

**EFFECTS OF METHANOL LEAF EXTRACT OF *Simarouba glauca* ON LIVER FUNCTION OF FEMALE WISTAR RATS.**

**BY**

**IDUBOR PRECIOUS ESEOSA  
LSC1705124**



**DEPARTMENT OF BIOCHEMISTRY,  
FACULTY OF LIFE SCIENCES  
UNIVERSITY OF BENIN,  
BENIN CITY.**

**DECEMBER, 2022**

**EFFECTS OF METHANOL LEAF EXTRACT OF *Simarouba glauca* ON LIVER FUNCTION OF FEMALE WISTAR RATS.**

**PRESENTED**

**BY**

**IDUBOR PRECIOUS ESEOSA  
LSC1705124**

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

**IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF  
THE AWARD OF THE DEGREE OF THE BACHELOR OF  
SCIENCE, B.Sc (HONS) IN BIOCHEMISTRY, UNIVERSITY OF  
BENIN.**

**DECEMBER, 2022**

## CERTIFICATION

We certify that this project work was carried out by Miss Idubor Precious Eseosa (LSC1705124) in partial fulfillment of the requirements for the award of the degree of bachelor of science (B.Sc) in the Department of Biochemistry, University of Benin, Benin City.

\_\_\_\_\_  
PROF. N.E.J. ORHUE.  
(Project supervisor)

\_\_\_\_\_  
DATE

\_\_\_\_\_  
DR. S. I. OJEABURU.  
(Project Coordinator)

\_\_\_\_\_  
DATE

\_\_\_\_\_  
PROF. (MRS.) K. I. IMAFIDON.  
(Head of Department)

\_\_\_\_\_  
DATE

\_\_\_\_\_  
EXTERNAL EXAMINER.

\_\_\_\_\_  
DATE

## **DEDICATION**

This project work is dedicated to God almighty who brought me this far and has been everything to me. To my parents; Mr. and Mrs. Idubor, Mr. Nosakhare Osagie and to all my other genuine supporters for the love and support that they have always given to me.

## **ACKNOWLEDGEMENTS**

I give my profound gratitude to the creator of the universe for his enormous love, sustenance and provision throughout my degree program, I remain eternally grateful to him. My eternal appreciation also goes to my parents; Mr. and Mrs. Idubor, Mr. Nosakhare Osagie, for their priceless love, advice and support all through my stay in the university. God richly bless you all.

I also want to also appreciate my project supervisor Prof. N. E. J ORHUE for his correctional, fatherly support and contribution all through the course of my undergraduate project experiments and documentation.

## TABLE OF CONTENTS

Cover	-	-	-	-	-	-	-	-	-	-	-	i
Title	-	-	-	-	-	-	-	-	-	-	-	ii
Certification	-	-	-	-	-	-	-	-	-	-	-	iii
Dedication	-	-	-	-	-	-	-	-	-	-	-	iv
Acknowledgement	-	-	-	-	-	-	-	-	-	-	-	v
Table of contents	-	-	-	-	-	-	-	-	-	-	-	vi
Abstract	-	-	-	-	-	-	-	-	-	-	-	viii

### CHAPTER ONE

1.0 Introduction / Literature Review	-	-	-	-	-	-	-	-	-	-	-	1
1.1 The liver	-	-	-	-	-	-	-	-	-	-	-	1
1.2 Anatomy of the Liver	-	-	-	-	-	-	-	-	-	-	-	7
1.3 Functions of the liver	-	-	-	-	-	-	-	-	-	-	-	8
1.4 The plant ( <i>Simarouba glauca</i> )	-	-	-	-	-	-	-	-	-	-	-	12

### CHAPTER TWO

2.0 Materials and equipments	-	-	-	-	-	-	-	-	-	-	-	17
2.1 Biochemical Assays	-	-	-	-	-	-	-	-	-	-	-	17

2.2 Preparation of plant extract - - - - - 19

2.3 Experimental Analysis - - - - - 20

**CHAPTER THREE**

3.0 Results - - - - - 21

3.1 Explanation of Results - - - - - 21

**CHAPTER FOUR**

4.0 Discussion and Conclusion - - - - - 22

References - - - - - 24

## ABSTRACT

The aim of the study is to evaluate the effect of methanol extract of *Simarouba glauca* on liver. The leaves, wood and stem bark of the *Simarouba glauca* plant are the portions that are reportedly most frequently used. In this study, the leaves were used. The *Simarouba glauca* leaves were procured, cleaned and allowed to dry for 28 days at room temperature. The dried leaves were ground into powder. Six hundred grams were weighed and dissolved in 2.5 L of methanol. The mixture was stirred continuously for seven days then the methanol extract was recovered from this combination by filtration and the extract was concentrated by freezing the filtrate. Thirty female wistar rats of average weight 80g were divided equally into six groups with five animals per group. Group I animals were used as the control, Group II, III, and IV, V and VI animals received methanol extract of *Simarouba glauca* at dose levels of 125, 250, 500, 750 and 1000 mg/kg body weight daily, orally for 28 days. The animals were then sacrificed and the blood obtained, centrifuged and the serum withdrawn for analysis. Total bilirubin, Alkaline phosphatase and Aspartate amino-transferase assays were carried out. The results of this study shows that there was no significant ( $P < 0.05$ ) difference in all the parameters assayed when compared with the control. This is indicative of the non-toxic effect of *Simarouba glauca* on the liver of female wistar rats.

