Pyrex, England)

Ethyl acetate (Pyrex, England)

Silymarin (Micro Labs Limited, India)

2.1.2 Methods

2.2.1 Plant Collection and Extract Preparation

Plant Collection

Fresh Stem bark of *Entandrophragma utile* was obtained from the forest area of Ogun State, Nigeria, and authenticated by Professor H. Akinibosun, a taxonomist at the Department of Botany and Biotechnology, University of Benin. After which, the stem bark was thoroughly washed and allowed to dry under shade. Then, it was ground into coarse particles with a mortar and pestle and subsequently stored in air-tight container.

Extraction and fractionation process

The extraction process was carried out by macerating 200g of pulverized extract of *Entandrophragma utile* in 1 litre of ethanol. The mixture was allowed to stand at room temperature for 72 hours with intermittent shaking to enhance solvent penetration. After maceration, the mixture was filtered using Whatmann No.1 filter paper and the filtrate was concentrated using a rotary evaporator under pressure at 40^0C to remove excess solvent. The resulting crude ethanol extract was collected and stored in an airtight container at 4^0C.

2.2.2Animal

30 Male albino Wistar rats with an average weight of 120-150 g were used for this study. The animals were obtained from the Animal House, Department of Anatomy, College of Basic Medical Science, University of Benin, Benin City, Edo State, Nigeria. The animals were kept in clean cages in a 12-hour light/dark cycle with daily litter change. The animals were acclimatized for two (2) weeks before the experiment commenced. The animals were fed rat chow and water *ad libitum*. The weight of the rats was monitored throughout the duration of the experiment. During the study, rats were maintained under standard ethical condition according to the protocol established by the National Research Council (US) Committee Guide for the Care and Use of Laboratory Animals (2011).

2.2.2 Experimental Design

Thirty (30) male albino wistar rats were divided into 6 groups of 5 rats each and treatments administered orally for twenty-eight days (28), while CCl_4 intoxication was done twice (within 3-days interval) within the last 7 days.

Animal grouping

Group I: (normal control). Fed only rat chow and water.

Group II: (experimental control); Rats were given CCl_4 twice (0.5ml/kg in olive oil in a ratio of 1:1, intraperitoneally) in the last 7 days of the experiment.

Group III: Rats were given a dose of CCl_4 twice (0.5ml/kg in olive oil in ratio of 1:1, intraperitoneally) in the last 7 days of the experiment and treated with silymarin (the reference drug) for 7 days.

Group IV: crude extract of *Entandrophragma utile* was administered for 28 days. Rats were given a dose of CCl_4 twice (0.5ml/kg in olive oil in a ratio of 1:1, intraperitoneally) in the last 7 days of the experiment.

Group V: Ethyl acetate fraction of *Entandrophragma utile* were administered to this group for 28 days. Rats were given a dose of CCl_4 twice (0.5ml/kg in olive oil in a ratio of 1:1, intraperitoneally) in the last 7 days of the experiment.

Group VI: Ethanol residue fraction of *Entandrophragma utile* was administered to this group for 28 days. Rats were given a dose of CCl_4 twice (0.5ml/kg in olive oil in a ratio of 1:1, intraperitoneally) in the last 7 days of the experiment.

2.2.4 Sample Collection & Tissue Preparation

At the end of the 28-day period, and following an over-night fast, the rats were euthanized. Thereafter, they were ventrally dissected and blood samples collected into EDTA bottles for biochemical assays. The liver sample was excised, fixed in 10% formalin and defatted in graded alcohol. Staining was done with hematoxylin-Eosin dye, while the liver architecture was viewed under x400 magnification using an Olympus microscope.

2.3 Biochemical Assays

2.3.1 Gamma-Glutamyl Transferase Assay

Plasma gamma-glutamyl transferase (GGT) was estimated by kinetic colorimetric method as described by Szasz and Persijn (1974).

- PRINCIPLE

  The rate of liberation of 5-amino-2-nitrobenzoate which is a yellow-coloured indicator is directly proportional to the gamma-glutamyl transferase activity in rat plasma which is quantified by increase in absorbance at 405 nm.

