ANALYSIS OF HEAVY METALS IN PLEUROTUS TUBERREGIUM SCLEROTIA,TOXICITY IN BLOOD, BONEMARROW AND SOME SELECTED ORGANS OF ALBINO RATS

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A DISSERTATION SUMBITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCES, FACULTY OF HEALTH SCIENCES AND TECHNOLOGY, COLLEGE OF MEDICINE, UNIVERSITY OF NIGERIA FOR THE AWARD OF A MASTER OF SCIENCE (M.Sc) DEGREE IN HAEMATOLOGY AND BLOOD TRANSFUSION SCIENCE

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DEDICATION

This work is dedicated to the loving memories of late Pa Ogbuabor Alphonsus.

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Ogbuabor Alphonsus Ogbonna

ABSTRACT

Pleurotus tuberregium is a common mushroom which is used as food or medicine, more commonly as a vegetable soup thickener. This study investigated the presence of heavy metals in wild samples of *pleurotus tuberregium* sclerotia consumed within our localities, compared the degree of heavy metal contamination of the samples, investigated the presence of heavy metals in the serum of albino rats due to its consumption and the effects of its consumption on blood, bone marrow, liver and kidney of the rats. Two wild samples of *pleurotus tuberregium* sclerotia were collected from Bomu village in Gokana Local Government Area of Rivers State a part of Nigeria rich in oil and mining activities and Agbudu in Orumba South Local Government Area of Anambra State a part of Nigeria relatively low in oil and oil mining activities. The samples were authenticated at the Department of Plants and biotechnological Sciences, University of Nigeria, Nsukka. A phytochemical screening of the samples was carried out as described by Trease and Evans. Samples were analyzed for the presence of heavy metals using an Atomic Absorption Spectrophotometer (AAS 240FS Varian) at the Springboard Research Laboratories, Awka, Anambra State. A crude aqueous extract of the samples was prepared. A total of 37 male albino rats 6-7 weeks old weighing 200g $\pm$ 20g were used for a protocol involving the oral acute toxicity and sub-chronic toxicity of the crude aqueous extract of the samples. The oral acute toxicity of the extract was determined using 12 of the rats according to the procedure for the two phases testing as described by Lorke. A repeated 14-days dosing oral toxicity was then applied for the sub-chronic study. The remaining 25 out of the 37 rats were divided into five groups (A – E) of five rats each. After a previous 14days period of acclimatization, Group A were fed with water and feed only and served as the control, Group B received 1500mg/kg of the sample extract from Rivers State, Group C received 1000mg/kg of the same sample extract while Groups D and E received 1500mg/kg and 1000mg/kg respectively of sample extract from Anambra State. Whole blood samples were analyzed for haematological profile using the fully automated analyser Mind Ray, Japan while serum was analyzed for biochemical parameters involving the liver and kidney indices using Boehringer knoll chemistry semi-automated analyzer 4010 system. The liver and kidney tissues were examined for histological changes and the bone marrow aspirates examined for effects of metal toxicity as well. Data was analyzed using SPSS version 17.0 and results expressed as mean $\pm$ standard error of the mean. Results were considered significant at $P<0.05$. Phytochemical screening of the samples revealed low presence of oils, tannins, moderate presence of glycosides, flavonoids, an abundance of proteins, alkaloids and saponnins. The crude aqueous extract was found to be non-toxic up to 5000mg/kg. Lead, mercury, aluminum, platinium, cadmium, arsenic and nickel were detected in both samples but there were no statistically significant differences ($P > 0.05$) between concentrations of metals. These metals were also detected in the serum of some of the test rats. There were no statistically significant difference ($P > 0.05$) in the serum concentrations of metals in test rats treated with the same doses of the different sample extracts except for the test Group B in which cadmium recorded high concentrations of (0.057 ± 0.006) as compared to Group D (0.010 ± 0.010) and test Group E in which Nickel recorded (0.055 ± 0.021) as compared to Group C (0.000 ± 0.000). There were no statistically significance differences ($p>0.05$) in the haematological parameters in all the groups and as compared to the controls . There were no statistically significant differences ($p>0.05$) in the biochemical parameters of the liver and kidneys in all the groups and as compared to the controls. The bone marrow aspirate revealed normal micrograph in all the groups while the liver and kidneys maintained their normal histoarchitectures. These result patterns are in accordance with the safety claims on the use of *p. tuberregium* sclerotia as food or medicine.

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CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND OF THE STUDY

Our health and well-being depend more than on any other factor on the food we eat every day. Whereas some foods are capable of preventing or even curing our disease conditions, others can be the source of our diseases. Their regular consumption can do as much for our health as most medical treatments or even poisons on the other hand.

In Nigeria particularly, a variety of unprocessed foods are eaten by different communities regardless of the nutritive value and potential toxic hazards posed to health by some of the constituents (Enechi and Odonwodo, 2003).

Although the adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues and is even increasing in some areas (British Medical Bulletin, 2003). The threat of heavy metal pollution in the environment is more serious than those of other pollutants due to their non bio-degradable nature, accumulative properties and long biological half lives (Aderinola et al, 2009). Exposure to heavy metals may occur through water, air and the foods we eat (Woodbury, 1993)

Edible wild and cultivated mushrooms have been shown to accumulate great concentrations of heavy metals (Ogbo and Okhuoya, 2011). Mushrooms have fruiting bodies that grow out of a mass of mycellium; a web of thread-like hyphae that grow underground or colonise a substrate such as dead wood. The mycelium excretes enzymes that breakdown complex substrates into simpler molecules and by so doing, they can also take up heavy metals into their fruiting bodies. The solubilization of soil components for the successful uptake of metals is therefore made possible by the ability of mushrooms enzymatic products to reduce protons, phosphates, organic acids and other metabolites to simpler and free states. This is an effective mechanism that enables them to readily take up some heavy metals from the ecosystem (Oghenekaro et al, 2008). Heavy metal concentrations in mushrooms are therefore considerably higher than those in agricultural crop plants, vegetables and fruits.