## CHAPTER ONE

### 1.0 INTRODUCTION/ LITERATURE REVIEW

#### 1.1 THE LIVER

The liver is a major organ only found in vertebrates which performs many essential biological functions such as detoxification of the organism and the synthesis of proteins and biochemicals necessary for digestion and growth (Nosek *et al.*, 2016; Elias *et al.*, 1952; Abdel-Misih *et al.*, 2010). In humans, it is located in the right upper quadrant of the abdomen, below the diaphragm. Its other roles in metabolism include the regulation of glycogen storage, decomposition of red blood cells, and the production of hormones. The liver is an accessory digestive organ that produces bile, an alkaline fluid containing cholesterol and bile acids, which helps the breakdown of fat. The gallbladder, a small pouch that sits just under the liver, stores bile produced by the liver which is afterwards moved to the small intestine to complete digestion (Tortora *et al.*, 2008). The liver's highly specialized tissue, consisting mostly of hepatocytes, regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Jean Hopkins *et al.*, 1993). Estimates regarding the organ's total number of functions vary, but is generally cited as being around 500 (Zakim *et al.*, 2002).

It is not known how to compensate for the absence of liver function in the long term, although, liver dialysis techniques can be used in the short term. Artificial livers have not been developed to promote long-term replacement in the absence of the liver. As of 2018, liver transplantation is the only option for complete liver failure.

The liver is a reddish-brown, wedge-shaped organ with two lobes of unequal size and shape. A human liver normally weighs approximately 1.5 kg (3.3 lb) (Ramzi *et al.*, 2005) and has a width of about 15 cm (6 in) (Mayo *et al.*, 2017). There is considerable size variation between individuals, with the standard reference range for men being 970–1,860 g (2.14–4.10 lb) (D. Kimberley *et al.*, 2012) and for women 600–1,770 g (1.32–3.90 lb) (D. Kimberley *et al.*, 2015). It is both the heaviest internal organ and the largest gland in the human body. Located in the right upper quadrant of the abdominal cavity, it rests just below the diaphragm, to the right of the stomach and overlies the gallbladder (Tortora *et al.*, 2000).

The liver is connected to two large blood vessels: the hepatic artery and the portal vein. The hepatic artery carries oxygen-rich blood from the aorta via the celiac trunk, whereas the portal vein carries blood rich in digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas. These blood vessels subdivide into small capillaries known as liver sinusoids, which then lead to lobules. Lobules are the functional units of the liver, each lobule is made up of millions of hepatic cells (hepatocytes) which are the basic metabolic cells. The lobules are held together by a fine, dense, irregular, fibro-elastic connective tissue layer extending from the fibrous capsule covering the entire liver known as Glisson's capsule. This extends into the structure of the liver by accompanying the blood vessels, ducts, and nerves at the hepatic hilum. The whole surface of the liver, except for the bare area, is covered in a serous coat derived from the peritoneum, and this firmly adheres to the inner Glisson's capsule.

On the diaphragmatic surface, apart from a triangular bare area where it connects to the diaphragm, the liver is covered by a thin, double-layered membrane, the peritoneum, that helps to reduce friction against other organs (Singh *et al.*, 2008). This surface covers the convex shape of the two lobes where it accommodates the shape of the diaphragm. The peritoneum folds back

on itself to form the falciform ligament and the right and left triangular ligaments (McMinn *et al.*, 2003).

These peritoneal ligaments are not related to the anatomic ligaments in joints, and the right and left triangular ligaments have no known functional importance, though they serve as surface landmarks (McMinn *et al.*, 2003). The falciform ligament functions to attach the liver to the posterior portion of the anterior body wall.

The visceral surface or inferior surface is uneven and concave. It is covered in peritoneum apart from where it attaches the gallbladder and the porta hepatis (Singh *et al.*, 2008). The fossa of gall bladder lies to the right of the quadrate lobe, occupied by the gallbladder with its cystic duct close to the right end of porta hepatis.

Several impressions on the surface of the liver accommodate the various adjacent structures and organs. Underneath the right lobe and to the right of the gallbladder fossa are two impressions, one behind the other and separated by a ridge. The one in front is a shallow colic impression, formed by the hepatic flexure and the one behind is a deeper renal impression accommodating part of the right kidney and part of the suprarenal gland (Skandalakis *et al.*, 2009).

The suprarenal impression is a small, triangular, depressed area on the liver. It is located close to the right of the fossa, between the bare area and the caudate lobe, and immediately above the renal impression. The greater part of the suprarenal impression is devoid of peritoneum and it lodges the right suprarenal gland (Dorland *et al.*, 2012).

Medial to the renal impression is a third and slightly marked impression, lying between it and the neck of the gall bladder. This is caused by the descending portion of the duodenum, and is known as the duodenal impression (Dorland *et al.*, 2012).

The inferior surface of the left lobe of the liver presents behind and to the left of the gastric impression (Dorland *et al.*, 2012). This is moulded over the upper front surface of the stomach, and to the right of this is a rounded eminence, the tuber omentale, which fits into the concavity of the lesser curvature of the stomach and lies in front of the anterior layer of the lesser omentum.

Microscopically, each liver lobe is seen to be made up of hepatic lobules. The lobules are roughly hexagonal, and consist of plates of hepatocytes, and sinusoids radiating from a central vein towards an imaginary perimeter of interlobular portal triads (Moore *et al.*, 2018). The central vein joins to the hepatic vein to carry blood out from the liver. A distinctive component of a lobule is the portal triad, which can be found running along each of the lobule's corners. The portal triad consists of the hepatic artery, the portal vein, and the common bile duct (Moore *et al.*, 2018). The triad may be seen on a liver ultrasound, as a Mickey Mouse sign with the portal vein as the head, and the hepatic artery and the common bile duct as the ears.