- PROCEDURE

  To 100 µL of plasma in test tubes was added 500 µL each of reagent R1 (120 mm/L Tris buffer pH 8.2, 300 mm/L glycylglycine and 12 mm/L sodium azide) and R2 (1.0 mm/L Modified L-gamma-glutamyl-3-carboxy-4-nitroanilide and 8mmol/L sodium azide). These were mixed together and initial absorbance read at 420 nm after 60 seconds. The timer was set and absorbance read after 1,2, and 3 minutes at 420 nm.

- CALCULATION

  Gamma-glutamyl transferase (u/L) = 1111 x Absorbance/mins

2.3.2 Alkaline Phosphatase (ALP) Assay

ALP activity was determined using the procedure elucidated by Young *et al* (1972).

- PRINCIPLE

  ALP present in the plasma sample catalyzes the conversion of p-nitrophenyl phosphate to p-nitrophenol, and its rate of formation is proportional to the level of alkaline phosphatase present in the sample (ref).

- PROCEDURE

  An aliquot of 10 mL of buffer R1 (containing 1.5 mmol/L diethanolamine, 0.5 mmol/L magnesium chloride and 0.1% w/v preservative) and one vial of R2 substrate (containing 10 mmol/L p-nitrophenyl phosphate) were mixed together to form the working reagent. To test tubes labelled blank and sample were added 20 µL of distilled water or plasma

accordingly. Afterwards, 1000 µL of working reagent was added to each tube, mixed and initial absorbance read at 405 nm against distilled water. Subsequent absorbances were read at 1, 2 and 3 minutes.

- CALCULATION

ALP activity = ΔAbsorbance/min x 2750 U/L

The factor (2750) represents the multiplier obtained from the standard curve generated from the Agappe diagnostic laboratory.

2.3.3 Alanine Amino-Transferase (ALT) Assay

- PRINCIPLE

ALT present in the plasma sample catalyzes the conversion of L-Alanine and alpha-ketoglutarate to pyruvate and L-glutamate which determine the kinetic activity of alanine aminotransferase (Kevin *et al.,* 2024).

- PROCEDURE

The working reagents were mixed in the ratio of 4:1 (R1 = 4 and R2= 1). The working reagent R1 containing (Tris buffer (pH 7.5), 110 mmol/L L-Alanine, 600 mmol/low density lipo-protein > 1500 U/L) and R2 containing (alpha-ketoglutarate 16 mmol/L, NADH 0.24 mmol/L). To 100 µL of plasma in test tubes were added 1000 µL of working reagent and mixed together and incubated for 1 min. The absorbance was read every 20 secs for 1 min at 380 nm. Distilled water was used as a blank. The reaction slope was in a decreasing order.

- CALCULATIO23N

ALT Activity (U/L) = (Absorbance/min) x 1745

The factor (1745) represents the multiplier obtained from the standard curve generated from the Agappe diagnostic laboratory.

2.3.4 ASPARTATE AMINO-TRANSFERASE (AST) ASSAY

- PRINCIPLE

Kinetic determination of aspartate aminotransferase (AST) is based on the conversion of L-Aspartate and alpha-ketoglutarate to Oxaloacetate and L-Glutamate (ref).

- PROCEDURE

To 40 mL of reagent 1 (R1) were mixed with 10 mL of reagent 2 (R2) in the ratio of 4:1, were reagent 1 (R1) contain (Tris Buffer (pH 7.8) 88 mmol/L, L-Aspartate 260 mmol/L, LDH > 1500 U/L, MDH> 900 U/L) and R2 contain (alpha-ketoglutarate 12 mmol/L and NADH-0.24 mmol/L). Portions 100 µL of plasma was pipetted into test tubes and added 1000µL of working reagent mixed and incubated for 1 min. The absorbance was read every 20 secs at 380 nm. The reaction slope was in a decreasing order, while the reaction blank contain distilled water.