While the presence of heavy metals in crude oils and other bituminous substances have been recognized worldwide (Nduka et al, 2006), their presence in some environments has been attributed to petroleum prospecting, mining and industrialization (Osuji and Onojake, 2004).

This suggests that heavy metal contamination of soil is widespread due to metal processing industries, tannery, combustion of wood, coal and mineral oil spillages. It is estimated that

more than four thousand incidents of crude oil spills have occurred in the Niger Delta region since 1960, releasing several million barrels of crude oil into the surrounding areas (Ogbo and Okhuoya, 2011). Mushrooms have also been used to evaluate the level of environmental pollution and to remediate metal polluted soil due to their metal uptake property (Oyetayo et al, 2010). And according to Ogbo and Okhuoya (2011), the use of fungi for the bioremediation of crude oil polluted sites can release metals into the environment increasing the bioavailability of such metals. The implication of this being that metals that are naturally less or unavailable could be made available thus increasing the risk of our exposure to their adverse effects.

The toxic effects of heavy metals results mainly from their interaction with cellular enzymes and inhibition of metabolic processes. Also, in contrast to organic pollutants, metals are not mineralized in the body by our usual microbial flora. They are therefore subject to bioaccumulation and posses risks to human health when transferred to the food chain (Jarup, 2003). A typical food chain in the context of this study involves increased mineralogy of our soils due to oil spillages, tannery, industrialization, effective mechanism of metal absorption by *pleurotus tuberregium sclerotia* and its life-long consumption as food or medicine. With growing public concern over the health hazards of heavy metal contamination of foods, many studies have detected the presence of heavy metals on samples of p. tuberregium sclerotia but to the best of our knowledge none has detected their presence and/or their effect on blood,bone marrow,kidney and liver due to its consumption.

1.1 AIMS AND OBJECTIVES

1. To investigate the presence of heavy metals in two different wild samples of *pleurotus tuberregium* sclerotia consumed within our localities.
2. To compare the degree of contamination of heavy metals in the samples.
3. To investigate the presence of heavy metals in blood due to their consumption.
4. To investigate the effect of their consumption on blood parameters, bone marrow, kidney and liver.

CHAPTER TWO

REVIEW OF LITERATURE

2.0 PLANT MATERIAL

Pleurotus tuberregium is a common mushroom which has been of great interest recently in most research studies (Agomuo, 2011). There are many varieties of mushroom species of which *pleurotus* are characterized by a white spore print, attached to the gills, often with an eccentric stipe or no stipe at all and are generally known as Oyester mushrooms. Being a sclerotial basidiomyte, the mushroom produces sclerotum or underground tuber, as well as a fruiting body. The sclerotum is spherical to ovoid and can be quite large, up to 30cm (11.8 inches) or larger in diameter (Oso, 1977). The mushroom when mature has the cap curved upwards to form a cup-like shape. The fungus infects dry or dead wood, where it produces the sclerotum which usually become buried in the soil but also found between the wood and its bark. The fungus is common in Nigeria where farmers usually lift the sclerotum out from the soil or wood while cultivating their farm. *Pleurotus tuberregium* is therefore unusual among traditionally accepted *pleurotus* species in that its fruiting bodies arise not just from the wood but from the underground true sclerotia (Hibbett and Thorn, 1994)

The sclerotum outer covering is brownish-black in color and the inner part is white. The sclerotum forms naturally in response to unconducive environmental conditions as an adaptive measure for survival. (Olukoya and Okogbo, 1990). They are commonly known as king tuber mushroom but called “USU or ero USU” in Igbo Language (Catherine and Jude, 2008).

CLASSIFICATION

Kingdom: Fungi

Division: Basidiomycota

Sub-class: Agaricomycetidae

Order: Agaricales

Family: Pleurotaceae

Genus: Pleurotus

Specie : Tuberregium

HABBITAT

Pleurotus tuberregium occurs in both tropical and subtropical regions of the world and are found distributed widely around most of equatorial Africa, India, Sri Lanka, South East Asia, North Australia as well as the Southern pacific areas (Isikhuamhen et al, 1999).

PHYTOCHEMISTRY

In a study carried out by Catherine and Jude (2008), the proximate composition of *pleurotus tuberregium* was found to be protein 64.31%, carbohydrate 20.00%, ash 2.2% and crude fiber 2.89% while the phytochemical screening revealed moderate phytate content and low alkaloids, flavonoids and tannins.

USES

Pleurotus species has been recognized as mushrooms with dual functions to humans; both as food and medicine (Chang and Buswell, 2003). In Nigeria, the sclerotum is peeled and ground for use as a thickener in a vegetable soup. In traditional medicinal practice, it is used in the preparation of cures for headache, stomach ailments, colds and fever, asthma, smallpox, heart problems and high blood pressure (Alobo, 2003, Idu et al 2007, Osenwegie et al, 2010). The effects of extracts from *pleurotus* species against some pathogenic organisms (Viral replication, bacterial growth), tumourogenesis and inflammation have also been widely reported by researchers (Adebayo et al, 2012, Jose et al, 2002, Hajhashmi et al, 2004). Their polysacharide extracts are biologically active and have been found to exhibit haematological and imunuomodulatory activities (Yap and Ng, 2001).

They have also been proved to be nutritive with good quality proteins, vitamins and minerals and have been recommended for obese persons and diabetic patients because of low calorie value, very low sugar content and no starch (Khanna and Garcha, 1984, Flagg and Maw, 1976). The mushroom has also biotechnological and environmental applications such as bioremediation of metal polluted soils due to oil spillages.

