EFFECTS OF ETHANOL EXTRACT OF *Tetrapleura tetraptera* LEAF ON THE LIVER OF ADULT WISTAR RATS

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A THESIS SUBMITTED TO THE DEPARTMENT OF HUMAN ANATOMY, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICINE, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

IN PARTIAL FUFILMENT OF THE REQUIREMENT FOR THE AWARD OF B.SC DEGREE IN HUMAN ANATOMY

MAY, 2024.

CERTIFICATION

This is to certify that the project work titled **"Effects of Ethanol Extract Of *Tetrapleura tetraptera* Leaf On The Liver Of Wistar Rats"** was carried out by me **OMORUYI-OSEMWINGIE IJESUROBO PEACE** with matriculation number **BMS1902078** and it meets the regulations governing the award of Bachelor of Science degree in Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria.

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(EXTERNAL EXAMINER) **DATE**

DEDICATION

I dedicate this project to the Almighty God for his guidance, provision, wisdom, understanding, strength and protection.

ACKNOWLEDGEMENT

First and foremost, I am grateful to the Almighty God for his infinite mercies, guidance and protection accorded me throughout my undergraduate studies.

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ABSTRACT

Tetrapleura tetraptera¸ commonly known as Aidan fruit, is a tropical plant indigenous to Western and Central Africa. Botanically, it belongs to the Fabaceae or Leguminosae family and is renowned for its distinctive four-winged fruit pods, which inspired its name *tetraptera*. This plant has been a prominent part of West African traditional medicine and culinary practices for generations. Several reports have documented that the various parts of the plant have various medicinal properties and it also possesses anti-inflammatory, anti-arthritic and antioxidant properties. This study examines The Effect of *Tetrapleura tetraptera* on the Livers of adult Wistar Rats. This work involved the use of an experimental study design, consisting of twenty-four (24) adult Wistar rats weighing 160-212g which were acclimatized for two (2) weeks, separated into four (4) groups; A, B, C and D with each group having six (6) Wistar rats of randomized patterns for administration and were all weighed prior to it. In **Group A** (control group), the rats were administered with 1ml distilled water, **Group B** were administered with 200mg/kg body weight of ethanol extract of *Tetrapleura tetraptera* (low dose), **Group C** were administered with 400mg/kg body weight of ethanol extract of *Tetrapleura tetraptera* (intermediate dose), **Group D** were administered with 800mg/kg body weight of ethanol extract of *Tetrapleura tetraptera* (high dose). After administration (twenty-eight (28) days), the animals were sacrificed, organs harvested and processed for assays according to established methods. Data from the animals were subjected to statistical analysis using GraphPad prism version 8.1 statistical package and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and data were presented as mean ± standard error of mean (SEM). Least significant difference (LSD) post-hoc test was used. Values of $P<0.05$ were considered statistically significant. The statistical values obtained were converted into graphical representation in form of bar charts. Histologically, **Group A**, the control group, showed liver tissue with normal architecture comprising of hepatocytes, sinusoids, bile ducts and the portal veins. **Group B**, showed liver tissue with periportal infiltrates of inflammatory cells, portal vasodilatation and vascular congestion. **Group C**, also showed the liver tissue with periportal infiltrates of inflammatory cells, portal vasodilatation and vascular congestion. **Group D**, also recorded the presence of periportal infiltrates of inflammatory cells, portal vasodilatation and congestion in the liver tissue. In conclusion, across the graded doses, the ethanol extract of *Tetrapleura tetraptera* can cause or induce liver toxicity leading to an inflammatory response which is observed by the presence of periportal inflammatory infiltrates in the liver.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Tetrapleura tetraptera¸ commonly known as Aidan fruit, is a tropical plant indigenous to Western and Central Africa. Botanically, it belongs to the Fabaceae or Leguminosae family, closely related to popular legumes such as *Glycine max* (Soybean), *Pisum sativum* (Beans), *Arachis hypogaea* (Peanut), and is renowned for its distinctive four-winged fruit pods, which inspired its name *tetraptera* [Olaniyi, 2020]. This plant has been a prominent part of West African traditional medicine and culinary practices for generations.

The various parts of *T. tetraptera*, including the fruit, seeds, leaves and bark, have been traditionally utilized for their medicinal properties [Korang *et al*, 2023]. In West African cultures, it has been employed to address a range of health issues such as digestive problems, inflammatory conditions, convulsion, leprosy, rheumatic pains, hypertension and parasitic infections. Beyond its traditional applications, *T. tetraptera* have also attracted attention for its potential health benefits, particularly its anti-inflammatory, anti-arthritic and antioxidant properties [Onda *et al*, 2017]. Antioxidants play a vital role in neutralizing harmful and free radicals in the body, contributing to overall health and well-being.

Based on different phytochemical analysis carried out on the plant, several bioactive compounds have been identified in *T. tetraptera*, enriching its therapeutic profile; these compounds include flavonoids, terpenoids, steroids, phenols, tannins, alkaloids and saponins [Adusei *et al*, 2019]. Flavonoids for example are known for their antioxidant and anti-inflammatory properties, potentially increasing the plant's medicinal value [Okwu, 2004].

The liver is the largest gland in the human body and after the skin; the largest single organ. It is responsible for a range of metabolic functions, these activities encompass the collection

and subsequent detoxification of nutrients/substances absorbed from the gastrointestinal tract [Moore *et al*, 2018]. It also possesses certain immunologic functions like; blood filtration, production of immunoglobins, detection and destruction of pathogens. The majority of the liver's metabolic processes are carried out by hepatocytes, which are six-surfaced (polyhedral) cells. These microscopic functional units cooperate to maintain the organ's overall, appropriate activity. [Rad, 2017].

Due to the liver being the centre of metabolic actions (collection, storage and detoxification) in the body, it is vulnerable to cellular damage and consequent scarring, especially from diseases like hepatitis, liver cirrhosis and Hepatocellular Carcinoma (HCC) which is the sixth most frequent cause of cancer incidences globally and the third most prevalent cause of cancer-related death [Wu *et al*, 2009].

1.2 STATEMENT OF PROBLEM

One of the body's most important organs, the liver is essential to metabolism, cleansing, and nutrient absorption. The ethanol extract from *Tetrapleura tetraptera* leaves, sometimes referred to as Aidan fruit, is said to provide possible health advantages [Odesanmi *et al,* 2011]. *Tetrapleura tetraptera* has been utilized traditionally for numerous therapeutic uses, so therefore, this study intends to fully comprehend the effects of *Tetrapleura tetraptera* leaf ethanol extract on the liver.

1.3 SIGNIFICANCE OF THE STUDY

This study is intended for the purpose of providing valuable insights on the hepatoprotective effects of the ethanol extract of *T. tetraptera* leaves on the liver in response to contributing its

understanding which is essential for the safe/effective use in traditional medicine or lead to the development of therapeutic strategies for liver diseases, or the hepatotoxic effects of the ethanol extract of *T. tetraptera* leaves in the implications and potential risk associated in its use, especially in cases where liver structure or function may be compromised.

1.4 AIM OF STUDY

The aim of this study is to determine the effects of the ethanol extract of *Tetrapleura tetraptera* leaves on the liver of Adult Wistar rats.

1.5 SPECIFIC OBJECTIVES

The objectives of this study are to determine the:

1. Effect of *Tetrapleura tetraptera* on the body weight of adult Wistar rats.
2. Effect of *Tetrapleura tetraptera* on the liver weight of adult Wistar rats.
3. Effect of *Tetrapleura tetraptera* on the hepatosomatic of adult Wistar rats.
4. Histological changes induced by *Tetrapleura tetraptera* on the liver.

CHAPTER TWO

LITERATURE REVIEW

2.1 PLANT OF STUDY: *Tetrapleura tetraptera*

2.1.1 Description

No.	Dialect	Local Name
1.	English	Aidan fruit
2.	Bini	Ighimiaka
3.	Yoruba	Aridan
4.	Igbo	Oshosho
5.	Hausa	Dawo
6.	Twi (Ghana)	Prɛkɛsɛ
7.	Dioula (Ivory Coast)	Osodru
8.	Fon (Benin Republic)	Kan
9.	Kabiye (Togo)	Kpakpati
10.	Wolof (Senegal)	Kanfo/Kani

Table 2.1: The Names given to *Tetrapleura tetraptera* in different languages/dialects.

Tetrapleura tetraptera is a deciduous tree that can grow up to 20-25m tall, with diameters ranging from 1.5-3m. The trunk is slender, and older trees may develop small, low, sharp buttresses. In forested areas, the crown is relatively small, thin and rounded, but becomes flatter with age. The bark is smooth, grey-brown, and thin, with a reddish, strongly scented inner layer. The twigs and young leaves are nearly hairless or have very fine hairs [Orwa *et al, 2009*]. It has pinkish flowers and its dark purplish-brown fruit display a curved structure characterized by four rib-like or wing-like ridges. While two of these ridges are woody, the

remaining two contain an oily, fragrant, sugary pulp [ABS Biotrade, 2024]. *Tetrapleura tetraptera* is frequently found along the edges of the West African rainforest region, abundant across tropical Africa; particularly in forested areas, including secondary forest where they thrive best in the rainforest environment [Orwa *et al*, 2009].