DATA ANALYSIS

The results were presented as mean ± Standard Error Mean (S.E.M) with a sample size of n = 4. Statistical analysis of data was carried out using the suitable analysis of variance (ANOVA) method, followed by post hoc test for multiple comparisons. A value of $p < 0.05$ was considered statistically significant. The statistical software employed for data analysis was SPSS version 20 and Graphpad version 10

CHAPTER THREE

3.0 Data Presentation

This chapter presents the results obtained from the biochemical analyses carried out to evaluate the ameliorative potential of *Entandrophragma utile* stem bark extracts and their fractions on liver enzymes in carbon tetrachloride (CCl_4)-intoxicated Wistar rats. The data are expressed as mean ± Standard Error of Mean (S.E.M) and statistically analyzed using Analysis of Variance (ANOVA), followed by post-hoc tests to determine significant differences among treatment groups at $p < 0.05$.

The results are illustrated graphically to show the comparative effects of the crude extract, ethyl acetate fraction, and ethanol residue fraction of *E. utile* on key hepatic biomarkers—alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). These enzymes serve as critical indicators of hepatocellular integrity and biliary function.

The control groups (normal and CCl_4-intoxicated) provide reference points for assessing the protective efficacy of the plant extracts, while the silymarin-treated group serves as a positive control for hepatoprotection. Each figure presented in this chapter depicts the effect of the various treatments on the enzymatic activities, highlighting significant variations that reflect the extent of hepatic injury or recovery following extract administration.

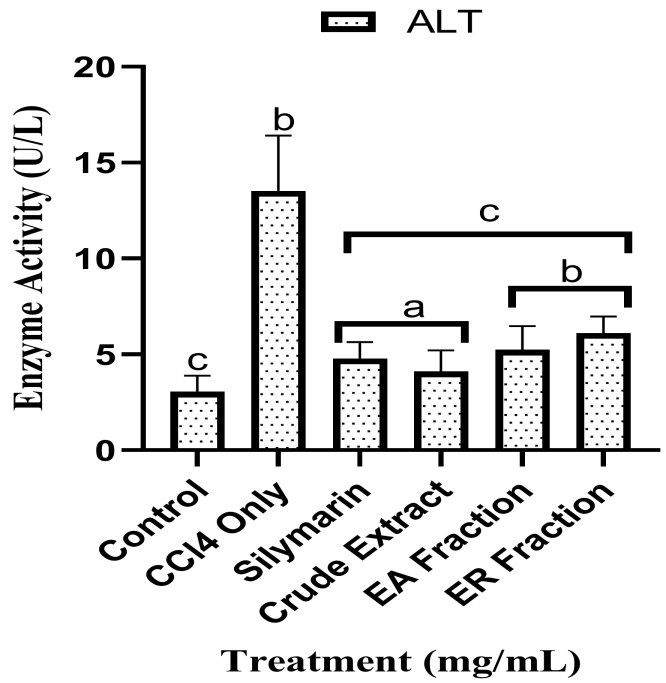

Figure 3.1: Effect of Crude Extract and Fractions of EU on Alanine Aminotransferase Activity in Carbon Tetrachloride-Intoxicated Wistar Rats. Values represent the mean ± Standard Error of Mean (n= 4). CCl_4: Carbon tetrachloride, EA: Ethylacetate, ER: Ethanol residue. a = non-significant compared to the control group, b = significant compared to the control group, c = significant compared to the CCl4 group.