2.1 HEAVY METALS

Heavy metals are natural constituents of the earth's crust. They are members of an ill-defined subset of elements that exhibit metallic properties. These include the transition metals, some metalloids, lanthanides and actinides (Reena et al, 2011). They are 35 in number, 23 of which occur at low concentration and are referred to as the trace metallic elements: antimony, arsenic, bismuth, cadmium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium and zinc. While some including lead, mercury, arsenic, cadmium, nickel,

aluminum and platinum has no important function in the human body and are referred to a non-essential elements, some others can be naturally occurring in the body and are essential to human health and thus referred to as essential elements. However, large amounts of any metal whether essential to human health or not can be a cause of acute or chronic pathological condition. However, there exist currently some debates as to what exactly constitute a heavy metal and which elements should be properly classified as such. Some authors have based their definition on atomic weight, and referred to it as those metals with a specific gravity that is at least 4.0 or 5.0 times greater than that of water; a property making them stable elements and not being able to be metabolized by the body. The specific gravity of water is 1 at 4^oC (39^oF). Specific gravity is a measure of density of a given amount of a solid substance when it is compared to an equal amount of water. Some well-known toxic metallic elements with a specific gravity that is 4 or 5 times or more than that of water are arsenic 5.7, cadmium 8.65, Iron 7.9, lead 11.34 and mercury 13.546. Therefore, heavy metals are trace elements with a density at least 4 or 5 times that of water and as Jarup (2003) has it, heavy metals refers to any metallic chemical element that has a relatively high density and is also toxic or poisonous at low concentrations. Simply put, Heavy metals are those metals having a specific density of more than $5g/cm^3$ (British Medical Bulletin, 2003). But according to Reena et al (2011) any toxic metal may be called a heavy metal, irrespective of its atomic mass or density.

2.1.2 HEAVY METALS AND ENVIRONMENTAL POLLUTION

Heavy metals are natural constituents of the earth's crust but indiscriminate human activities alters their geochemical nature and biochemical balance making them a major environmental Reena et al (2011) pollutant. Metal pollution of the environment is made possible due to the use of sludge or municipal compost, pesticides, fertilizers, and emissions from municipal wastes incinerates, exudates, residues from metalliferous mines and smelting industries. (Halin et al, 2002). Ogbo and Okhuoya have estimated that more than four thousand incidents of crude oil spills have occurred in the Niger Delta region of Nigeria since 1960 releasing several million barrels of crude oil into the surrounding areas. The implicaitn of this is that metals are naturally unavailable biologically could be made biologically available thus increasing the risk of environmental pollution. Metal pollution of the environment can result in soil quality degradation, crop yield reduction and poor quality of agricultural products thereby posing significant hazards to humans, animals and ecosystems health (Long et al, 2002).

2.1.3 HEAVY METALS CONTAMINATION OF PLANTS

There are a small number of plants that easily absorb high levels of metals from the surrounding soil. These are known as hyper accumulators. Plants take up heavy metals by absorbing them from deposits on the part of the plants exposed to the air from polluted environment as well as from contaminated soil. (Osuocha et al, 2013). Heavy metal contamination of plants cannot be underestimated as plants and their products are important components of human diet. They provide rich sources of vitamins, minerals and fibers and also have beneficial antioxidative effects. Heavy metal contamination of food is one of the most important aspects of food quality assurance or safety (Reena et al, 2011). Monitoring and assessment of heavy metals concentrations in the plant foodstuffs such as vegetables form the market sites have been carried out in some developed and developing countries (Reena et al, 2011).

2.1.4 HEAVY METALS AND HUMAN REQUIREMENTS

Humans require varying amounts of some heavy metals (Lane and Morel, 2009). Iron, cobalt, copper, manganese, molybdenum and zinc are required by humans and are therefore referred to as essential metals. However, all metals are toxic at high concentrations. Zinc is an important cofactor in over hundred enzymatic reactions in the human body but high levels of zinc can readily result in a deficiency of copper another metal required by the body also. Iron is a major constituent of haemoglobin and is required by the body for the synthesis of Red Blood Cells, yet higher levels. Of iron in the blood can result in a fatal condition known as hemochromatosis. a condition i which patient may present with cirrhosis of the liver, arthritis due to iron deposition in the joints, testicular failure, diabetes due to pancreatic islet cell failure or cardiomyopathy.

2.2 HUMAN HEALTH HAZARDS OF EXPOSURE TO HEAVY METALS

Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels and damage to blood composition, lungs, kidneys, liver and other organs of the body. Long-term exposure may result in slowly progressing physical, muscular and neurological degenerative processes that mimic Alzheimers disease, Parkinsons disease, muscular dystrophy and multiple sclerosis. The type of symptoms usually reflect the degree of exposure, including exposure overtime (Domingo, 2006; Mellick, 2006; Zintaras and Hajigeorgiou, 2004).

In the 1950s, industrial effluent was consistently dumped into Japans Minimata Bay and Mercury bioaccumulated to exceedingly high concentration in local fish. Although some adults did develop signs and symptoms of toxicity as described above, the greatest impact was on the next generation into which many were born with severe neurologic deficits (Eto, 2000). Hippocrates described abdominal colic in a man whom he extracted metals and the pernicious effects of arsenic and mercury among smelters were known even to Theophrastus of Erebus (370-287Bc).

The classic acute occupational heavy metal toxicity , "metal fume fever or Monday morning fever" as it is variously known, is a self limiting inhalation syndrome seen in workers exposed to metal oxide fumes characterized by fever, headache, fatigue, dyspnea, cough and a metallic taste occurring within 3-10 hours after exposure (kaye et al, 2002).

Studies have also proved that even minute levels of heavy metals have negative health effects, However, these vary from person to person (Casdorph and walker, 1995). Nutritional status, metabolic rate, the integrity of detoxification pathways, the mode and degree of heavy metal exposure all affect how an individual responds to heavy metal toxicity. Children and the elderly whose immune systems are either underdeveloped or age-compromised are more vulnerable to toxicity (weiner,1981)

HAZARDS IN MALES

In addition to general toxic metal symptoms, males may experience symptoms of benign prostatic hypertrophy (BPH) with its inherent discomfort. Also heavy metal can also produce male infertility due to their deposition and bioaccumulation in the prostrate.