Histology, the study of microscopic anatomy, shows two major types of liver cell: parenchymal cells and non-parenchymal cells. About 70–85% of the liver volume is occupied by parenchymal hepatocytes. Non-parenchymal cells constitute 40% of the total number of liver cells but only 6.5% of its volume (Kmiec *et al.*, 2001). The liver sinusoids are lined with two types of cell, sinusoidal endothelial cells, and phagocytic Kupffer cells (Pocock *et al.*, 2006). Hepatic stellate cells are nonparenchymal cells found in the perisinusoidal space, between a sinusoid and a hepatocyte (Kmiec *et al.*, 2001). Additionally, intrahepatic lymphocytes are often present in the sinusoidal lumen (Kmiec *et al.*, 2001). The central area or hepatic hilum, includes the opening known as the porta hepatis which carries the common bile duct and common hepatic artery, and

the opening for the portal vein. The duct, vein, and artery divide into left and right branches, and the areas of the liver supplied by these branches constitute the functional left and right lobes. The functional lobes are separated by the imaginary plane Cantlie's line, joining the gallbladder fossa to the inferior vena cava. The plane separates the liver into the true right and left lobes. The middle hepatic vein also demarcates the true right and left lobes. The right lobe is further divided into an anterior and posterior segment by the right hepatic vein. The left lobe is divided into the medial and lateral segments by the left hepatic vein.

The hilum of the liver is described in terms of three plates that contain the bile ducts and blood vessels. The contents of the whole plate system are surrounded by a sheath (Kawarada *et al.*, 2000). The three plates are the hilar plate, the cystic plate and the umbilical plate and the plate system is the site of the many anatomical variations to be found in the liver (Kawarada *et al.*, 2000).

### **Couinaud classification system:**

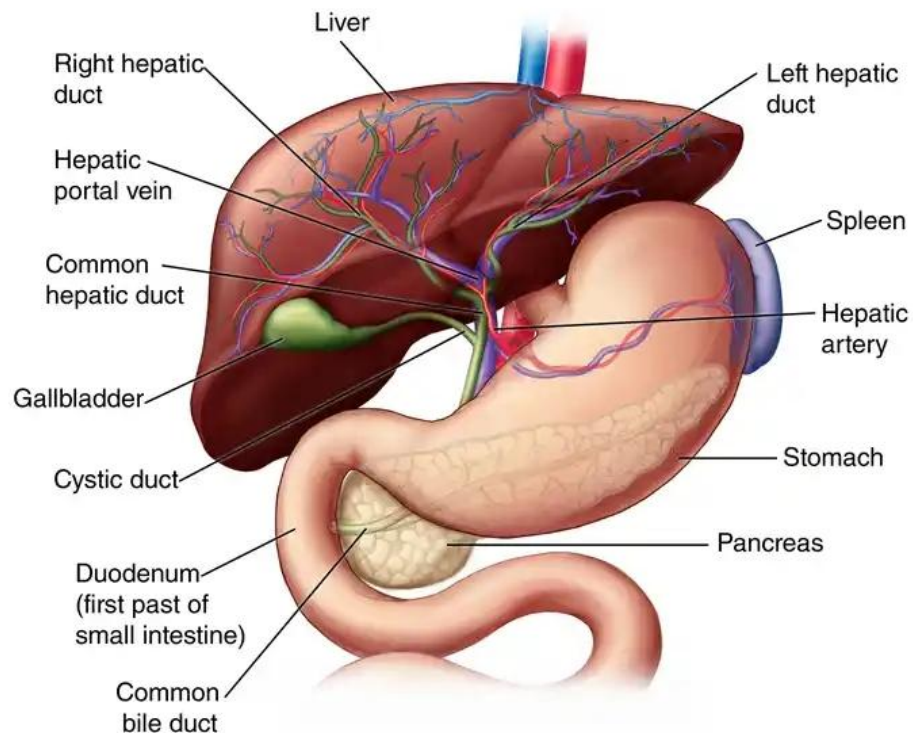
In the widely used Couinaud system, the functional lobes are further divided into a total of eight subsegments based on a transverse plane through the bifurcation of the main portal vein. The caudate lobe is a separate structure that receives blood flow from both the right- and left-sided vascular branches (Stuckman *et al.*, 2003). The Couinaud classification divides the liver into eight functionally independent liver segments. Each segment has its own vascular inflow, outflow and biliary drainage. In the centre of each segment are branches of the portal vein, hepatic artery, and bile duct. In the periphery of each segment is vascular outflow through the hepatic veins. The classification system uses the vascular supply in the liver to separate the functional units (numbered I to VIII) with unit 1, the caudate lobe, receiving its supply from

both the right and the left branches of the portal vein. It contains one or more hepatic veins which drain directly into the inferior vena cava. The remainder of the units (II to VIII) are numbered in a clockwise fashion.

**Gene and protein expression:**

About 20,000 protein coding genes are expressed in human cells and 60% of these genes are expressed in a normal, adult liver (Mathias *et al.*, 2015). Over 400 genes are more specifically expressed in the liver, with some 150 genes highly specific for liver tissue. A large fraction of the corresponding liver-specific proteins are mainly expressed in hepatocytes and secreted into the blood and constitute plasma proteins and hepatokines. Other liver-specific proteins are certain liver enzymes such as HAO1 and RDH16, proteins involved in bile synthesis such as BAAT and SLC27A5, and transporter proteins involved in the metabolism of drugs, such as ABCB11 and SLC2A2. Examples of highly liver-specific proteins include apolipoprotein A II, coagulation factors F2 and F9, complement factor related proteins, and the fibrinogen beta chain protein (Kampf *et al.*, 2014).