Figure 2.1: *Tetrapleura tetraptera* tree with fruits [Adesina *et al*, 2016]

The genus name comes from a Greek term signifying “four ribs”, alluding to the ribbed fruit. The specific epithet denotes “four winged” [Orwa *et al*, 2009]. Fruit hangs at the extremities of branches on sturdy 25 cm long stalks, and it is highly persistent. It is dark purple-brown, glossy, glabrous, and typically somewhat curved. Its dimensions are 15–25 cm long by 5 cm wide, with four longitudinal, wing-like ridges that are over 3 cm broad. Two of the wings have a woodsy flavor, while the other two are stuffed with an aromatic, soft, sweet pulp. The little, firm, black seeds are stuck in the pod's body, which does not split open, and they rattle within the pods. The seeds are around 8 mm long. There is oil in the kernel [Orwa *et al*,

2009]. The tree sheds its leaves in December. Flowering commences in late February and concludes in early April. The non-splitting pods reach maturity from September to December. Upon falling, the pods emit a scent that attracts small animals, likely aiding in seed dispersal [Orwa *et al*, 2009].

Figure 2.2: *Tetrapleura tetraptera* fruit and seed [IITA Forest Center, 2024]

Figure 2.3: *Tetrapleura tetraptera* leaves and flower [Harris & Wortley, 2008]

Aidan fruit Plant can provide a number of products and services including;

No.	Raw Material	Use
1.	Food	The fruit pulp is rich in sugars and may be used in flavouring food

2.	Timber	Reddish to brown, fairly hard heartwood and white sapwood
3.	Tannin or dyestuff	Tannin is obtainable from the fruit pulp
4.	Medicine	Leaves, bark, roots and the kernels are used for medicinal purposes
5.	Other Products	Fruits and flowers are used as perfumes and in pomades prepared from palm oil.

Table 2.2: Raw materials that can be gotten from *T. tetraptera* and their uses [Orwa *et al*, 2009].

2.1.2 Scientific Classification

Kingdom: Plantae

Division: Angiospermatophyta

Class: Eudicotidae

Order: Fabales

Family: Fabacae

Subfamily: Caesalpinioideae

Genus: *Tetrapleura*

Species: *T.tetraptera*

Preferred Scientific name: *Tetrapleura tetraptera* (Schum. & Thonn. Taub)

2.1.3 Phytochemical properties of *Tetrapleura tetraptera*

T. tetraptera, possesses a good amount of phytochemical properties, as several bioactive compounds have been identified in its different parts. From research and analysis carried out by [Akintola *et al*, 2015], it was seen that it contains high levels of saponins which are known to be able to lyse red blood cells by damaging their membranes, therefore which results in

hepatotoxicity [Odesanmi *et al*, 2009], its low tannin concentrations indicate its high nutritional value, while its notable vitamin content suggests their antioxidant roles, their high ash levels shows the availability of high mineral contents. As reported by [Irondi *et al*, 2016], *T. tetraptera* contain a high concentration of flavonoids (quercetin, rutin, apigenin, catechin, luteolin and epicatechin) as well as phenolic acids. These two types of natural phenolic compounds are thought to be of pharmacological importance because they provide a variety of health benefits, including anti-inflammatory activity. *T. tetraptera* also contain aridanin, which is a tripterpenoid glycoside, which is a lead compound in the development of new drugs [Sikam *et al*, 2023]

PHYTOCHEMICALS	MINIMUM(%)	MAXIMUM(%)	MEAN(%)
Saponin	16.33	17.78	16.78
Alkaloids	1.88	2.22	2.01
Tannins	0.16	0.25	0.21
Phenols	0.04	0.13	0.09
Sterols	0.06	0.12	0.08

Table 2.3: Summary of the basic statistical results of the phytochemical composition of *T. tetraptera* [Akintola *et al*, 2015].

2.1.4 Pharmacological effects of *Tetrapleura tetraptera*

Tetrapleura tetraptera, one of the native fruit species, has been used for food and as traditional medicine in the treatment of human illnesses. Nevertheless, little is known about its active ingredient [Akintola *et al*, 2015]. It is majorly consumed by the African populace as herbal tea, food flavouring because of its aromatic properties, soap making.

The effects of *T. tetraptera* on different organ systems in the body includes;

Nervous System:

Studies and findings from research carried out by Ishola *et al*, 2016, shows that *T. tetraptera* possesses neuroprotective, anticataleptic, memory and cognitive enhancement properties, which is due to its antioxidant systems present within it. Also, its anti-inflammatory properties suggests that it helps in the reduction of inflammation in the nervous system, having the potentials to alleviate symptoms of conditions like multiple sclerosis. Furthermore, it has the potential to protect against stroke by modulating redox and electrolyte imbalances, as well as reducing neurotransmitter dysregulation and other neurochemical dysfunctions [Saliu *et al*, 2021].

Respiratory System:

As a result of being rich in flavonoids and phenolic acids, it possesses anti-inflammatory activity benefits [Irondi *et al*, 2016], which helps in remediating inflammatory conditions in the respiratory system, such as asthma and bronchitis.

Digestive System:

The plant's extract has been found to protect against gastric ulcers and inflammation. Being that it possesses a high concentration of flavonoids, it is able to stimulate the mucus and counteract the deteriorating effects of reactive oxidants species in the gastrointestinal lumen, as well as also having the ability to be anti-ulcerogenic [Oloyede *et al*, 2018].

2.2 ORGAN OF STUDY: Liver

2.2.1 GROSS ANATOMY

The liver is the body's largest gland and, following the skin, the largest single organ. It weighs around 1500 g and accounts for roughly 2.5% of adult body weight. Bile is continuously produced by the liver; however, between meals, it accumulates and is retained

in the gallbladder. When food enters the duodenum, the gallbladder delivers concentrated bile via the biliary ducts to it. [Moore *et al*, 2018].

The liver is located mostly in the right upper quadrant of the abdomen, where it is protected by the thoracic (rib) cage and diaphragm. The normal liver is located deep to ribs 7-11 on the right side and crosses the right and left costal margins (its inferior border) at the tips of the right 9th and left 8th costal cartilages respectively towards the left nipple [Akinola *et al*, 2018]. The liver takes up the majority of the right hypochondrium and upper epigastrium, extending into the left hypochondrium [Garg *et al*, 2020]. The liver comprises four lobes and two supporting ligaments. The liver has a convex diaphragmatic surface (anterior, superior, and some posterior) which is smooth and dome-shaped, resembling the concavity of the pleurae, lungs, pericardium & heart and a relatively flat or concave visceral surface (postero-inferior), separated anteriorly by a strong inferior border that follows the right costal margin, inferior to the diaphragm [Moore *et al*, 2015].

Recesses Of The Liver

1. Subphrenic recesses are located in the superior peritoneal cavity, between the diaphragm and the liver's anterior and superior diaphragmatic surfaces. The falciform ligament connects the liver to the anterior abdominal wall and separates the right and left subphrenic recesses [Moore *et al*, 2015].
2. The hepatorenal recess, also known as the Morison pouch, is a posterosuperior extension of the subhepatic space that connects the right part of the liver's visceral surface to the right kidney and suprarenal gland. The hepatorenal recess is a gravity-dependent region of the peritoneal cavity in the supine posture, where fluid drains from the omental bursa [Moore *et al*, 2015].

Anatomical Lobes Of The Liver

The liver is split into two anatomical and two auxiliary lobes based on peritoneal reflections, fissures, and arteries that serve the liver and gallbladder. The falciform ligament and left sagittal fissure form a midline plane that separates the large right and tiny left lobes. The sloping visceral surface has two auxiliary lobes: the quadrate lobe anteriorly and inferiorly and the caudate lobe posteriorly and superiorly, separated by the transverse porta hepatis and the right and left sagittal fissures [Moore *et al*, 2015].

Functional Subdivisions Of The Liver

The liver has functionally separate right and left portal lobes that are more equal in size than the physical lobes, despite the parenchyma being continuous. Each portion of the liver has its own hepatic artery, portal vein, and duct for drainage [Moore *et al*, 2015]. The caudate lobe functions as a third liver, receiving arteries from both bundles and emptied by one or two tiny hepatic veins that enter the IVC distant to the main hepatic veins.

Blood Supply Of The Liver

The liver, like the lungs, has two blood supplies (afferent vessels): a main venous source and a minor arterial one. The hepatic portal vein delivers 75-80% of blood to the liver.

The liver parenchyma is sustained by portal blood, which contains approximately 40% more oxygen than blood returning to the heart via the systemic circuit. The hepatic portal vein transports almost all nutrients from the digestive system to the liver's sinusoids [Moore *et al*, 2015]. The liver receives around 20-25% of its blood from the hepatic artery, which is originally dispersed to non-parenchymal structures such the intrahepatic bile ducts. The hepatic portal vein is generated from the superior mesenteric and splenic veins posterior to the pancreas neck. It is short and wide. The hepatic artery, a branch of the celiac trunk, is divided into two parts: the common hepatic artery (from the celiac trunk to the gastroduodenal artery origin) and the hepatic artery proper (from the gastroduodenal artery origin to the hepatic artery bifurcation) [Moore *et al*, 2015].