HAZARDS IN FEMALES

Females under the influence of heavy metal toxicity may be infertile. They may have symptoms of widely fluctuating hormonal levels that are actually due to heavy metal poisoning. Heavy metals are able to cross the placental barrier on pregnant women and get deposited on the fetus. This is why some authors have proposed that female dental personnel exposed to mercury vapor in their work have a higher incidence of spontaneous abortion, premature labor and prenatal mortality (Casdorph and Walker,1995). Fetuses are susceptible to the toxic effects of high methyl mercury, a common cause of environmental mercury toxicity especially in the first trimester because their developing nervous systems are so sensitive and because methyl mercury easily crosses the placenta. The mercury

concentration in the baby's red blood cells can rise to 30 percent higher than those of its' mother.

HAZARDS IN CHILDREN

Heavy metal exposure has been reported to be one of the major causes of autism in genetically predisposed children. A study by Natal et al (2006) has demonstrated significant porphynuria in autistic children resulting from defect in the heme synthetic pathway due to heavy metal toxicity.

Autism is a complex neurodevelopment disorder that is usually diagnosed in early childhood and is characterized by deficits in social behaviors, impairment in verbal and non-verbal communication. It is a well established fact that the developing human brain is much more sensitive to low level of environmental neurotoxins than the adult brain (Shannon et al, 2008). Bioaccumulation of heavy metals in the brain induces oxidative stress in the developing brain which leads to development of neurological symptoms (James et al, 2004). Based on this, a redox-methylation hypothesis of autism is advanced, proposing that environmental insults initiates autism in genetically sensitive individuals by promoting cellular oxidative stress and initiating adaptive responses that includes reduced methylation activity. Impaired methylation in turn leads to developmental delay and deficits in attention and neuronal synchronization that are hallmarks of autisim.

2.3 HEAVY METALS AND THEIR EFFECT ON HUMAN HEALTH WITH THEIR PERMISSIBLE LIMITS (INDIAN PHARMACOPOEIA 2007 GUIDELINES; Adopted from Reena et al, 2011)

POLLUTANT	MAJOR SOURCES	EFFECT ON HUMAN HEALTH	PERMISSIBLE LEVEL (mg/L)
Arsenic	Pesticides, fungicides, metal smelters	Bronchitis, dermatitis, poisoning	0.02
Cadmium	Wielding, electroplating, pesticides fertilizers, Cd batteries, nuclean fission plant	Renal dysfunction, lung disease, lung cancer, Bone defects (osteomalacia, osteoporosis), increased blood pressure, kidney damage, bronchitis,	0.06

		gastrointestinal disorder, bone marrow, cancer	
Lead	Paint, pesticide, smoking, automobile emission, mining, burning of coal	Mental retardation in children, developmental delay, fatal infant encephalopathy, congenital paralysis, sensor neural deafness and acute or chronic damage to the nervous system, epilepticus, liver, kidney, gastrointestinal damage.	0.1
Magnanese	Weilding, fuel addition, ferromangenese production	Inhalation or contact causes damage to central nervous system	0.26
Mercury	Pesticides, batteries, paper industry.	Tremors, gingivitis, minor psychological changes, acrodynia chracterised by pink hands and feet. Spontaneous abortion, damage to nervous systems, protoplasm poisoning.	0.01
Zinc	Refineries, brass manufacture metal plating, plumbing.	Zinc fumes have corrosive effect on skin, cause damage to nervous membrane.	15
Chromium	Mines, mineral sources	Damage to the nervous system, fatigue irritability.	0.05
Copper	Mining, pesticide, production, chemical industry, metal	Anemia, liver and kidney damage stomach and	0.1

	piping.	intestinal irritation.	

2.4 MECHANISMS OF HEAVY METALS INDUCED HEALTH HAZARDS

A number of biological events have been identified as the major processes involved in the adverse effects produced by exposure to heavy metals. These include induction of oxidative stress, intracellular ion homeostasis disruption, sulfur metabolism, plasma membrane disruption and mitochondria function disruption.

INDUCTION OF OXIDATIVE STRESS

Oxidative stress is the disturbance in the pro-oxidant and antioxidant balance in the body in favor of the pro-oxidants (Edward, 1997). Generally, production of reactive oxygen species is associated with normal metabolic processes of the body. Under normal physiological conditions, these toxic species are destroyed by antioxidant defense systems of the body. Normal metabolism is therefore characterized by steady formation of pro-oxidants balanced by their similar rate of consumption by anti-oxidants. Cells therefore encounter oxidative stress when (1) production of reactive oxygen species overwhelms the antioxidant defense systems (2) compromised antioxidant or the combination of both conditions.

The reactive oxygen species of biological system includes superoxide anion (O^{-}_{2}), perhydroxyl radical (HO^{-}_{2}), Hydrogen peroxide (H_2O_2), Hydroxyl radical (HO), alkoxyl radical (R0), peroxy radical (R00), organic hydroperoxide (R00H) and singlet molecular oxygen. Accumulation of these substances results in oxidative stress and subsequent oxidative damage to cells (Valko et al, 2005).

The antioxidant in biological system includes the enzymatic and non-enzymatic scavangers of reactive oxygen species. The enzymatic group includes superoxide dismustase (SOD), Glutathione peroxidase (GSHpx), Glutathione Reductase (GSH_R) and Catalase while the non-enzymatic components includes ascorbate, alpha tocopherol, Glutathione (GSH) and metallothionein (MT).

INTRACELLULAR ION HOMOEOSTASIS DISRUPTION

Metal induced alteration in intracellular ion homoeostasis is a major mechanism for eliciting their toxic effects. Disturbances in ion homoeostasis would result in impaired phosphorylation/dephosphorylation reactions along the various signal transduction pathways of the body, altering cellular metabolism and vital functions.

MITOCHONDRIAL FUNCTION DISRUPTION

Occurs in most, if not all toxic metal insults; the mitochondria being a major source of energy for cellular functions. The mitochondria is also a major source of free radicals

but contains a number of antioxidant molecules such as superoxide dismustase (SOD), Glutathione (GSH), Glutathione reductase (GSH_R) and Glutathione peroxidase ($GSHp_R$). Metal induced dysturution of the mitochondria therefore results in oxidative stress and subsequent cellular damages as well due to imbalance in oxidants and antioxidants present in the mitochondria in favor of the oxidants.