## 1.2 ANATOMY OF THE LIVER



**John Hopkins *et al.*, (1993)**

The liver is located in the upper right –hand portion of the abdominal cavity, beneath the diaphragm, and on top of the stomach right kidney, and intestines.

Shaped like a cone, the liver is a dark reddish- brown organ that weighs about 3 pounds.

- Oxygenated blood flows in from the hepatic artery.
- Nutrient- rich blood flows in from the hepatic portal vein.

The liver holds about one pint (13%) of the body’s blood supply at any given moment. the liver consists of 2 main lobes. both are made up of 8 segments that consist of 1,000 lobules (small lobes). these lobules are connected to small ducts (tubes) that connect with larger ducts to form

the common hepatic duct. the common hepatic duct transports the bile made by the liver cells to the gall bladder and duodenum (the first part of the small intestine) via the common bile duct.

### **1.3 FUNCTIONS OF THE LIVER**

The various functions of the liver are carried out by the liver cells or hepatocytes. The liver is thought to be responsible for up to 500 separate functions, usually in combination with other systems and organs. Currently, no artificial organ or device is capable of reproducing all the functions of the liver. Some functions can be carried out by liver dialysis, an experimental treatment for liver failure. The liver also accounts for about 20% of resting total body oxygen consumption.

#### **Blood supply:**

The liver receives a dual blood supply from the hepatic portal vein and hepatic arteries. The hepatic portal vein delivers around 75% of the liver's blood supply and carries venous blood drained from the spleen, gastrointestinal tract, and its associated organs. The hepatic arteries supply arterial blood to the liver, accounting for the remaining quarter of its blood flow. Oxygen is provided from both sources; about half of the liver's oxygen demand is met by the hepatic portal vein, and half is met by the hepatic arteries. The hepatic artery also has both alpha- and beta-adrenergic receptors; therefore, flow through the artery is controlled, in part, by the splanchnic nerves of the autonomic nervous system.

The liver plays a major role in carbohydrate, protein, amino acid, and lipid metabolism.

#### **Carbohydrate metabolism:**

The liver performs several roles in carbohydrate metabolism:

The liver synthesizes and stores around 100g of glycogen via glycogenesis, the formation of glycogen from glucose. When needed, the liver releases glucose into the blood by performing glycogenolysis, the breakdown of glycogen into glucose.

The liver is also responsible for gluconeogenesis, which is the synthesis of glucose from certain amino acids, lactate, or glycerol. Adipose and liver cells produce glycerol by breakdown of fat, which the liver uses for gluconeogenesis.

Liver also does glyconeogenesis which is synthesis of glycogen from lactic acid.

#### Protein metabolism:

The liver is responsible for the mainstay of protein metabolism, synthesis as well as degradation. All plasma proteins except Gamma-globulins are synthesised in the liver. (Miller *et al.*, 1954) It is also responsible for a large part of amino acid synthesis. The liver plays a role in the production of clotting factors, as well as red blood cell production. Some of the proteins synthesized by the liver include coagulation factors I (fibrinogen), II (prothrombin), V, VII, VIII, IX, X, XI, XII, XIII, as well as protein C, protein S and antithrombin. The liver is a major site of production for thrombopoietin, a glycoprotein hormone that regulates the production of platelets by the bone marrow. (Jelkmnan *et al.*, 2001)

#### **Lipid metabolism:**

The liver plays several roles in lipid metabolism: it performs cholesterol synthesis, lipogenesis, and the production of triglycerides, and a bulk of the body's lipoproteins are synthesized in the liver. The liver plays a key role in digestion, as it produces and excretes bile (a yellowish liquid) required for emulsifying fats and help the absorption of vitamin K from the diet. Some of the bile

drains directly into the duodenum, and some is stored in the gallbladder. The liver produces insulin-like growth factor 1, a polypeptide protein hormone that plays an important role in childhood growth and continues to have anabolic effects in adults.

### **Breakdown:**

The liver is responsible for the breakdown of insulin and other hormones. The liver breaks down bilirubin via glucuronidation, facilitating its excretion into bile. The liver is responsible for the breakdown and excretion of many waste products. It plays a key role in breaking down or modifying toxic substances (e.g., methylation) and most medicinal products in a process called drug metabolism. This sometimes results in toxication, when the metabolite is more toxic than its precursor. Preferably, the toxins are conjugated to avail excretion in bile or urine. The liver converts ammonia into urea as part of the ornithine cycle or the urea cycle, and the urea is excreted in the urine. Due to the fact that the liver is an expandable organ, large quantities of blood can be stored in its blood vessels. Its normal blood volume, including both that in the hepatic veins and that in the hepatic sinuses is about 450 milliliters, or almost 10 percent of the body's total blood volume. When high pressure in the right atrium causes backpressure in the liver, the liver expands, and 0.5 to 1 liter of extra blood is occasionally stored in the hepatic veins and sinuses. This occurs especially in cardiac failure with peripheral congestion. Thus, in effect, the liver is a large, expandable, venous organ capable of acting as a valuable blood reservoir in times of excess blood volume and capable of supplying extra blood in times of diminished blood volume. (Laatt *et al.*, 1976)

### **Lymph production:**

Because the pores in the hepatic sinusoids are very permeable and allow ready passage of both fluid and proteins into the perisinusoidal space, the lymph draining from the liver usually has a protein concentration of about 6 g/dl, which is only slightly less than the protein concentration of plasma. Also, the high permeability of the liver sinusoid epithelium allows large quantities of lymph to form. Therefore, about half of all the lymph formed in the body under resting conditions arises in the liver.