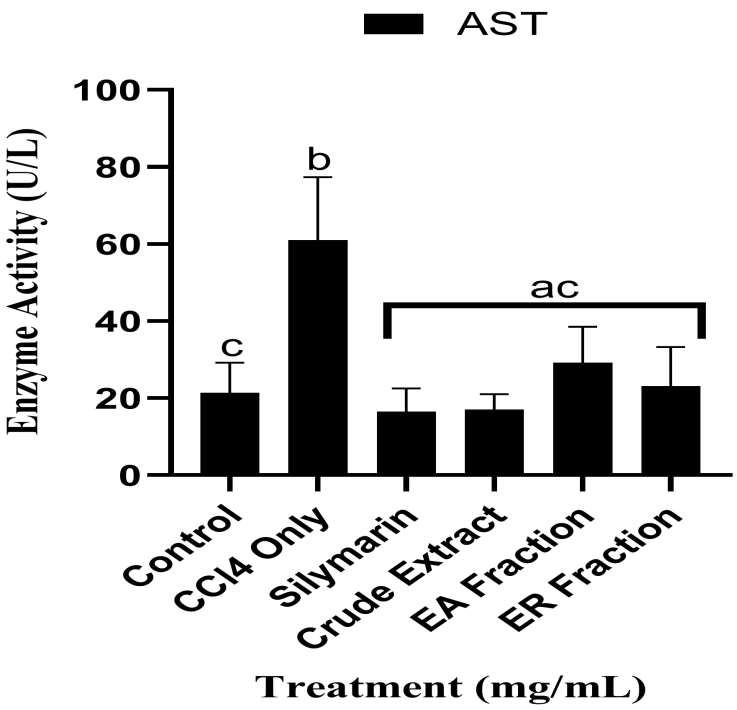

Figure 3.2: Effect of Crude Extract and Fractions of EU on Aspartate Aminotransferase Activity in Carbon Tetrachloride-Intoxicated Wistar Rats. Values represent the mean ± Standard Error of Mean (n= 4). CCl_4: Carbon tetrachloride, EA: Ethylacetate, ER: Ethanol residue. a = non-significant compared to the control group, b = significant compared to the control group, c = significant compared to the CCl4 group.

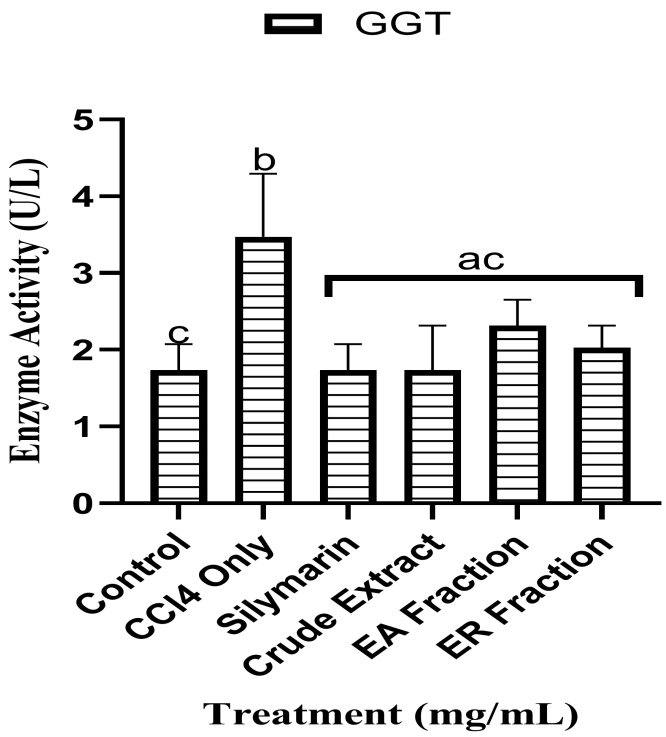

Figure 3.3: Effect of Crude Extract and Fractions of EU on Gamma glutamyl transferase Activity in Carbon Tetrachloride-Intoxicated Wistar Rats. Values represent the mean ± Standard Error of Mean (n= 4). CCl_4: Carbon tetrachloride, EA: Ethylacetate, ER: Ethanol residue. a = non-significant compared to the control group, b = significant compared to the control group, c = significant compared to the CCl4 group.