PLASMA MEMBRANE FUNCTION DISRUPTION

Metal induced plasma membrane damage occurs directly through interaction with membrane components such as ion-dependent ATPases and ion channels. In general, toxic irreversible plasma membrane disruption is the last event of cell injury or death. Most metals interact with intra cellular membrane target molecules to cause changes in cellular metabolism and function which eventually leads to cell damage such as lysis. Cadmium, lead and Aluminum can directly interact with the cell membrane to alter its functions. The targets on the cell membrane with these metals are primarily the ion channels and pumps. The interaction between metals, ion channels and/or pumps does not necessarily result in structural damage to the cell membrane. Rather, it causes functional alterations. These in turn, lead to changes in cellular metabolism and function and eventually structural damage to the cell membrane (Edward, 1997). Therefore, the mode of membrane damage induced by many metals is initial and direct functional damage followed by delayed and indirect structural damage. In the case of some metals, more commonly the essential metals (iron, copper and zinc) their primary targets are not the membrane but the inner cell environment. These metals first cause overt cellular changes before actual cell membrane damage.

DISRUPTION OF SULFUR METABOLISM

According to Richard et al (2008), the ability of heavy metals to bind to thiol groups and to disrupt pathways of sulfur metabolism is well established. Sulfur metabolism has been proved to be important for the excretion of xenobiotics (sulfation) and their oxidized metabolites which results in oxidative assault. And according to Winyard et al (2005), sulfur metabolism can be considered a final common pathway of toxicity, reflecting the summed effect of diverse environmental metal insults and its accumulation in the body.

Generally, metal disruption of cellular and molecular events results in cell death; an irreversible loss of vital cellular structure and function. There are two fundamental types of cell death namely Necrosis and Apoptosis. Apoptosis is a programmed cell death that occurs during the normal development of life. It is major process responsible for cell death

in various physiological states such as embryonic remodeling, adult cell turnover and differentiation but also occurs in various pathologic conditions, such as infective state, tumuorigenesis, tissue intolerance and other unidentified conditions. Necrosis on the other hand occurs as a consequence of an injurious environment. This mode of cell death is characterized by irreversible loss of metabolic functions such as loss of ion gradients, adenosine triphosphate, oxidative damage to cells, mitochondrial dysfunction and loss of structural integrity of the plasma membrane.

It is well established that at their toxic concentrations metals cause necrosis in a variety of tissues. Studies have equally demonstrated that metals also induce apoptosis as a mode of toxicity (Buja et al, 1993). The mechanisms involved in apoptosis and necrosis differ in many ways. The same mode of cell death involves different mechanisms in various cell types. A most distinct process that differs between apoptosis and necrosis is ATP generation and protein synthesis; which are inhibited in necrosis but preserved in apoptosis. There are, however some common mechanisms involved in toxicant – induced apoptosis and necrosis especially the cell death induced by metals. Such mechanisms include alteration in intracellular ion homoeostasis and oxidative stress.

2.5 HEAVY METAL BIOACCUMULATION AND BIOMAGNIFICATION

According to Extension Toxicology Network EXTOXNET primary file (1993), bioaccumulation refers to an increase in the concentration of a chemical in a biological organism over time compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are metabolized or excreted. Understanding the dynamic processes of bioaccumulation, therefore, is important in protecting human beings and other organisms from the adverse effects of chemical exposure.

Biomagnifications on the other hand describes a process that results in the accumulation of a chemical in an organism at higher concentrations than are found in its food.

Bioaccumulation and Biomagnifications begins when a chemical passes from the environment into an organisms body (bloodstream and subsequently deposited in its cells, and tissues organs).

FACTORS AFFFECTING METAL BIOACCUMULATION AND BIOMAGNIFICATION

An important factor in the uptake and absorption of chemical substances in the body is water solubility; the ability of a chemical to dissolve in water. Usually, Compounds that are highly water soluble have a low potential to bioaccumulate or biomagnificate and do not leave water readily to enter the cells of an organism. Once inside, they are easily excreted unless the cells have a specific mechanism for retaining them. Compounds that dissolve readily in fat but not in water tend to be more slowly eliminated by the body and thus have a greater potential to bioaccumulate and biomagnificate.

Another factor affecting bioaccumulation and biomagnifications is whether an organism can break down and/or excrete a given chemical. Many metabolic reactions change a chemical into more water soluble metabolites, that are readily excreted. Such metals therefore have low potential to bioaccumulate and biomagnificate.

Individual risks with exposure to heavy metals and its accumulation and magnification in the body is likely to be determined also by genetic susceptibility factors. (Shannon et al, 2008). A major genetic susceptibility factor in this regard is genetic variations otherwise known as polymorphisms, more importantly single nucleotide poly morphisms (SNPs). Single nucleotide polymorphisms are prevalent forms of variation that involves change of one base pair in DNA and may alter expression levels or functions of encoded proteins. Deletion and insertion polymorphisms, ranging from one base pair to an entire gene making the human body prone to metal toxicity are now common.

Patients suffering from symptoms like fatigue, instability, mood disorders, poor concentrations and insomnia have been shown to most likely have the apolipoprotein E_4 (ApoE4) gene otherwise known as Alzhemiers gene. Thus, those with the genetic allele ApoE4 protein in their blood has been found to detoxify metals poorly and to be much more genetically susceptible to chronic neurological conditions than those with types ApoE2 or E3 (Grether et al, 2004). It has also been proved that genetic carriers of the brain protein ApoE2 are protected against Alzheimer's disease. ApoE proteins are synthesized in the brain with the assigned physiological function of carrying metabolic waste from the brain to the cerebrospinal fluid, across the blood brain barrier into the plasma where they are cleared by the liver. The biochemical difference between ApoE2 and ApoE4 is that ApoE2 has two additional thiol groups capable of binding and removing oxidants and as a result has the ability to protect the brain from metal toxic effects. Similarly, single-nucleotide polymorphisms (SNPs) in genes that encode the enzyme for the rate-limiting step in glutathione synthesis (glutamate cysteine ligase) and that which catalyze

glutathione conjugation (glutathione- S- transferase) has been able to explain to a reasonable extent why some people develop the oxidative stress linked to heavy metals toxicity while others who are similarly exposed do not (Custodio et al, 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.0 PLANT COLLECTION AND IDENTIFICATION

A fresh sclerotum of *Pleurotus Tuberregium* was harvested from a farm land at Agbudu in Orumba south Local Government Area of Anambra state and Bomu village, Gokana Local Government Area of Rivers State respectively. The plants were identified by the taxonomist Dr. C.C Onyeneke of the Department of Plants and Biotechnological Sciences, University of Nigeria, Nsukka.