The liver stores a multitude of substances, including vitamin A (1–2 years' supply), vitamin D (1–4 months' supply), vitamin B12 (3–5 years' supply), vitamin K, vitamin E, iron, copper, zinc, cobalt, molybdenum, etc.

The formation of blood cells is called haemopoiesis. In embryonic stage RBC and WBC are formed by liver. In the first trimester fetus, the liver is the main site of red blood cell production. By the 32nd week of gestation, the bone marrow has almost completely taken over that task. (Timens *et al.*, 1990)

Liver helps in purification of blood. The Kupffer cells of liver are phagocytic cells, helps in phagocytosis of dead blood cells and bacteria from the blood. (Nguyen – Lefebvre *et al.*, 2015). The liver is responsible for immunological effects – the mononuclear phagocyte system of the liver contains many immunologically active cells, acting as a 'sieve' for antigens carried to it via the portal system.

The liver produces albumin, the most abundant protein in blood serum. It is essential in the maintenance of oncotic pressure, and acts as a transport for fatty acids and steroid hormones.

The liver synthesizes angiotensinogen, a hormone that is responsible for raising the blood pressure when activated by renin, an enzyme that is released when the kidney senses low blood pressure. The liver produces the enzyme catalase to break down hydrogen peroxide, a toxic oxidising agent, into water and oxygen.

### **1.5 THE PLANT (*Simarouba glauca*)**

*Simarouba glauca* is a flowering tree that is native to Florida, South America, and the Caribbean. Common names include paradise-tree, dysentery-bark, bitterwood. The tree is well suited for warm, humid, tropical regions. Its cultivation depends on rainfall distribution, water holding capacity of the soil and sub-soil moisture. It is suited for temperature range of 10 to 40 °C (50 to 104 °F). It can grow at elevations from sea level to 1,000 m (3,300 ft). It grows 40 to 50 ft (12 to 15 m) tall and has a span of 25 to 30ft (7.6 to 9.1 m). It bears yellow flowers and oval elongated purple colored fleshy fruits.



***Simarouba glauca* Plant**

**Scientific Classification:**

Kingdom: Plantae

Clade: Angiosperms

Order: Sapindales

Family: Simaroubaceae

Genus: *Simarouba*

Species: *S. glauca*

**PHARMACOLOGICAL AND BIOCHEMICAL IMPORTANCE:**

Traditional medicines are defined as the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses (WHO, 2005). From time immemorial, humans have relied on traditional medicines for the treatment of diseases. The most popular and widely used form of traditional medicines are the herbal medicines which include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients (WHO, 2005). *Simarouba glauca* ( SG), commonly known as ‘Laxmitaru’ or ‘Paradise tree’ or ‘Bitter wood’ belongs to the family *Simaroubaceae* and is native to Southern Florida, South America and the Caribbean (Manasi, 2011). It is well known for its various medicinal and pharmacological properties. The bark and leaf extracts of SG have pharmacological properties such as

haemostatic, antihelminthic, antiparasitic, antidysenteric, antipyretic and anticancerous properties (Patil and Gaikwad, 2011). The leaves, fruits, pulps and seeds of SG are known to possess medicinal properties such as analgesic, antimicrobial, antiviral, astringent, emmenagogue, stomachic, tonic and vermifuge (Joshi and Joshi, 2002). SG is a rich source of quassinoids such as glaucarubol, glaucarubolone and the two esters of glaucarubolone, ailanthinone and glaucarubinone (Almeida *et al.*, 2007). The main active constituents in SG include: ailanthinone, benzoquinone, canthin, dehydroglaucarubinone, glaucarubine, glaucarubolone, glaucarubinone, holacanthone, melianone, simaroubidin, simarolide, simarubin, simarubolide, sitosterol, tirucalla etc. (Patil and Gaikwad, 2011). Oral acute and sub-chronic toxicity tests of the aqueous extract of SG leaves have been shown to be non-toxic at doses of up to 2000 mg/kg (Osagie-Eweka *et al.*, 2021). Because the crude extracts contain so many different chemicals, some yet unknown, research has to be undertaken to not just identify these chemicals, but also to understand their effects on biological systems.

### **Medicinal Properties of *Simarouba glauca* :**

**Antimalarial Activity:** Studies have shown SG extract to be effective against chloroquine-resistant malaria in vitro and in vivo (Rodríguez *et al.*, 2006). **Antibacterial Activity:** Fresh and dried SG leaf extracts have antibacterial activity against both Gram-positive and Gram-negative bacteria. They inhibit microorganisms such as *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Rajurkar, 2011). **Antiamoebic Activity:** In vitro studies showed that glaucarubin from SG was active against *Entamoeba histolytica* (Assendelft *et al.*, 1956).

**Antioxidant Activity:** SG leaves extract were reported to have antioxidant activity. Chloroform extracts of SG were capable of scavenging H<sub>2</sub>O<sub>2</sub> in a concentration dependent manner. The extracts were highly effective in scavenging free radicals such as DPPH and chelating ferrous iron (Umesh, 2015).