At or near the porta hepatis, the hepatic artery and hepatic portal vein divides into right and left branches, which serve the respective livers [Moore *et al*, 2015].

Lymphatic Drainage And Innervation Of The Liver

The lymphatic vessels in the liver are superficial in the subperitoneal fibrous (Glisson capsule) and deep in the connective tissue, following the branches of the portal triad and hepatic.

Disse's perisinusoidal spaces produce the majority of lymph, which then drains to the deep lymphatics in the intralobular portal triads [Moore *et al*, 2015].

Lymphatics from the liver's diaphragmatic and visceral surfaces, as well as those from the portal triads, connect to the porta hepatis. The superficial lymphatics connect to the hepatic lymph nodes located in the lesser omentum [Moore *et al*, 2015].

Hepatic lymphatic arteries feed into celiac lymph nodes, which then drain into the cisterna chyli (chyle cistern), a dilated sac located at the lower end of the thoracic duct. Lymphatics flow from the liver's posterior diaphragmatic and visceral surfaces to the bare region. Lymphatics drain into phrenic lymph nodes or join deep lymphatics that accompany hepatic veins converging on the IVC. They then flow through the diaphragm to the posterior mediastinal lymph nodes. Efferent lymphatic vessels from these nodes connect to the right lymphatic and thoracic ducts [Moore *et al*, 2015].

The nerve supply to the liver comes from the hepatic plexus, which comprises both sympathetic and parasympathetic or vagal fibres. Nerves also reach the liver via its numerous peritoneal ligaments [Garg *et al*, 2020].

2.2.2 LIVER HISTOLOGY

The liver can be thought of as a modified exocrine gland that also serves other functions. It is primarily composed of liver cells, also known as hepatocytes. Hepatocytes are big cells with a spherical, open nucleus and conspicuous nucleoli. Bile refers to the exocrine secretion of

liver cells. Bile drains from the canaliculi into bigger channels, ultimately leading to the bile duct itself. This duct transports bile to the duodenum, where it aids in the digestion of fat. The liver looks to be composed into hexagonal parts, known as the hepatic lobes [Singh *et al*, 2011].

The connective tissue in the human liver is thin, and the lobules frequently overlap. Transverse sections show that each lobule consists of cords of liver cells divided by sinusoids. The cells are organized in plates (one cell thick) that branch and anastomose, forming a network. Sinusoids occupy spaces inside the network. Each lobule has angular intervals filled with connective tissue. Portal canals comprise a connective tissue network that runs throughout the liver. Each 'canal' includes a branch of the portal vein, a branch of the hepatic artery, and an interlobular bile duct. The three structures together constitute the portal triad [Singh *et al*, 2011].

The area of liver tissue (parts of three hepatic lobules) supplied by one branch of the portal vein to be the genuine functional unit of liver tissue, and it is referred to as a portal lobule. A smaller element, known as the portal acinus, is also present, and it is made up of liver tissue fed by a single hepatic arteriole that runs along the confluence of two hepatic lobules. A connective tissue-based capsule (Glisson's capsule) protects the liver. The capsule surrounds the portal triads and reaches into the liver through the portal canal [Singh *et al*, 2011].

The interlobular ductules are lined by cuboidal epithelium, with smooth muscle in the walls of larger ducts. The cytoplasm of liver cells contains numerous mitochondria, rough and smooth endoplasmic reticulum, a well-developed Golgi complex, lysosomes, and vacuoles containing various enzymes. Many hepatocytes have two nuclei or a single polyploid nucleus [Singh *et al*, 2011].

Despite the liver's many functions, all liver cells have the same appearance. Every cell has the ability to carry out every task. On the other hand, lobule peripheral cells receive more highly

oxygenated blood than lobule center cells. Hepatocytes in various areas differ in terms of function. Liver cells are arranged into one-cell-thick anastomosing plates, which create a network of gaps between which sinusoids are located. Each liver cell has a sinusoid on two sides in this manner. There are many pores (fenestrae) in the endothelium that lines the sinusoids. One cannot see the basement membrane. Hepatic macrophages (Kupffer cells) are scattered among the endothelial cells. A small perisinusoidal gap (of Disse) divides the surface of the liver cell from the sinusoid's endothelial lining. The liver cells' microvilli extend into this area [Singh *et al*, 2011].

2.2.3 LIVER EMBRYOLOGY

By the end of the third week, the liver bud, also known as the hepatic diverticulum, emerges as an endodermal epithelial protrusion from the ventral wall of the distal end of the foregut [Sadler, 2012]. The liver bud enlarges quickly in week four and forms the septum transversum. Parts of the liver are formed by the integration of the septum transversum and the liver bud. The liver bud develops in the ventral mesentery while maintaining the bile duct's future link to the foregut [Webster *et al*, 2012]. The liver will be formed by the pars hepatica, a cranial portion of the liver bud, and the gallbladder by the pars cystica, a caudal bud [Krishnamurthy, 2009].

Different types of cells combine to produce the liver. Hepatocytes and the bile duct's epithelial lining are formed by the liver bud that emerges from the foregut. The hepatic sinusoids are formed by the vitelline and umbilical veins. Septum transversum cells will also generate the hematopoietic cells, Kupffer cells, smooth muscle, and connective tissue of the biliary system in addition to the stroma and capsule (connective tissues) of the liver. The adult structures of the ventral mesentery are the smaller omentum, which lies between the

stomach and the liver, and the falciform ligament, which connects the liver to the front abdominal wall [Webster *et al*, 2012].

By week ten of development, the liver makes up around 10% of the weight of the embryo. This drops to 5% of the total body weight at birth [Webster *et al*, 2012]. Marker enzymes escape from the cells as a result of acute liver injury, which also changes the permeability of the liver's membranes and hepatic transport function.

2.2.4 FUNCTIONS OF THE LIVER

As the largest gland in the body and one of its essential organs, the liver carries out a variety of critical metabolic and homeostatic tasks, some of which are listed below:

1. METABOLIC FUNCTION: The liver is the organ that performs the majority of metabolic reactions, including the metabolism of several hormones, proteins, lipids, carbs, and vitamins [Sembulingam *et al*, 2012].
2. STORAGE FUNCTION: The liver stores a variety of materials, including iron, folic acid, amino acids, glycogen, and vitamins A, B12, and D [Sembulingam *et al*, 2012].
3. SYNTHETIC FUNCTION: Gluconeogenesis is how the liver makes glucose. All of the plasma proteins as well as additional proteins including clotting factors, complement factors, and hormone-binding proteins are synthesized by it, with the exception of immunoglobulins. Heparin, somatomedin, and steroids are also synthesized by it [Sembulingam *et al*, 2012].
4. BILE SECTION: Bile is secreted by the liver and comprises lecithin, cholesterol, fatty acids, and bile salts. Bile salts are primarily responsible for the bile's functions. In the GI tract, bile salts are necessary for the breakdown and absorption of lipids. Bile aids in the removal of waste materials and the breakdown of fats that are expelled in urine or faeces [Sembulingam *et al*, 2012].

5. EXCRETORY FUNCTION: The liver uses bile to excrete many substances, including bacteria, viruses, heavy metals (such as lead, arsenic, and bismuth), poisons, cholesterol, and bile colours [Sembulingam *et al*, 2012].
6. HEAT PRODUCTION: The liver produces a tremendous quantity of heat as a result of metabolic processes. The organ that produces the most heat is the liver [Sembulingam *et al*, 2012].
7. HEMOPOIETIC FUNCTION: The liver creates blood cells during the foetal (hepatic) stage. It stores iron and vitamin B12 required for erythropoiesis, and it produces thrombopoietin, which stimulates the production of thrombocytes [Sembulingam *et al*, 2012].
8. HEMOLYTIC FUNCTION: The liver's reticuloendothelial cells, or Kupffer cells, destroy senile red blood cells (RBCs) after a 120-day lifespan [Sembulingam *et al*, 2012].
9. INACTIVATION OF HORMONES AND DRUGS: The liver catabolizes hormones like growth hormone, parathormone, cortisol, insulin, glucagon, and oestrogen. It also inactivates medications, especially those that are fat-soluble, which are then changed into water-soluble substances and eliminated through urine or bile [Sembulingam *et al*, 2012].
10. DETOXIFICATION AND DEFENSIVE FUNCTIONS: The liver's reticuloendothelial cells, also known as Kupffer cells, are crucial to the body's defence mechanism. The liver has a role in the foreign body detoxification process as well.
    i. The liver's reticuloendothelial cells use phagocytosis to ingest and break down foreign substances like bacteria or antigens.
    ii. The liver's reticuloendothelial cells also secrete chemicals that stimulate the body's immune system, such as interleukins and tumour necrosis factors.
    iii. The elimination of toxic properties from a variety of dangerous chemicals is facilitated by liver cells. Detoxification is the process of eliminating a hazardous agent's poisonous properties. There are two ways that liver detoxification happens:

a. Complete breakdown of the compounds through metabolic processes.
b. Conversion of toxic substances into non-toxic materials by means of conjugation with glucuronic acid or sulphates [Sembulingam *et al*, 2012].