PREPARATION OF PLANT EXTRACT

The sand particles on the body of the plant were washed off with distilled water. The plant was dried under a shade and later milled into fine powder using Thomas wiltey milling machine.

500g of the powder was soaked in one litre of distilled water for 24 hours with intermittent agitation and the mixture sieved with a muslim cloth to obtain a fine filtrate extract. The water in the extract was evaporated under a shade to get the concentrated crude extract. This was reconstituted with distilled water by weighing out l0g of the dried extract using a sensitive electric weighing balance and dissolving it in 100mls of distilled water with vigorous shaking.

3.1 EXPERIMENTAL ANIMALS

A total of 37 male albino rats 6-7 weeks old weighing (200±20g) were purchased and housed in the animal house of college of medicine, University of Nigeria Enugu Campus. They were allowed to acclimatize for a period of two weeks and fed with commercially available rat feed and distilled water ad libitium under standard conditions.

twelve of them were used for the determination of the acute oral toxicity of *p. tuberregium* sclerotia extract while the remaining 25 were used for the rest of the study.

3.2 ACUTE TOXICITY STUDIES (LD_{50})

The LD_{50} (median lethal dose) of the extract was determined using the procedure for the two phases testing as described by Lorke (1983).

In phase I:

Nine young adult male Albino rats were randomly divided into three groups of three each. The animals were deprived of food for 16-18hrs prior to administration of extract. They were weighed and grouped into treatment groups as follows:

Group A (for low dose of 10mg/kg)

Group B (for medium dose of 100mg/kg) and

Group C (for high dose of 1000mg/kg)

And observed for signs of toxicity immediately and up to 72hrs after administration

In phase II:

Three young adult male Albino rats were divided into three groups of one animal each. The animals were deprived of food for 16-18hrs prior to administration of the extract. They were also weighed to form a treatment group as follows:

Group A (for low dose of 1500mg/kg)

Group B (for medium dose of 3000mg/kg) and

Group C (for high dose of 5000mg/kg)

3.3 SUB-ACUTE TOXICITY STUDIES

A sub-acute study of the presence of heavy metals in blood and its effect due to consumption of *pleurotus tuberrregium* sclerotia was carried out as described by Organization for Economic Cooperation and Development (OECD) Test Guidelines (TG) which described short-term repeat-dose toxicity testing; Repeated dose 14-day oral toxicity study in rodents was used.25 of the 37 rats were divided into five groups (A-E) of five rats per group. After the previous 14days of the animal in the animal house for acclimatization with the new environment, the next 14days that followed was used for the experiment and the last day (the 29th day) was for sample collection and animal sacrifice.

Group A served as normal control which was fed with water and feed only. Group B received 1500mg/kg of extract of *p. tuberregiun* from Rivers State by oral gavage using an oral cannula. Group C received 1000mg/kg of the same sample while group D and E received 1500mg/kg and 1000mg/kg respectively of extract of *p. tuberregium* from Anambra.

On the 29th day, blood was collected from all the groups by retro-orbital sinus puncture of the median cantus vein using a capillary tube. The animals were sacrificed under chloroform anaesthesia.

3.4 SAMPLES AND SAMPLING TECHNIQUES

Seven milliliters of blood sample were collected from each of the study animal and dispensed into different tubes; EDTA vials for the analysis of the haematological

parameters, and plain vials for serum analysis of heavy metals, liver and kidney function. The sample vials for serum analysis were allowed to clot at room temperature and aliquoted into clean eppendorf tubes and stored frozen until analysed.

The liver and kidney were harvested using surgical blades. The bones were broken and the bone marrows smear made on a scrupulously grease –free slide within five minutes of animal sacrifice and stained for examination.

3.5 ANALYSIS OF SAMPLES

3.5.1 HISTOLOGICAL ANALYSIS

Method as described by Baker & Silverton (1998)

The liver and kidney tissue samples removed from the experimental rats were fixed in 10% formol saline solution for 42 hours and then passed through 70%, 95% and absolute alcohols for 1½ hours each. The tissues were then infiltrated through molten paraffin wax in the oven maintained at 60% for 1 hour using Leukhart embedding boxes. They were cut with a Cambridge rotary microtome at five microns thick for light microscopy. The Haematoxylene and Eosin method of staining was applied. The sections were stained in haematoxylene for 15 minutes, differentiated in 1% acidified alcohol by rinsing twice and then counter stained with Eosin for 3 minutes and dehydrated in 70%, 95% and absolute alcohol. The sections were cleared in xylene and mounted with DPX moutant for microscopy.

3.5.2 BONE MARROW ANALYSIS

Method based on modification of that described by Baker & Silverton (1998)

A thin film of the bone marrow from the broken bones of the rats from each study group (A-E) were made on a grease-free slide using a fine smooth edge glass spreader. The films were allowed to air –dry for 1 hour before flooding the slides with Lieshman stain for 8 minutes. The slides were diluted after the 8 minutes with equal volumes of water and allowed to stain for another 30 minutes. They were washed and allowed to air dry.

MICROSCOPY AND PHOTOMICROGRAPHY

The section were examined using Olympus binocular microscope with an in- built lighting system. They were photographed using a HP digital microscope camera (model: CB 350) attached to an eyepiece of the Olympus binocular microscope.

3.5.3 DETERMINATION OF THE HAEMATOLOGICAL PROFILE

The anticoagulated whole blood samples were investigated for a complete blood count involving Red blood cell count, haemoglobin, packed cell volume, Mean cell Haemoglobin concentration, mean cell volume, Platelet count, Total leucocyte count and Differential Leucocyte counts using the Mind Ray fully Automated Haematology Analyser, Japan.