**Antiulcer Activity:** Chloroform extracts of SG showed dose dependent inhibition of ethanol-induced gastric lesions in albino rats causing 82.63% protection at 400 mg/kg and 53.48 % protection at 200 mg/kg. Chloroform extracts of SG also showed dose dependent inhibition of indomethacin induced gastric lesions in albino rats, causing 62.65 % protection at 400 mg/kg and 54.86 % protection at 200 mg/kg. Chloroform Extracts of the leaves of SG decreased the acidity of mucosal secretions (Shankara and Sriram, 2014).

**Antifungal Activity:** Methanol and ethanol extract of SG exhibited antifungal activity against *Fusarium oxysporum* and *Aspergillus parasiticus*. Ethanolic extracts of fresh leaves were found to be more effective than the methanolic extracts of the fresh leaves against the growth of the fungi by agar well diffusion method (Mikawlawng *et al.*, 2014). **Hemostatic Activity:** The bark of SG has been used as a hemostatic agent to avoid bleeding. Externally, it is used for wounds and cuts (Valdés *et al.*, 2008). The bark is boiled in water and is occasionally used to give a strong astringent and tonic to cleanse your skin and cure dysentery, diarrhoea, bowel, blood bleeding, and internal bleeding (Sharma and Dwivedi, 2016).

**Antihypertensive Activity:** Methanol leaf extracts of SG at 50 and 100 mg/kg have been shown to reverse hypertension induced coronary artery enlargement. At 25 and 100 mg/kg, methanol leaf extracts of SG reversed hypertension-induced renal changes. This is similar to the effect of the antihypertensive drug, amlodipine (Osagie-Eweka *et al.*, 2021). **Poison:** The

residual cake left after processing the crude fat from the seed contains two toxic quassinoid glucosides namely 15-o- $\beta$ -D-glucopyranosyl glaucarubolone 3 and 15-o- $\beta$ -Dglucopyranosyl glaucarubol (Bhatnagar, 1984).

**Anticancer Activity:** SG extracts contain canthin-6-one, 2-hydroxycanthin-6-one, limonoid and melianodiol which have been shown to inhibit the growth of human cancer cell lines (Rivero-Cruz *et al.*, 2005). Scopoletin, canthin-6-one, and canthine-6-one dimethoxy derivatives from SG wood extracts showed the potential to inhibit human breast cancer cell lines at 2.0 mg/ml and 5.5 mg/ml respectively (Reynertson *et al.*, 2011).



blank and sample tubes respectively. The mixtures were further incubated for 10 minutes at 25°C and the absorbance was measured at 578 nm.

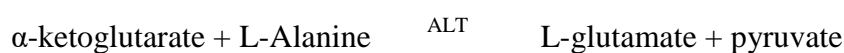
### Calculation

Total bilirubin:

$$10.8 \times \text{Abs (mg/dl)}.$$

Determination of ALT Activity

### Principle



The activity of ALT was assayed by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine.

### Assay Procedure

Plasma sample (0.1 mL) and 0.5 mL of reagent 1 were pipetted into a test tube. The blank contained 0.1 mL of distilled water and 0.5 mL of reagent 1. Each tube was mixed and incubated for 30 min at 37 °C. Portions of reagent 2 (0.5 mL) were added to each tube, and the contents were mixed and incubated for another 20 min at 25 °C. Then, 5.0 mL of 0.4 mol/L NaOH solution was added to each tube. The tubes were mixed and absorbance read at 540 nm against reagent blank after 5 min. The activities of ALT corresponding to the absorbance values obtained were extrapolated from ALT standard calibration curve.

### Determination of Activity of AST

### Principle



→

The activity of AST was assayed by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

### **Assay Procedure**

Two tubes were arranged in duplicate on rack and labelled “sample blank” and “sample”, respectively. Plasma (0.1 mL) and 0.5 mL of reagent 1 were added to each tube. The contents of the tubes were mixed and allowed to stand for 20 min at 25 °C. Then, 5.0 mL NaOH solution was added to each tube, and the contents were mixed and absorbance read at 540 nm against the blank after 5 min. The activities of ALT corresponding to the absorbance values obtained were extrapolated from ALT standard calibration curve.

## **2.2 PREPARATION OF PLANT EXTRACT**

Collection of leaves and preparation of the extract:

Leaves of SG were harvested from Cercobela Farms, Ubiaja, Esan South East Local Government Area of Edo State, Nigeria. The leaves were rinsed with tap water and airdried for twenty-eight days at room temperature. Leaves were pulverized and sifted off a mesh to obtain fine particles. Weighed quantities of the fresh, finely ground powder were mixed with methanol (99.55%) and subjected to maceration with intermittent shaking for two days. The extract obtained were collected by filtration using muslin cloth. The filtrate obtained were subjected to rotary evaporation to obtain solid extract which were weighed and stored in an air-tight container (Sreedhar et al., 2015).

### **2.3 EXPERIMENTAL ANALYSIS:**

Female albino Wistar rats weighing ~80g were used for the study. The animals were maintained under controlled conditions of temperature ( $28 \pm 2^{\circ}\text{C}$ ), humidity ( $50 \pm 5\%$ ) and 12-hour light-dark cycles. All the animals were acclimatized for fourteen days before commencement of the study. The animals were housed in metabolic cages. They had free access to a normal pelletised commercial feed and water ad libitum. The initial and final body weight of animals were recorded. Blood samples were collected by cardiac puncture. The animals were sacrificed by cervical dislocation and then the uterus and ovaries were harvested, freed from adherent tissue and weighed (Okafor et al., 2021). The ovaries and uterus were removed and their weights (absolute and relative to body weight) were measured (Montagnini et al., 2016).