CHAPTER THREE

MATERIALS AND METHOD

3.1 EQUIPMENT

Weighing balance (Wetter PGN), Rotary Microtome (Bright B5143, Huntungton, England), Microscope (Leica DM750 research microscope), British Milling Machine, Camera (LeicaCC50), Whatman filter paper, Water bath (Gallenkamp, England), Paraffin Dispenser (Bright B5143, Huntungton, England), Orogastric tube, Refrigerator, Beaker, Dissecting board, Disposable gloves, Cotton wool, Dissecting scissors, Sample bottles, Plain bottles, Embedding mould, Slides and cover slips, Dye, Plastic cages for animal housing.

3.2 PLANT COLLECTION AND IDENTIFICATION

The leaves of *T. tetraptera* used in this research work were authenticated and identified by the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

3.3 EXTRACT PREPARATION

The collected leaf samples were chopped into particles and air-dried (at room tmperature) for about a week. It was then oven-dried at a temperature of 40•c for about 30 minutes and then pulverized into powder using the British Milling Machine. The weight of the powdered sample was then actualized to 100g.

From the powdered sample, ethanolic extract were prepared by weighing out 20 g of the powder dissolving it in 200 ml of freshly prepared 70% ethanol, and the mixture stirred vigorously. The conical flask holding the mixture was wrapped with a foil and stored away from direct sunlight for 48 hours at room temperature. The mixture was then filtered using Whatman filter paper and the filtrate heated at 60°C to evaporate the water until slurry was

left behind. The slurries were then stored in sterile containers until further use. All process was done as followed from [Ebana *et al*, 2020]

3.4 EXPERIMENTAL ANIMALS

Twenty-four (24) adult Wistar rats of 160-212g weight were procured from the Animal House, Department of Anatomy, University of Benin, Benin City, Edo State, Nigeria, and were utilized for this experimental research. The rats were acclimatized for fourteen (14) days before commencement of the experiment. During this period, the animals were allowed free access to at Chikun Food Grower Mash (Olam Agri Holdings Pte. Ltd., Lagos State, Nigeria) and clean water ad libitum (as often as necessary).

3.5 METHOD OF ADMINISTRATION/CHOICE OF DOSAGE

Administration of extract was done using an orogastric tube to ensure accuracy in treatment. The dosage of 200 mg/Kg, 400 mg/Kg and 800 mg/Kg of ethanol *T. tetraptera* extract was used as earlier reported by Fokou *et al*, (2023) which was done for a period of twenty-eight (28) days.

3.6 EXPERIMENTAL DESIGN

Twenty-four (24) experimental adult Wistar rats of either sexes were randomly assigned into six (4) groups; Groups A_D comprising of six (6) rats per group.

Group A: Rats served as control. They were administered 1ml of distilled water.

Group B: Rats were treated daily with oral administration of 200mg/kg body weight of ethanolic extract of *T. tetraptera* (Low dose).

Group C: Rats were treated daily with oral administration of 400mg/kg body weight with ethanolic extract of *T. tetraptera* (Intermediate dose).

Group D: Rats were treated daily with oral administration of 800mg/kg body weight of ethanolic extract of *T. tetraptera* (High dose).

3.7 METHOD OF SAMPLES COLLECTION

At the end of the treatment, the rats were weighed and then sacrificed under chloroform anaesthesia. The livers of each rat were harvested and immediately fixed on 10% formalin to avoid autolysis.

3.8 HISTOLOGICAL PROCEDURE

Paraffin Tissue Processing

Following the fixation of the harvester tissue in 10% formal saline, the tissue were processed as follows;

- Dehydration of tissues in an increasing gradient of 70% to 90% alcohol and absolute alcohol using ethanol as the choice of alcohol.
- Clearance of alcohol was done using xylene as a clearing agent. The tissues were allowed to pass through two changes for total removal of alcohol.
- The tissues were infiltrated in three changes of molten paraffin wax in an oven at a temperature of 65-70•c. The changes were done for 15 minutes each, and the last changes of paraffin wax for 30 minutes.
- Embedding was carried out using an embedding mound, into which the molten paraffin wax

was poured and the infiltrated tissues were placed in it in a longitudinal orientation to produce longitudinal sections.

- The molten paraffin wax was allowed to cool resulting in solidification to form tissue blocks.
- After trimming, sectioning of the tissue blocks was done using the rotary microtone to cut tissue into thin ribbon like sections of thickness of 5microns.

3.9 HEMATOXYLIN AND EOSIN STAINING METHOD

- Satisfactory and good tissue sections which came out as ribbon were selected and placed in 20% alcohol for spreading of the paraffin sections which are then cut and floated in a water bath at a temperature of 30°C.
- The sectioned tissues were picked with slides and allowed to dry.
- The tissue sections were placed in xylene for 15 minutes to remove excess paraffin wax from the tissues and were then subjected to hydration by passing them through defending grades of alcohol(100%, 90% and 70%) and then into water, all of which lasted for 5 minutes each.
- Staining of the tissue was done using H&E dyes. The tissues were stained in hematoxylin for 10 minutes.
- Tissues were washed in running tap water (blueing)
- Sections were counter stained with 1% Eosin for 5-10 minutes.
- Tissues were rinsed in water.
- Tissues were dehydrated rapidly through 70% graded alcohol to absolute alcohol for 5 minutes.
- Tissues were then finally cleared using xylene for 5 minutes and the slides were mounted with glass cover slip using a suitable mountant, distrene plasticizer and Xylene (DPX).

3.10 PHOTOMICROGRAPHY

The sections of the heart were obtained and examined under Leica DM750 research microscope with a digital camera (LeicaCC50) attached. Digital photomicrographs of the tissue sections were taken at x100 and x400 objective magnifications.

3.11 STATISTICAL ANALYSIS

Data were subjected to statistical analysis using GraphPad prism version 8.1 statistical package and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and data were presented as mean ± standard error of mean (SEM). Least significant difference (LSD) post hoc test was used. Values of $P<0.05$ were considered statistically significant. The statistical values obtained were converted into graphical representation in form of bar chats.

CHAPTER FOUR

RESULTS

4.1 EFFECT OF TREATMENT ON WEIGHT

Table 4.1 shows the mean values of body weight, liver weight and hepatosomatic index across experimental groups.

Figure 4.1 shows the body weight change across the experimental groups. There was no significant difference (p>0.05) in the body weight change of rats across the treated group when compared to the control.

Figure 4.2 shows the liver weight across the experimental groups. There was no significant difference (p>0.05) in the liver weight of rats across the treated groups when compared to the control.

Figure 4.3 shows the liver/body weight ratio (hepatosomatic index) across the experimental groups. There was a significant increase in 200mg/kg compared with control. However, there were no significant changes in the 400mg/kg and 800mg/kg doses compared with control respectively.

Table 4.1: Comparing the mean values of body weight, liver weight and Hepatosomatic index following an administration of *Tetrapleura tetraptera* of different doses in Wistar rats.

Parameters	Control	200mg/kg *Tetrapleura tetraptera*	400mg/kg *Tetrapleura tetraptera*	800mg/kg *Tetrapleura tetraptera*
Body weight	212.1 ± 9.465	205.4 ± 5.997	183.8 ± 8.417	210.7 ± 11.16
Liver weight (g)	7.933 ± 0.482	8.960 ± 0.3076	7.540 ± 0.4377	8.550 ± 0.3106
Hepatosomatic index	0.03727 ± 0.00084	0.04379 ± 0.00199×	0.0413 ± 0.00285	0.04087 ± 0.00141

×P < 0.05 indicates significant difference compared with control.