Procedure

Whole blood samples were collected in EDTA bottles. They were gently mixed. The machine was switched on and allowed to stabilize for 5 minutes. The sample bottles were inserted into the sample aspirator and the aspiration button pressed. Samples were aspirated and the result of the various haematological parameters displayed on the screen.

3.5.4 DETERMINATION OF THE BIOCHEMICAL PROFILE

The serum samples were investigated for biochemical indices for liver function including Alaninine transaminase, Aspartate transaminase, Alkaline phosphatase, total protein and albumin. And kidney function involving creatinine, electrolytes, (sodium, potassium, chloride) using a Boehringer knoll chemistry semi-automated analyzer 4010 system, Boehringer, Mannheim, Germany and analytical kits were obtained from Randox Ltd, UK.

Procedure

The various protocols for the serum determination of these analytes including use of control samples and known standards were observed as stated in Randox kits Limited UK. The result were determined after observing the protocols by inserting the tubes containing a mixture of the appropriate reagents and the serum sample into the sample aspirator of the machine, selecting the required analyte on the run test menu of the screen, the aspiration button pressed and the result displayed on the screen.

ALKALINE PHOSPHATASE (ALP)

Method based on modification of Reitman and Frankel as described by Tietz (1995).

Principle

Alkaline phosphatase hydrolyses the substrate, disodium phenylphosphate to release phenol. The quantity of phenol released under standard conditions of time, temperature and pH, is measured by the absorbance of the red colour it assumes in alkaline solution. The phenol reacts with 4-aminophenazone in the presence of alkaline potassium ferricyanide to produce the red colour.

Procedure

1. 2.0ml of the buffered substrate was pipette into a test tube and incubated at 37^{o}c for 3 minutes.
2. 0.1ml of serum sample was added and the mixture incubated for 15 minutes.

3. 0.8ml of 0.5m sodium hydroxide 1.2ml of 0.5m sodium carbonate, 1.0ml of 4-aminophenazone and 1.0ml of potassium ferricyanide were added to the mixture and the tube shaked gently.
4. ALP was selected in the run test screen of the machine.
5. The test tube containing the mixture was inserted into a flow cell aspirator of the machine and the press button tapped for sample aspiration. Result was displayed on the screen.

ALANINE TRANSAMINASE (ALT)

Method based on modification of Reitman and Frankel as described by Tietz (1995).

Principle

ALT catalyses an equilibrium transfer of amino group from the amino acid alanine to the corresponding keto acid, α-ketoglutarate to form glutamate and itself converted to pyruvate. The pyruvate increase is measured in a subsequent indicator reaction which is catalysed by lactate dehydrogenase in the presence of NADH oxidized to NAD with lactate as a product. The rate of decrease in NADH is directly proportional to the rate of formation of pyruvate and thus the ALT activity.

Procedure:

1. 0.5ml of the buffered substrate was incubated at 37^{o}c for 3 minutes.
2. 0.1ml of serum sample was added and the mixture further incubated for 30 minutes.
3. 0.5ml of dinitrophenylhydrazone (DNPH) was added and the resultant mixture incubated at room temperature for further 20 minutes.
4. 5.0ml of 0.4N NaoH was added and the mixture incubated for further 10 minutes.
5. ALT was selected in the run test screen of the machine.
6. The test tube containing the mixture was inserted into the flow cell aspirator of the machine and the press button tapped for sample aspiration. Result was displayed on the screen.

ASPARTATE TRANSAMINASE (AST)

Method based on the modification of Reitman and Frankel as described by Tietz (1995).

Principle

When AST is incubated at 37^{o}c for exactly 60 minutes in a pH 7.5 buffered substrate containing aspartate and 2-ketoglutarate, it catalyses the transfer of amino acid group from aspartate to ketoglutarate forming oxaloacetate and glutamate. The

oxaloacetate reacts with 2, 4 dinitrophen-l-hydrazine to form 2, 4, dinitrophenyl hydrazone which in alkaline pH is red-brown and is measured spectrophotometrically.

Procedure

1. 0.5ml of the buffered substrate was incubated at 37^{o}c for 3 minutes.
2. 0.1ml of serum sample was added and the mixture further incubated for 60 minutes.
3. 0.5ml of dinitrophenylhydrazone (DNPH) was added and the mixture incubated at room temperature for further 20 minutes.
4. 5.0ml of 0.4N NaoH was added and the mixtures incubated for another 10 minutes.
5. AST was selected in the run test screen menu of the machine.
6. The test tube containing the mixture was inserted into the flow cell aspirator of the machine and the press button tapped for sample aspiration. Result was expressed in the screen.

BILIRUBIN

Method based on modification of Jendrassik and Grof as described by Tietz (1995).

Principle

Direct (conjugated) bilirubin reacts with diazotised sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin by the reaction with diazotized sulphanilic acid.

REAGENTS

Reagent 1 (R_1): a mixture of 29mmol/L sulphanilic acid and 0.17N HCL.

Reagent 2 (R_2): 38.5mmol/L sodium Nitrite.

Reagent 3 (R_3): a mixture of 2ml each of 0.26mol/L caffeine and 0.52mol/L sodium benzoate.

Reagent 4 (R_4): a mixture of 2ml each of 0.93mol/L Tartrate and 1.9N sodium Hydroxide.

Procedure

1. 200ul of R_1, 50ul of R_2, 1000ul of R_3 and 2000ul of serum sample was mixed and the mixture incubated for 10 minutes at 24^{o}c.
2. 1000ul of R_4 was then added and the mixtures incubated further for 15 minutes at same temperature.
3. T_{BILL} was selected on the run test menu in the screen of the machine.

4. The test tube containing the mixture was inserted into a flow cell aspirator of the machine and the press button tapped for sample aspiration. Result was expressed in the screen.

CREATININE

Method based on that described by Schwartz and Work (2009).