### **ADMINISTRATION OF PLANT EXTRACTS:**

animals were divided equally into six groups with 5 animals per group. Group I animals were used as the control, Group II, III, and IV, V and VI animals received methanol extract of *Simarouba glauca* at dose levels of 125, 250, 500, 750 and 1000mg/kg body weight daily, orally for 28 days.

## CHAPTER THREE

### RESULTS

#### 3.1 EFFECT OF METHANOL EXTRACT OF *SIMAROUBA GLAUCA* LEAVES ON LIVER TOTAL BILIRUBIN, ALP AND AST ACTIVITIES IN FEMALE RATS

##### 3.1.1 Effect on Liver Total Bilirubin Concentration

The effect of methanol extract of *Simarouba glauca* (MESG) leaves on liver total bilirubin concentration is presented in Table 3.1. There was no significant difference ( $p > 0.05$ ) between liver bilirubin levels of the extract treated female rats (G1, G2, G3, G4 and G5) when compared to the control rats.

##### 3.1.2 Effect on Liver ALP and AST Activities

The effect of methanolic extract of *Simarouba glauca* (MESG) leaves on liver AST and ALT is presented in Table 3.1. There was no significant difference ( $p > 0.05$ ) between liver bilirubin levels of the extract treated female rats (G1, G2, G3, G4 and G5) when compared to the control rats.

**Table 3.1:** Liver Bilirubin, ALP and AST Levels in Normal and Extract Treated Rats.

GROUPS	BIL (g/L)	ALP (U/L)	AST (U/L)
CONTROL	137.6 ± 39.3 <sup>a</sup>	0.16 ± 0.00 <sup>a</sup>	0.20 ± 0.01 <sup>a</sup>
G1	125.6 ± 15.1 <sup>a</sup>	0.20 ± 0.03 <sup>a</sup>	0.20 ± 0.00 <sup>a</sup>
G2	135.7 ± 12.4 <sup>a</sup>	0.16 ± 0.01 <sup>a</sup>	0.20 ± 0.00 <sup>a</sup>
G3	146.2 ± 3.7 <sup>a</sup>	0.16 ± 0.00 <sup>a</sup>	0.20 ± 0.00 <sup>a</sup>
G4	127.0 ± 12.3 <sup>a</sup>	0.16 ± 0.00 <sup>a</sup>	0.20 ± 0.01 <sup>a</sup>
G5	139.0 ± 6.0 <sup>b</sup>	0.17 ± 0.01 <sup>a</sup>	0.20 ± 0.01 <sup>a</sup>

Values are in mean ± SEM. Data with the same superscripts are considered not statistically different ( $p > 0.05$ ).

## CHAPTER FOUR

### DISCUSSION AND CONCLUSION

The use of plants as complementary or alternative medicine recently has been encouraged over the years. A reason for this is the belief that these botanicals contribute to the improvement of human health. The aim of this study was to observe the effects of treatment with the methanolic extract of *Simarouba glauca* (doses at 200, 400, 600, 800 and 1,000 mg/kg bw.) on the liver of normal rats. Total bilirubin, ALP and AST concentrations were used as the biomarker for the liver function test.

Total bilirubin (TB) is made up of direct bilirubin (DB) and indirect bilirubin (IB). A rise in hepatic DB in an adult signals compromised liver function, however, this is not the case with IB (Saranraj and Sivasakthi, 2014). An increase in IB (>90%) is suggestive of acute red blood cell hemolysis or Gilbert syndrome, as well as possibly an accelerated rate of heme breakdown (Osagie-Eweka *et al.*, 2021).

Relative to the control rats (137.6 g/L), results indicated a slight decrease in hepatic TB levels in G1 (125.6 g/L), G2 (135.7 g/L) and G4 (127 g/L), albeit not statistically significant ( $p > 0.05$ ). Total bilirubin concentrations in MESH treated rats (G3= 146.2 g/L and G5 = 139 g/L) is also not statistically different ( $p > 0.05$ ). This correlates with the study of Osagie-Eweka *et al.*, (2021), whose study also indicated that MESH did not demonstrate a significant hepatotoxic or therapeutic effect when using TB as the marker for the liver function exam.

Some pharmacological and therapeutic properties found in plants, have reportedly been related to hepatotoxicity, which is defined by a considerable rise in ALP and AST; this is especially true if delivered in high dosages (McCombs, 2019). Hepatic ALP and AST activities in this experiment

between the control and treated rats were plainly not statistically different ( $p > 0.05$ ), proving that MESHG did not induce hepatic damages nor improve liver function in these treated animals.

Treatment with MESHG showed no statistical difference ( $P > 0.05$ ) in liver AST activity in the treated rats compared to the control rats. . This result shares relevant features with the experiment of Imam and Wudil, (2011). Their work found a slight but insignificant decline in AST activity ( $P > 0.05$ ). Multiple papers drew similar results but the specifics of how MESHG alter AST levels was not clearly expressed in most (MedlinePlus, 2020).

Low AST levels are expected and normal – they are just uncommon in the general population. However, in these cases, low AST may signify vitamin B6 deficiency because the AST enzyme requires vitamin B6 to function (McKenzie *et al.*, 2016). Vitamin B6 deficiency is uncommon but it is known to likely occur in the elderly, alcoholics. According to Schmidt and Schmidt, (2019), animals with underlying health conditions such as liver, kidney, or inflammatory diseases also showed lower levels of AST. AST may also be low in vitamin B6 unrelated chronic liver diseases and in hormone replacement therapeutics (McKenzie *et al.*, 2016).