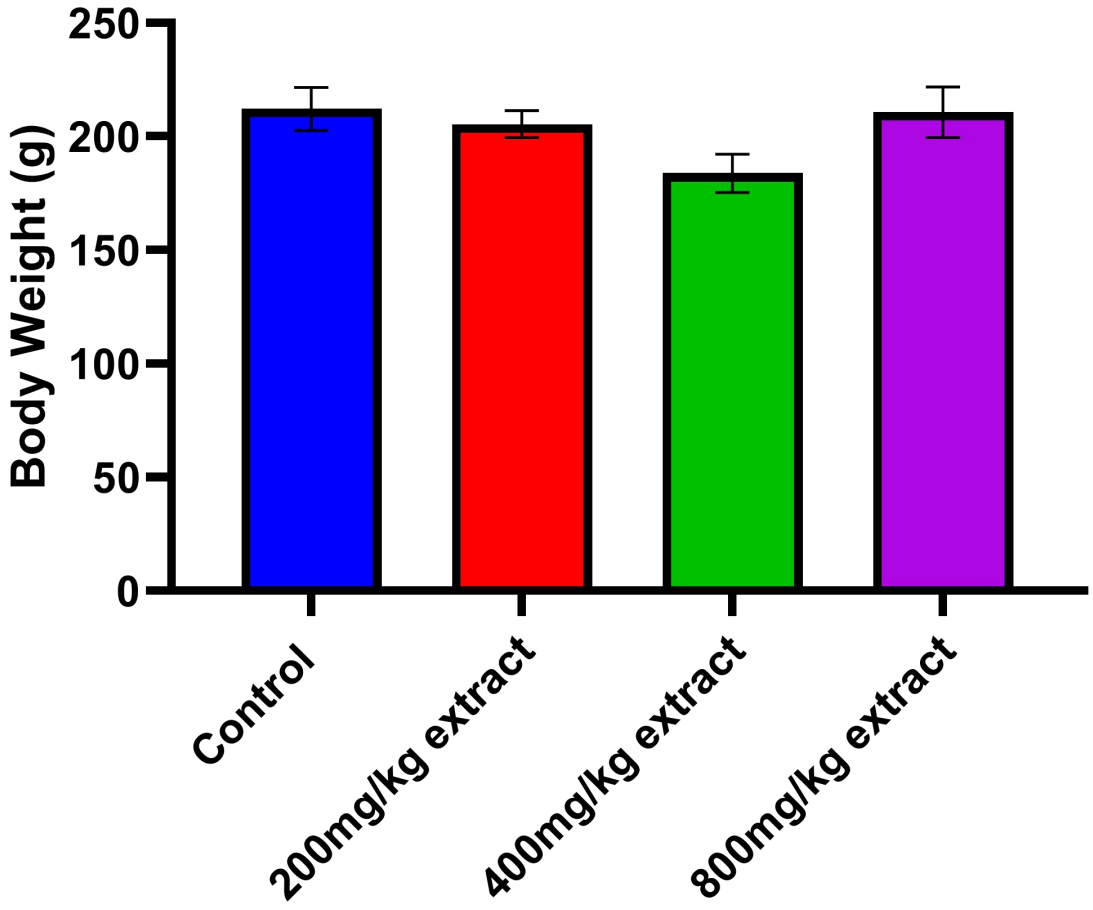

Figure 4.1: The effect of the administration of *Tetrapleura tetraptera* of different doses on total body weight in Wistar rats.

There were no significant changes in the different doses compared with control respectively.

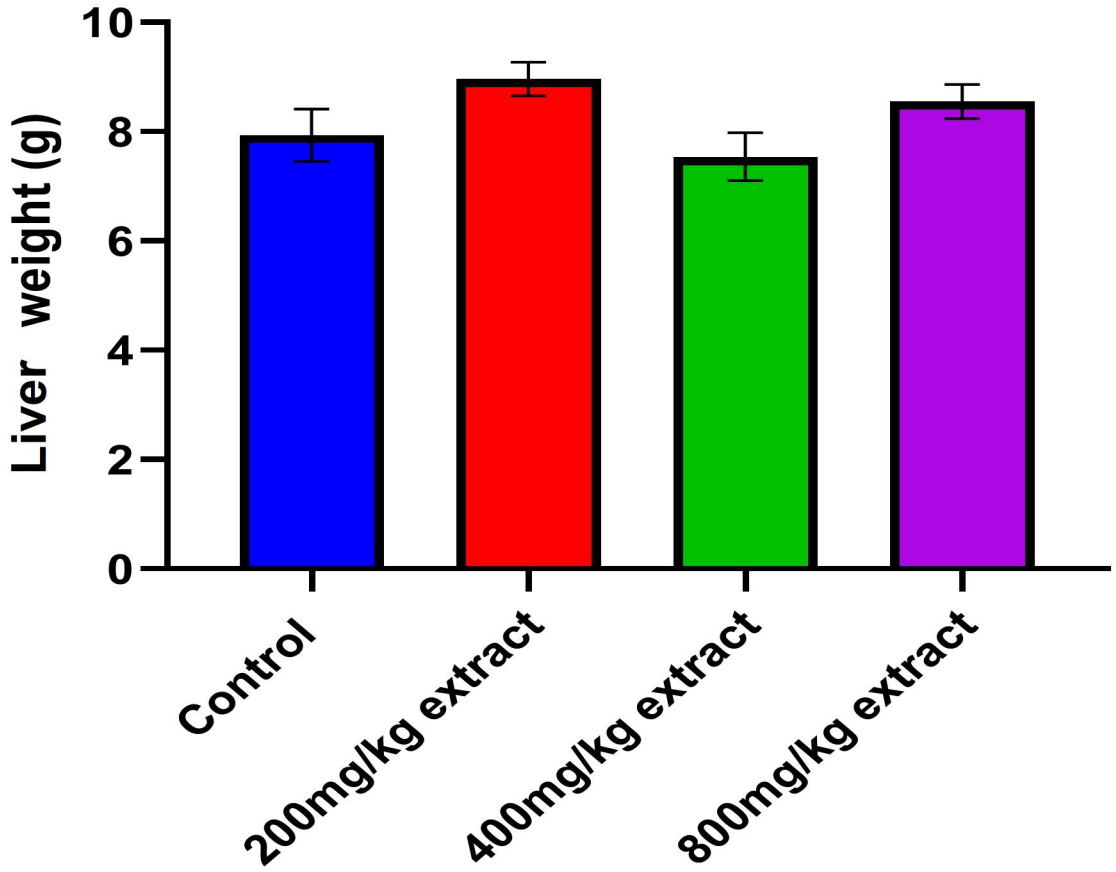

Figure 4.2: The effect of the administration of *Tetrapleura tetraptera* of different doses on liver weight in Wistar rats.

There were no significant changes in the different doses compared with control respectively.

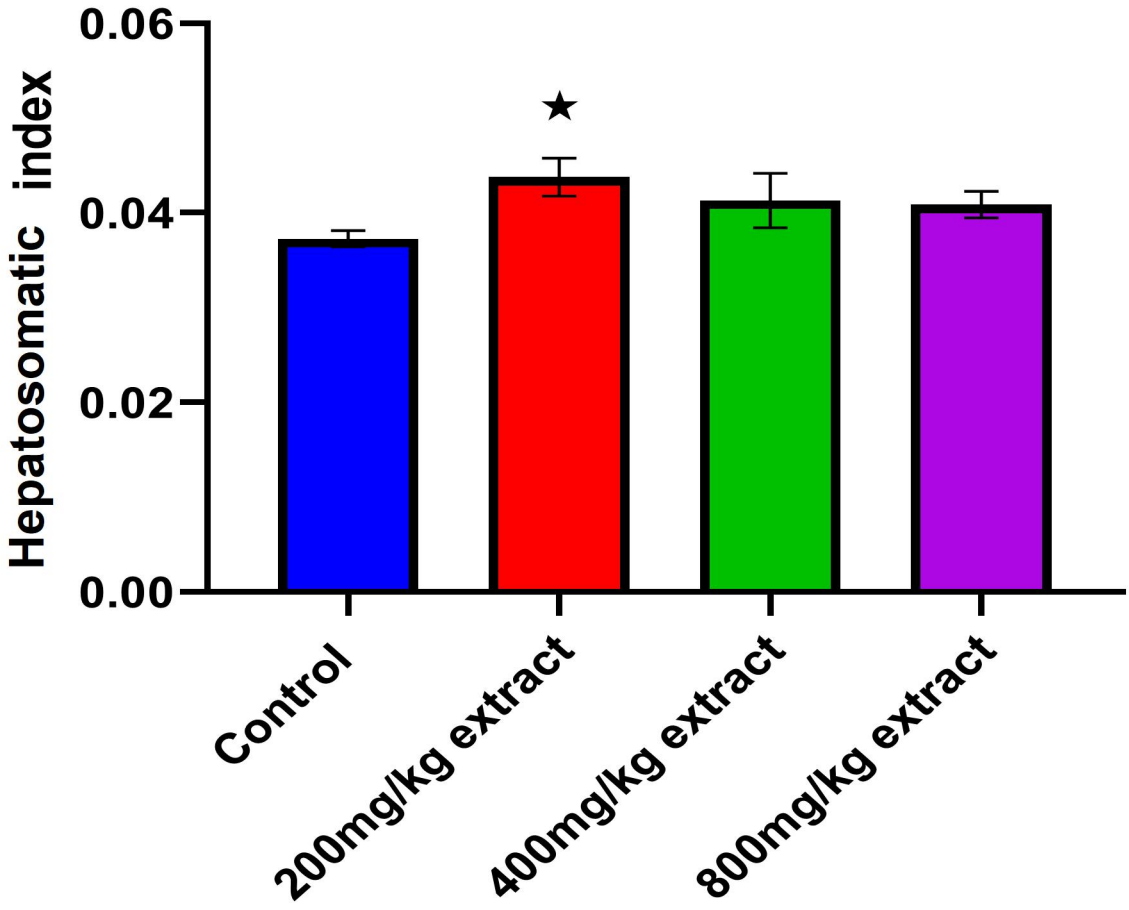

Figure 4.3: The effect of the administration of *Tetrapleura tetraptera* of different doses on hepatosomatic index in Wistar rats.

There was a significant increase in 200mg/kg compared with control. However, there were no significant changes in the 400mg/kg and 800mg/kg doses compared with control respectively.