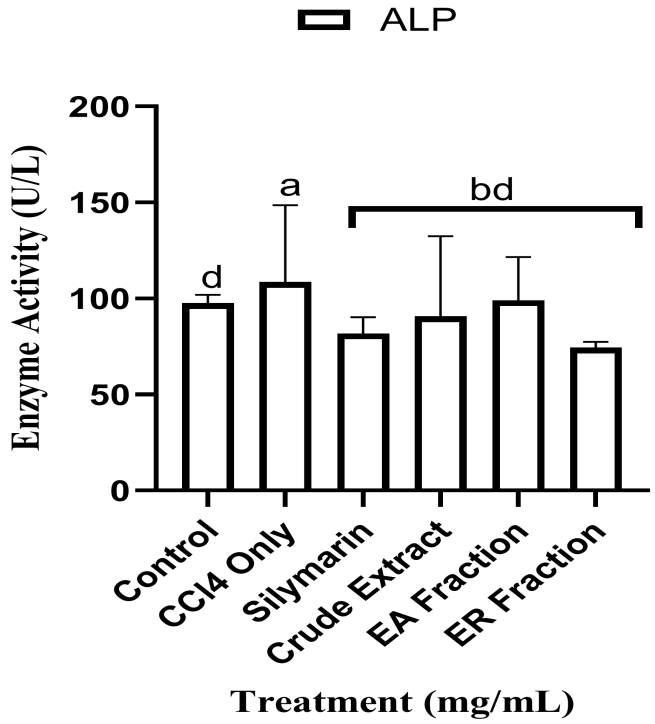

Figure 3.4: Effect of Crude Extract and Fractions of EU on Alkaline Phosphatase Activity in Carbon Tetrachloride-Intoxicated Wistar Rats. Values represent the mean ± Standard Error of Mean (n= 4). CCl_4: Carbon tetrachloride, EA: Ethylacetate, ER: Ethanol residue. a = non-significant compared to the control group, b = significant compared to the control group, d = non-significant compared to the CCl4 group.

CHAPTER FOUR

4.0 Discussion

This study investigated the ameliorative potential of Entandrophragma utile extracts on liver enzymes in carbon tetrachloride (CCl_4)-intoxicated Wistar rats. The parameters evaluated—alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT)—serve as key biochemical markers of hepatocellular integrity and function.

The results showed that administration of CCl_4 caused a significant increase in the plasma levels of ALT, AST, ALP, and GGT in the experimental control group compared with the normal control. This agrees with the well-established mechanism of CCl_4-induced hepatotoxicity, where reactive metabolites such as trichloromethyl ($CCl_3\cdot$) and trichloromethyl peroxy ($CCl_3OO\cdot$) radicals lead to lipid peroxidation and destruction of hepatocyte membranes, resulting in the leakage of intracellular enzymes into the bloodstream (Conner et al., 1986; De Groot et al., 1988).

Treatment with *Entandrophragma utile* crude extract and its fractions (ethyl acetate and ethanol residue) markedly reduced the elevated enzyme levels, showing a dose-dependent hepatoprotective effect similar to the reference drug, silymarin. The significant reduction in ALT and AST levels suggests that the extracts stabilized the hepatocellular membrane and prevented enzyme leakage into the plasma. Similarly, the reduction in ALP and GGT activities implies the restoration of normal biliary function and membrane integrity, indicating potential protection against cholestatic injury.

The hepatoprotective effect observed may be attributed to the phytochemical constituents of Entandrophragma utile such as triterpenoids, methyl angolensate, ergosterol derivatives, and flavonoids (Ngouana et al., 2024; Adesanwo et al., 2022). These compounds are known for their strong antioxidant and free radical scavenging abilities, which can suppress lipid peroxidation and enhance endogenous antioxidant defenses. The antioxidant mechanism aligns with the activation of the NRF2/ARE pathway, which regulates the expression of cytoprotective genes like catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), thereby counteracting oxidative stress in hepatocytes (Wang et al., 2021).

Histological observations (as described in the methodology) would likely reveal that the CCl_4 group exhibited hepatic necrosis, cytoplasmic vacuolation, and sinusoidal congestion, while the

groups treated with E. utile extracts showed a near-normal liver architecture with reduced degenerative changes. This histological improvement corroborates the biochemical findings, confirming the hepatorestorative role of the extracts.