In this study, treatment of rats with MESHG caused no statistical difference ( $P < 0.05$ ) in liver ALP levels. This result correlates with the findings of Imam and Wudil, 2011. Their experiments also indicated that treatment with MESHG did not significantly affect the activity of liver function enzymes nor alter ALP levels, even when the rats in the experiments were treated with a far higher dose of MESHG. High ALP levels is a sign of a liver problem or a bone disorder and a four-fold of the upper limit of normal or greater elevation occurs in up to 75% of the patients with cholestasis, either intrahepatic or extrahepatic (Lowe *et al.*, 2022). Therefore, the liver ALP levels in this experiment, suggests that MESHG was not toxic to the liver of the rats.

## REFERENCES

Evans, G.O. (2009). "Animal Clinical Chemistry: A Practical Handbook for Toxicologists and Biomedical Researchers". Second Edition. CRC Press. Retrieved on December 15th, 2022.

Imam, A.A. and Wudil, A.M. (2011). "Sub-Acute Hepatotoxicity of Aqueous Leaf Extract of *Eucalyptus camaldulensis* in Rats". *Bayero Journal of Pure and Applied Sciences*. **4**:20.

Lowe, D., Sanvictores, T. and John, S., (2022). "Alkaline Phosphatase". *Statpearls publishing*.

McComb, R.B., Bowers, G.N. and Posen, S. (2019). "Alkaline Phosphatase". Plenum Publishing Corporation; New York, USA.

McKenzie, J., Fisher, B.M., Jaap, A.J., Stanley, A., Paterson, K. and Sattar, N. (2016). "Effects of HRT on liver enzyme levels in women with type 2 diabetes: a randomized placebo-controlled trial". *Clinical Endocrinology (Oxford)*. **65**(1):40-4.

MedlinePlus, (2020). "AST Test". *National Library of Medicine (US)*. Accessed on August 25, 2022.

Osagie-Eweka, S.D.E., Orhue, N.E.J., Omogbai, E.K.I. and Amaechina, F.C. (2021). "Oral acute and sub-chronic toxicity assessment of aqueous leaf extract of *Simarouba glauca* DC (Paradise tree)". *Toxicology Reports*. **8**:239–247.

Saranraj, P. and Sivasakthi, S. (2014). "Medicinal Plants and its Antimicrobial Properties: A Review". *Global Journal of Pharmacology*. **8**(3): 316-327. Retrieved on December 15th, 2022.

Schmidt, E. and Schmidt, F.W. (2019). "Enzyme Diagnosis in Disease of the liver and biliary

system". *Advanced Clinical Enzymology*. **1**:239 – 242.

Skandalakis, Lee J.; Skandalakis, John E.; Skandalakis, Panajiotis N. (2009). "Liver". *Surgical Anatomy and Technique: A Pocket Manual*. pp. 497–531.

Moore, K (2018). *Clinically oriented anatomy* (Eighth ed.). p. 501. .

Moore, K (2018). *Clinically oriented anatomy* (Eighth ed.). p. 494.

Kmieć Z (2001). Cooperation of liver cells in health and disease. *Adv Anat Embryol Cell Biol*. *Advances in Anatomy Embryology and Cell Biology*. Vol. 161;1-151

Pocock, Gillian (2006). *Human Physiology* (Third ed.). Oxford University Press. p. 404.

Kawarada, Y; Das, BC; Taoka, H (2000). "Anatomy of the hepatic hilar area: the plate system". *Journal of Hepato-Biliary-Pancreatic Surgery*. **7** (6): 580–586.

Strunk, H.; Stuckmann, G.; Textor, J.; Willinek, W. (2003). "Limitations and pitfalls of Couinaud's segmentation of the liver in transaxial Imaging". *European Radiology*. **13** (11): 2472–2482.

Uhlén, Mathias; Fagerberg, Linn; Hallström, Björn M.; Lindskog, Cecilia; Oksvold, Per; Mardinoglu, Adil; Sivertsson, Åsa; Kampf, Caroline; Sjöstedt, Evelina (2015-01-23). "Tissue-based map of the human proteome". *Science*. **347** P. 6

Kampf, Caroline; Mardinoglu, Adil; Fagerberg, Linn; Hallström, Björn M.; Edlund, Karolina; Lundberg, Emma; Pontén, Fredrik; Nielsen, Jens; Uhlen, Mathias (2014-07-01). "The human

liver-specific proteome defined by transcriptomics and antibody-based profiling". *The FASEB Journal*. 28 (7): 2901–2914.

Lade AG, Monga SP (2011). "Beta-catenin signaling in hepatic development and progenitors: Gilbert SF (2000). *Development* which way does the WNT blow?". *Dev Dyn*. 240 (3): 486–500.

Berg T, DeLanghe S, Al Alam D, Utley S, Estrada J, Wang KS (2010). " $\beta$ -catenin regulates mesenchymal progenitor cell differentiation during hepatogenesis". *J Surg Res*. 164 (2): P 276–285.

Clemente, Carmin D. (2011). *Anatomy a Regional Atlas of the Human Body*. Philadelphia: Lippincott Williams & Wilkins. p. 243.

Shneider, Benjamin L.; Sherman, Philip M. (2008). *Pediatric Gastrointestinal Disease*. Connecticut: PMPH-USA. p. 751.