4.2 EFFECT OF TREATMENT ON HISTOLOGY OF THE HEART

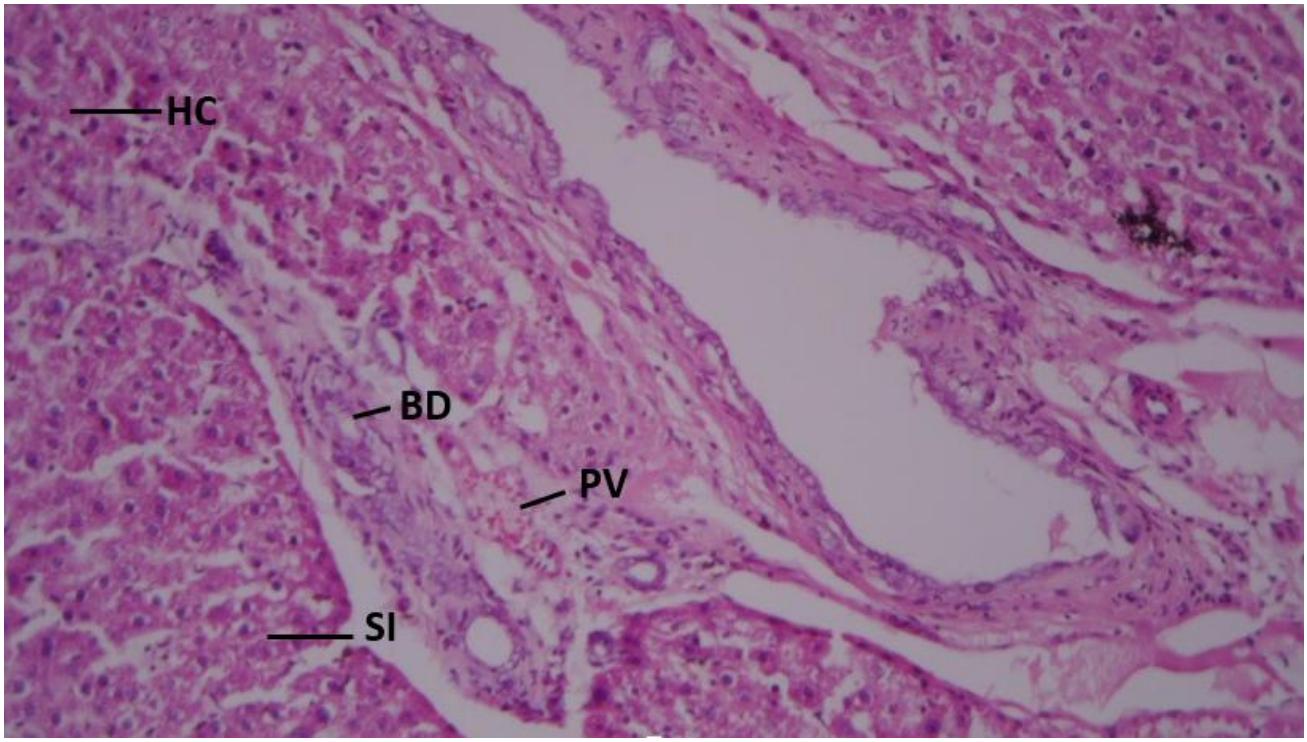

Plate 4.1: Rat liver control, composed of normal architecture: hepatocytes (HC), sinusoids (SI), bile ducts (BD), portal vein (PV): H&E x 100

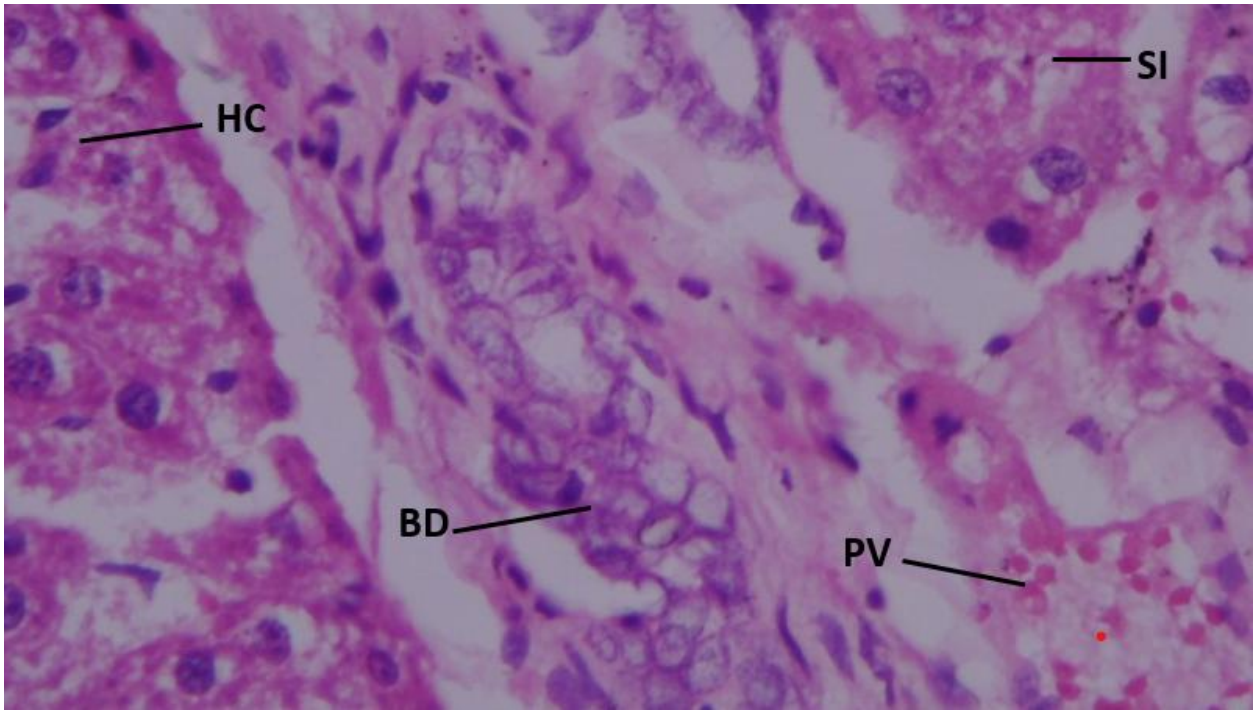

Plate 4.2: Rat liver control, composed of normal architecture: hepatocytes (HC), sinusoids (SI), bile ducts (BD), portal vein (PV) : H&E x 400

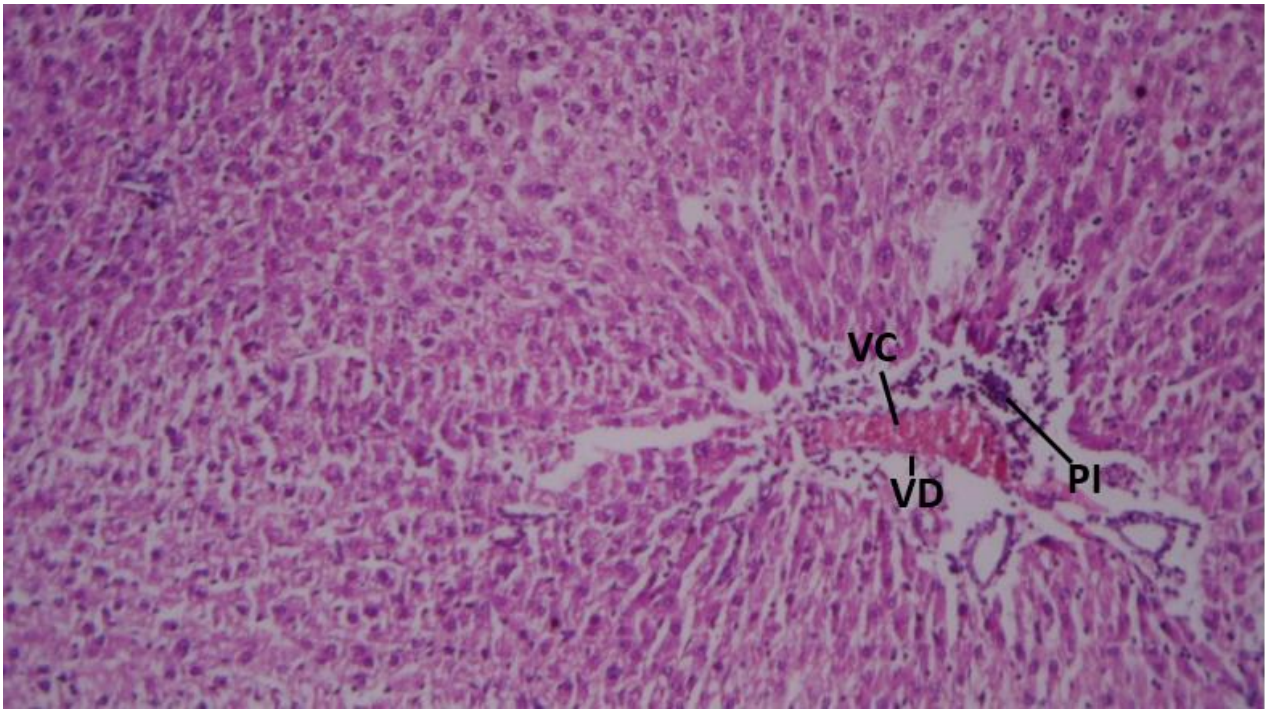

Plate 4.3: Rat liver given 200mg Tetraptera showing: periportal infiltrates of inflammatory cells (PI), portal vasodilatation (VD) and congestion (VC): H&E x 100