Overall, the findings suggest that Entandrophragma utile possesses bioactive compounds capable of mitigating oxidative damage and restoring liver function following toxin-induced injury. Its mechanism of action appears comparable to that of standard hepatoprotective drugs, underscoring its ethnopharmacological relevance and therapeutic potential.

The present study demonstrates that Entandrophragma utile extracts exert significant hepatoprotective effects against carbon tetrachloride-induced liver injury in Wistar rats. The extracts effectively normalized elevated liver enzyme levels (ALT, AST, ALP, GGT) and likely improved hepatic histoarchitecture, indicating restoration of liver function. These effects are attributed to the antioxidant and free radical scavenging activities of the phytochemicals present in the plant, which reduce oxidative stress and lipid peroxidation.

Therefore, E. utile has potential as a natural therapeutic agent in the prevention and management of chemically induced hepatotoxicity. The results scientifically validate the traditional use of this plant in managing liver-related ailments.

4.1 **Conclusion**

The findings of this study demonstrated *Entandrophragma utile* possesses significant hepatoprotective and restorative properties against carbon tetrachloride (CCl_4)-induced liver injury in Wistar rats. The crude extract, as well as its ethyl acetate and ethanol residue fractions, markedly reduced elevate plasma levels of liver enzymes-alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) which are well-established indicators of hepatocellular and cholestatic damage.

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APPENDIX

RAW DATA OF ENZYME ACTIVITY

ALANINE AMINOTRANSFERASE

Calculation:
ALT activity (U/L) = Absorbance/min x 1745

ALT- SGPT @ 380nm

GROUP 1	0secs	1min	2mins	3mins	Enzyme Activity
H	0.374	0.297	0.288	0.000	15.705
T	0.444	0.428	0.416	0.440	17.450
ABD	0.416	0.401	0.399	0.394	3.490
S	0.334	0.331	0.331	0.329	—
FL	0.234	0.236	0.235	0.233	1.745
B	0.563	0.537	0.512	0.499	43.625
HL	0.478	0.475	0.474	0.471	1.745
GROUP 2	0secs	1min	2mins	3mins	Enzyme Activity
T	0.813	0.805	0.779	0.735	45.370
ABD	0.468	0.466	0.462	0.460	6.980
FL	0.586	0.576	0.564	0.561	20.940
B	0.137	0.127	0.120	0.112	12.215
GROUP 3	0secs	1min	2mins	3mins	Enzyme Activity
H/S	0.231	0.234	0.231	0.227	5.235
H/S/FL	0.439	0.438	0.434	0.431	6.980
ABD/FL	0.387	0.379	0.371	0.363	13.960
S	0.096	0.094	0.092	0.092	3.490
FL	0.350	0.349	0.347	0.347	3.490
GROUP 4	0secs	1min	2mins	3mins	Enzyme Activity
ABD	0.106	0.104	0.102	0.099	3.490
HL	0.223	0.206	0.110	0.109	169.265
GROUP 5	0secs	1min	2mins	3mins	Enzyme Activity
H	0.387	0.382	0.385	0.380	—
S	0.100	0.084	0.086	0.087	—
HL	0.093	0.097	0.099	0.100	—
GROUP 6	0secs	1min	2mins	3mins	Enzyme Activity

H/FL	**0.154**	**0.151**	**0.149**	**0.145**	**3.490**
ABD	**0.163**	**0.161**	**0.157**	**0.154**	**6.980**
S	**0.178**	**0.175**	**0.171**	**0.166**	**6.980**
B	**0.160**	**0.158**	**0.154**	**0.150**	**6.980**
NEW GROUP 4	**0secs**	**1min**	**2mins**	**3mins**	**ENZYME ACTIVITY**
H	**0.205**	**0.189**	**0.166**	**0.152**	**40.135**
ABD	**0.253**	**0.131**	**0.127**	**0.125**	**6.980**
S	**0.457**	**0.436**	**0.387**	**0.338**	**85.505**
FL	**0.251**	**0.228**	**0.218**	**0.194**	**17.450**
HL	**0.234**	**0.214**	**0.211**	**0.208**	**4.235**

ALKALINE PHOSPHATASE

Calculation:
ALP activity = absorbance/min x 2750 U/L

ALP @ 405nm

GROUP 1	0secs	1min	2mins	3mins	Enzyme Activity
H	0.376	0.399	0.405	0.461	154.00
T	0.348	0.385	0.422	0.457	93.50
ABD	0.367	0.400	0.440	0.482	115.50
S	0.372	0.374	0.411	0.448	101.75
FL	0.338	0.371	0.407	0.439	88.00
B	0.401	0.442	0.493	0.541	132.00
HL	0.353	0.382	0.421	0.460	107.25
GROUP 2	0secs	1min	2mins	3mins	Enzyme Activity
T	0.331	0.360	0.382	0.408	71.50
ABD	0.383	0.465	0.547	0.630	228.25
FL	0.318	0.339	0.363	0.388	68.75
B	0.308	0.332	0.356	0.377	57.75
HL	0.304	0.332	0.350	0.371	57.75
GROUP 3	0secs	1min	2mins	3mins	Enzyme Activity
H/S	0.300	0.328	0.347	0.368	57.75
H/S/FL	0.315	0.349	0.382	0.417	96.25
T	0.302	0.336	0.372	0.403	85.25
ABD/FL	0.313	0.355	0.400	0.440	110.00
S	0.297	0.333	0.366	0.401	96.25
FL	0.290	0.326	0.362	0.394	88.00
GROUP 4	0secs	1min	2mins	3mins	Enzyme Activity
ABD	0.291	0.318	0.347	0.375	77.00
HL	0.504	0.579	0.658	0.735	211.75
GROUP 5	0secs	1min	2mins	3mins	Enzyme Activity
H	0.306	0.338	0.372	0.405	90.75
S	0.336	0.405	0.470	0.530	165.00
HL	0.298	0.327	0.353	0.380	74.25
GROUP 6	0secs	1min	2mins	3mins	Enzyme Activity
H/FL	0.305	0.339	0.362	0.389	74.25
ABD	0.286	0.313	0.339	0.364	68.75

S	**0.290**	**0.320**	**0.355**	**0.385**	**82.5**
B	**0.367**	**0.472**	**0.574**	**0.673**	**272.25**
NEW GROUP 4	**0secs**	**1min**	**2mins**	**3mins**	**Enzyme Activity**
H	**1.341**	**1.370**	**1.382**	**1.393**	**30.25**
ABD	**0.809**	**0.837**	**0.864**	**0.890**	**71.50**
S	**1.360**	**1.388**	**1.402**	**1.413**	**30.25**
FL	**1.310**	**1.326**	**1.339**	**1.348**	**24.75**
HL	**1.288**	**1.325**	**1.336**	**1.345**	**49.50**

ASPARTATE AMINOTRANSFERASE

AST @ 380nm

GROUP 1	0secs	1min	2mins	3mins	Enzyme Activity
H	0.457	0.454	0.447	0.441	12.215
T	0.317	0.322	0.323	0.322	—
ABD	0.344	0.345	0.344	0.343	1.745
S	0.224	0.221	0.217	0.213	6.98
FL	0.685	0.671	0.646	0.625	43.625
B	0.566	0.560	0.559	0.546	1.745
HL	0.652	0.610	0.490	0.579	20.94
GROUP 2	0secs	1min	2mins	3mins	Enzyme Activity
T	0.424	0.426	0.426	0.426	—
ABD	0.689	0.736	0.710	0.642	45.37
FL	0.712	0.696	0.661	0.645	61.075
B	0.692	0.677	0.659	0.650	31.41
HL	0.469	0.400	0.339	0.398	106.445
GROUP 3	0secs	1min	2mins	3mins	Enzyme Activity
H/S	0.521	0.508	0.478	0.472	52.35
H/S/FL	0.722	0.688	0.670	0.652	31.41
T	0.410	0.408	0.405	0.398	5.235
ABD/FL	0.530	0.513	0.440	0.431	127.385
S	0.469	0.467	0.455	0.454	20.94
FL	0.487	0.481	0.476	0.472	8.725
GROUP 4	0secs	1min	2mins	3mins	Enzyme Activity
ABD	0.537	0.533	0.525	0.520	13.96
HL	0.861	0.803	0.798	0.781	8.725
GROUP 5	0secs	1min	2mins	3mins	Enzyme Activity
H	0.558	0.547	0.522	0.512	43.625
S	0.526	0.520	0.513	0.504	12.215
HL	0.876	0.856	0.829	0.829	47.115
GROUP 6	0secs	1min	2mins	3mins	Enzyme Activity
H/FL	0.770	0.749	0.719	0.678	52.35
ABD	0.421	0.422	0.420	0.429	3.49
S	0.407	0.390	0.379	0.365	19.195
B	0.459	0.458	0.459	0.456	—