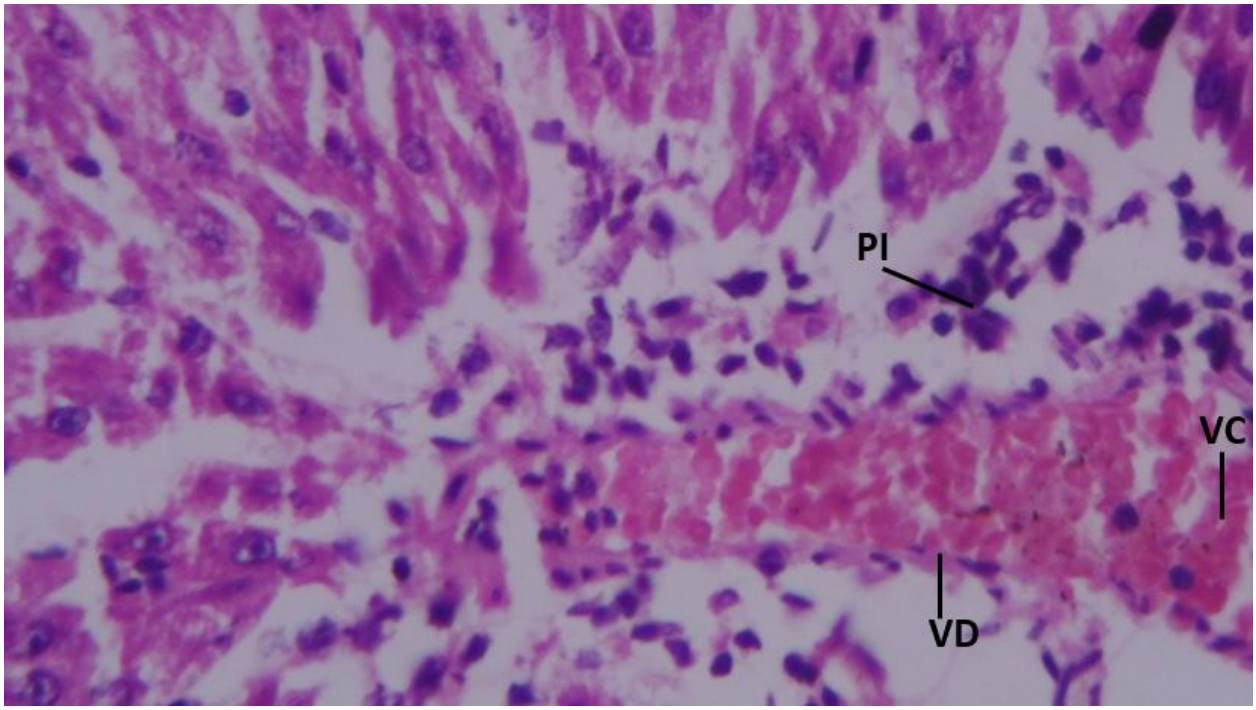

Plate 4.4: Rat liver given 200mg Tetraptera showing: periportal infiltrates of inflammatory cells (PI), portal vasodilatation (VD) and congestion (VC): H&E x 400

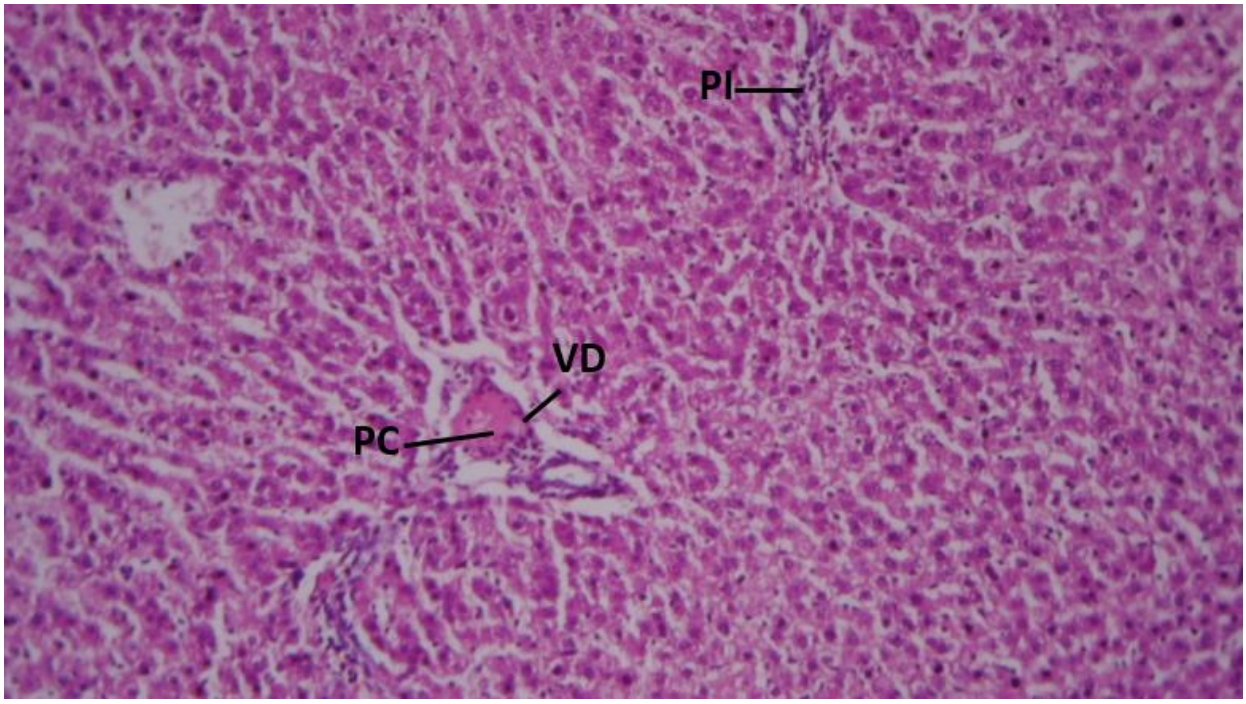

Plate 4.5: Rat liver given 400mg Tetraptera showing: periportal inflammatory infiltrates (PI), portal congestion (PC) and dilatation (VD): H&E x 100

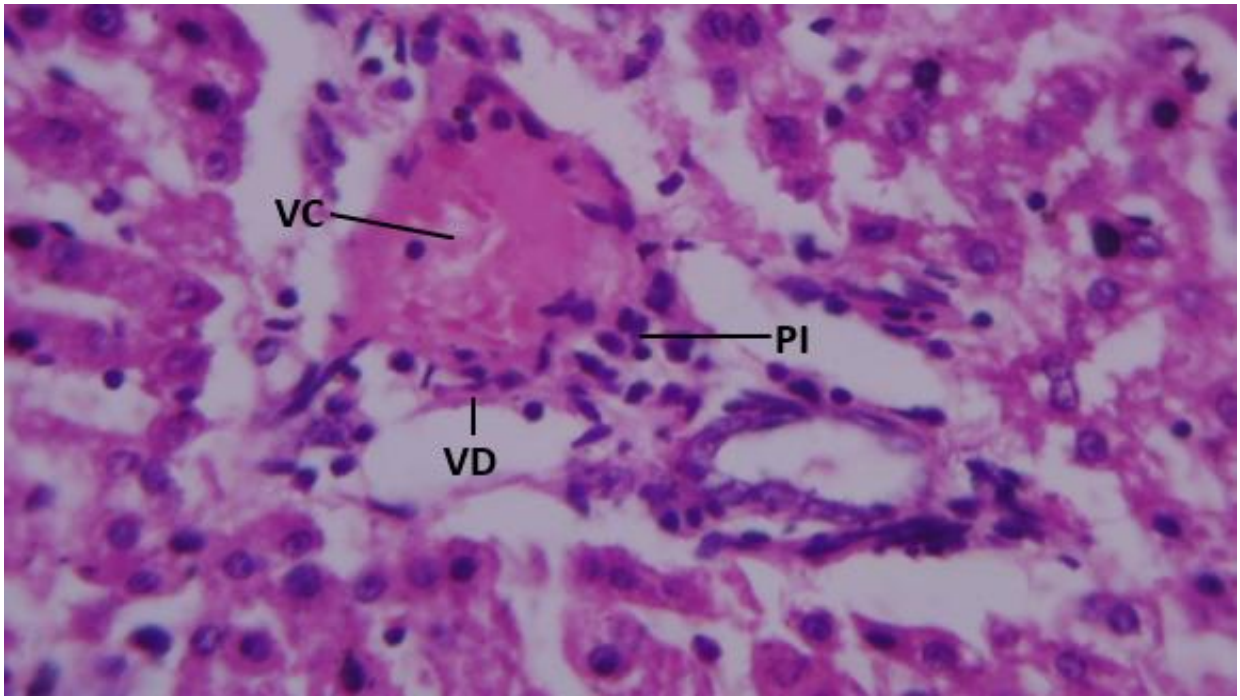

Plate 4.6: Rat liver given 400mg Tetraptera showing: periportal inflammatory infiltrates (PI), portal congestion (PC) and dilatation (VD) : H&E x 400

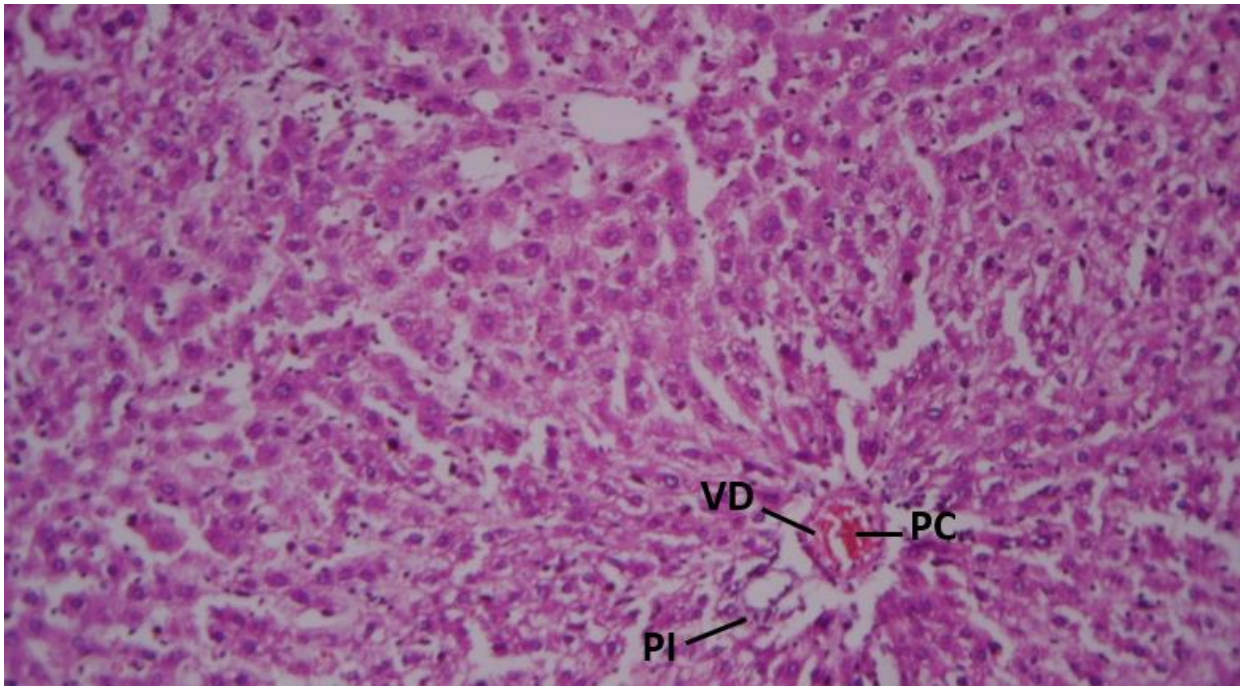

Plate 4.7: Rat liver given 800mg Tetraptera showing: periportal inflammatory infiltrates (PI), portal congestion (PC) and dilatation (VD): H&E x 100

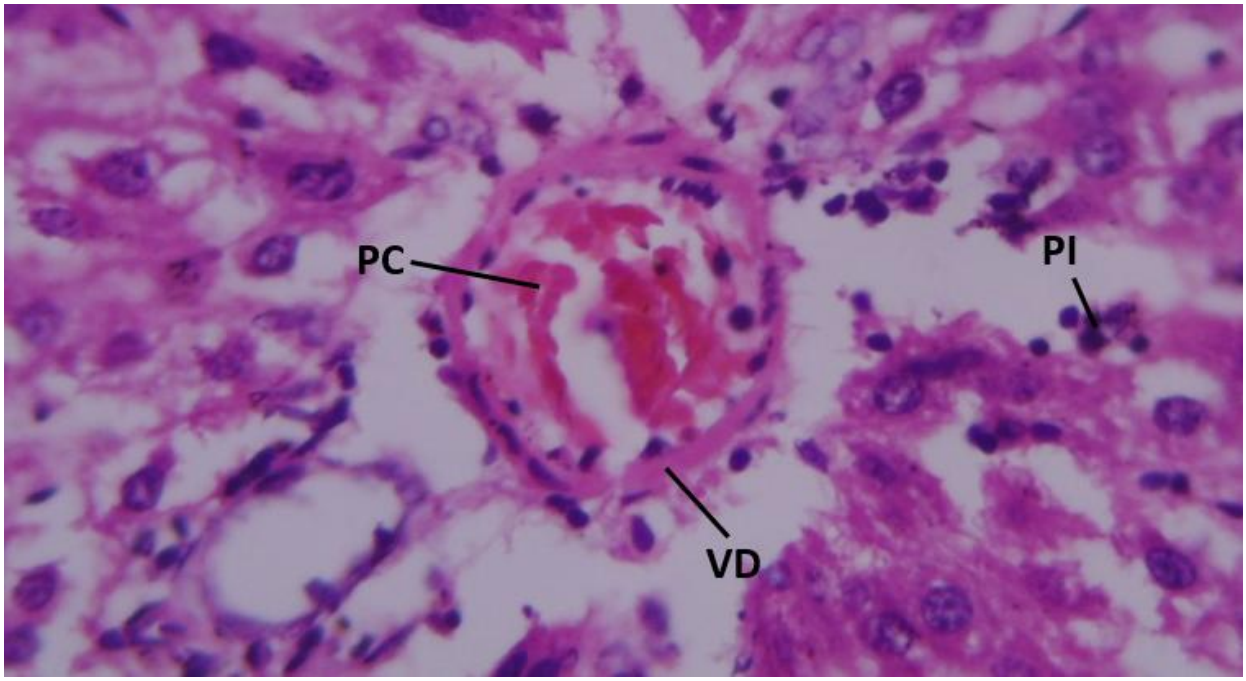

Plate 4.8: Rat liver given 800mg Tetraptera showing: periportal inflammatory infiltrates (PI), portal congestion (PC) and dilatation (VD): H&E x 400

CHAPTER FIVE

DISCUSSION

5.1 DISCUSSION

The various parts of *T. tetraptera*, including the fruit, seeds, leaves and bark, have been traditionally utilized for their medicinal properties [Korang *et al*, 2023], and this study has been directed at ascertaining the effects of the leaves of *T. tetraptera* on the liver, as it is an important organ of metabolization in the body. Statistical analysis from this study returned at the administration of the ethanol *T. tetraptera* leaves extract, that there was no significant changes in the body weight and liver weights across the doses administered compared to the control, which infers that extract has no effect on overall body and liver weight. These results, are in tandem with the study carried out by Bonsou *et al*, (2022) which also showed that at a dose of 1000mg/kg body weight, there was a small gain in total body weight, suggesting that *T. tetraptera*'s effect on the body weight can be influenced at higher doses. The hepatosomatic index, showed a significant increase in the case of the low dose (200mg/kg), however, the intermediate and high doses, showed no significant changes, inferring that the low dose might have stimulated or promoted an adaptive effect in the liver, suggesting a hepatoprotective potential in this dose.

Histological study demonstrated that even at the lowest dose of ethanol extract from *Tetrapleura tetraptera* leaves given to rats, significant changes were found in the liver tissue. These changes included the buildup of inflammatory cells in the periportal areas, dilatation of the portal blood arteries, and congestion in the liver's vascular network. Similarly, when the intermediate dose (400mg/kg) was given, the liver tissue exhibited similar histological alterations, including periportal infiltration of inflammatory cells, portal vasodilation, and vascular congestion. Furthermore, the extract's high dosage (800mg/kg) produced similar

effects, with periportal inflammatory cell infiltrates, portal vasodilation, and congestion within the liver tissue becoming noticeable.

These findings collectively indicate that the administration of *Tetrapleura tetraptera* leaf extract induced consistent histopathological alterations in the liver tissue across different dosage levels. The observed periportal infiltrates of inflammatory cells suggest an immune response to certain phytochemicals in *T. tetraptera*; For example, saponins present in large concentrations (such as in *T. tetraptera*) are known to be able to lyse red blood cells by damaging their membranes, therefore resulting in hepatotoxicity [Odesanmi *et al*, 2009]. Concurrently, the portal vasodilatation and vascular congestion suggest disturbances in hepatic blood flow dynamics, potentially compromising liver function. These results also tally with the study done by Odesanmi *et al*, (2009) which concluded that the ethanol extract of *T. tetraptera* can show selective toxicity and that doses higher than 50mg/kg should be avoided.

5.2 CONCLUSION

The Ethanol extract of *Tetrapleura tetraptera* leaf has shown to be able to induce liver damage (hepatotoxicity). Further studies are recommended to be carried out in order to ascertain the underlying mechanisms of the responses shown in the tissues.

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