NEW GROUP 4	**0secs**	**1min**	**2mins**	**3mins**	**Enzyme Activity**
H	**0.293**	**0.270**	**0.270**	**0.262**	**17.45**
ABD	**0.250**	**0.239**	**0.234**	**0.229**	**8.725**
S	**0.490**	**0.454**	**0.438**	**0.418**	**27.92**
FL	**0.357**	**0.347**	**0.323**	**0.309**	**41.88**
HL	**0.275**	**0.228**	**0.225**	**0.222**	**5.235**

Gamma-glutamyl transferase

Calculation:

Gamma-glutamyl transferase (u/L) = 1111 X Absorbance/mins

GGT @ 420nm

GROUP 1	0secs	1min	2mins	3mins	CONCENTRATION (g/dL)
H	0.122	0.124	0.126	0.127	2.316
T	0.016	0.021	0.023	0.025	2.316
ABD	0.062	0.065	0.066	0.066	1.158
S	0.074	0.087	0.092	0.094	5.790
FL	0.026	0.031	0.029	0.031	—
B	0.024	0.029	0.029	0.031	—
HL	0.267	0.266	0.270	0.274	4.632
GROUP 2	0secs	1min	2mins	3mins	CONCENTRATION (g/dL)
T	0.085	0.087	0.090	0.091	3.474
ABD	0.056	0.063	0.065	0.065	2.316
FL	0.062	0.067	0.069	0.069	2.316
B	0.054	0.057	0.058	0.060	1.158
HL	0.091	0.092	0.090	0.091	—
GROUP 3	0secs	1min	2mins	3mins	CONCENTRATION (g/dL)
H/S	0.024	0.030	0.030	0.032	—
H/S/FL	0.040	0.050	0.049	0.048	—
T	0.028	0.037	0.036	0.035	—
ABD/FL	0.041	0.045	0.045	0.047	—
S	0.042	0.047	0.050	0.052	3.474
FL	0.035	0.041	0.045	0.046	4.632
GROUP 4	0secs	1min	2mins	3mins	CONCENTRATION (g/dL)
ABD	0.068	0.075	0.078	0.082	3.474
GROUP 5	0secs	1min	2mins	3mins	CONCENTRATION (g/dL)
H	0.028	0.035	0.037	0.037	2.316

S	**0.074**	**0.072**	**0.074**	**0.075**	**2.316**
HL	**0.192**	**0.198**	**0.198**	**0.198**	**—**
GROUP 6	**0secs**	**1min**	**2mins**	**3mins**	**CONCENTRATION (g/dL)**
H/FL	**0.111**	**0.114**	**0.113**	**0.111**	**—**
ABD	**0.072**	**0.076**	**0.078**	**0.078**	**2.316**
S	**0.094**	**0.101**	**0.105**	**0.105**	**4.632**
B	**0.091**	**0.099**	**0.103**	**0.107**	**4.632**

NEW GROUP 4	**1min**	**2mins**	**3mins**	**CONCENTRATION (g/dL)**
H	**0.075**	**0.076**	**0.077**	**1.158**
ABD	**0.068**	**0.069**	**0.071**	**1.158**
FL	**0.132**	**0.135**	**0.139**	**3.474**
HL	**0.090**	**0.091**	**0.092**	**1.158**