Principle

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Procedure

1. 500ul of reagents one 'R_1' (35mmol/L of picric acid) was mixed with 500ul of reagent two 'R' (0.32mol/L of sodium Hydroxide) to obtain a working reagent.
2. 500ul of the working reagent was then mixed with 500ul of serum sample.
3. CREA was selected on the run test menu in the screen of the machine.
4. The mixture was inserted into the flow cell aspirator of the machine and the press button tapped for sample aspiration. Result was expressed in the screen.

UREA

Method based on that described by Tietz (1995)

Principle

Ammonia produced by the action of Urease on urea is estimated by measuring the blue colour of indophenol formed with phenol and hypochlorite in the presence of sodium nitroprusside as catalyst.

Procedure

1. 200ul of buffered Urease reagent was mixed with 20ul of serum sample and incubated at 37^{o}c for 15 minutes.
2. 1.0ml phenol colour reagent was added to mixture with gentle agitation.
3. 1.0ml hypochlorite reagent was then added and the resultant mixture incubated at 37^{o}c for 20 minutes.
4. 5ml of distilled water was added to the mixture.
5. Urea was selected in the run test menu in the screen of the machine.

6. The test tube containing the mixtures was inserted into a flow cell aspirator of the machine and the press button tapped for sample aspiration. Result was expressed in the screen.

SODIUM

Method based on modification of that described by Tietz (1995).

Principle

Sodium is precipitated as a triple salt, sodium magnesium uranyl acetate, with the excess uranium being reacted with ferrocyanide producing a chromophone whose absorbance varies inversely as the concentration of sodium in the test specimen.

REAGENTS

1. Acid reagent: a dilute acetic acid.
2. Sodium colour reagent: Potassium ferrocyanide, non-reactive stabilizers and filters.

Procedure

Filtrate Preparation

1. 1.0ml of filtrate reagent was pipette into a test tube containing 50ul of serum sample.
2. The tube was shaken vigorously continuously for 3 minutes and centrifuged at high speed of 1500G for 10 minutes to obtain a supernatant.

Colour Development

3. 1.0ml Acid reagent was mixed with 50ul of the supernatant.
4. 50ul of the colour reagent was added to it.
5. Sodium was selected in the run test menu in the screen of the machine.
6. The test tube containing the mixtures was inserted into a flow cell aspirator of the machine and the press button tapped for sample aspiration. Result was expressed in the screen.

POTASSIUM

Method based on that described by Tietz (1995)

Principle

The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension.

Procedure

1. 1.0ml of sodium tetraphynylboron reagent was mixed with 0.025ml of serum sample.
2. The mixture was incubated at 30^{o}c for 10 minutes.

3. Potassium was selected in the run test menu on the screen of the machine.
4. The test tube containing the mixture was inserted into a flow cell aspirator of the machine and the press button tapped for sample aspiration. Result was expressed in the screen.

CHLORIDE

Method based on modification of that described by Tietz (1995).

Principle

When serum is mixed with a solution of undissociated mercuric thiocyanate, the chloride preferentially combines with the mercury to form mercuric chloride. The thiocyanate released combines with ferric ions present in the reagent to form ferric thiocyanate which can be measured spectrophotometically.

Reagents

1. Mercuric Thiocyanate in methanol 1.3mmol/L.
2. Ferric Nitrate 59mmol/L.
3. Mercuric Nitrate 0.26mmol/L.

Procedure

1. 1.0ml each of the reagents was mixed.
2. 1.0ml of the resultant mixture was added to 0.01ml of the sample and the mixture incubated at 37^{o}c for 5 minutes.
3. Chloride was selected in the run test menu of the screen in the machine.
4. The test tube containing the mixture was inserted into a flow cell aspiration of the machine and the press button tapped for sample aspiration. Result was expressed in the screen.

3.5.5 HEAVY METAL ANALYSIS OF SAMPLES

The sample of *pleurotus tuberregium* sclerotia and the serum samples obtained from the rats treated with extracts of *pleurotus tuberregium* sclerotia and those of the controls were analysed using an Atomic Absorption Spectrophotometer (AA 240FS varian) according to the manufacturer's operational guidelines. All reagents used for the study were of analytical grade. All glass wares were soaked in 10% HNO_3 for 2 hours and later rinsed with distilled water prior to use for metal analysis. Distilled water was used for the preparation of all solutions.

The samples were analysed for Nickel, Lead, Cadmium, Mercury, Aluminum, platinum and Arsenic at wavelengths of 232,217, 228.8, 253.7,309.3,239.6 and 193.7 respectively against a working standard solution of 100mg/L of each metal.

PROCEDURE

The AAS was switched on and the oxidizing gas (nitrous oxide-acetylene) chamber of the machine was fed to supply the flame needed to heat the cathode lamps. A blank cell (containing distilled water) was inserted into the machine and run in other to zero it. The standard for the various metals were also run to ensure that the curve corresponds to the standard curve for the individual metals after which the digested samples were run using the same standard as the metal in question to determine the concentration of each metal in the different samples of *pleurotus tuberregium* and the diluted serum samples for the determination of serum concentrations of each metal in rats due to sample extracts consumption. The running was done by dipping a capillary tube into the required sample in the bottle and little of the sample was aspirated, result displayed on the screen, showing the corresponding concentration of the metal. This was repeated two times for a particular heavy metal and the mean taken. This was done for the various metals in the various samples for consideration.

PREPARATION OF METAL WORKING STANDARD SOLUTION

100mg/L working standard solution for each metal was first prepared in 100ml of distilled water from a standard stock solution containing water, Nitrous oxide and 1000mg/L of metal to be measured and calculated as follows:

$$C1V1=C2V2$$

C1= concentration of the working solution = 1000mg/L

V1=volume of distill water used to prepare the working solution =100ml

C2= concentration of each metal in the stock standard =100mg/L

V2= volume of stock standard that will be used

$$V2 = \frac{100mg/L \times 100m1}{1000mg/L} = 10ml$$

10mg/L therefore, were prepared from 100mg/L working solution by collecting 10mls of working solution and adding 90mls of distilled water.

DILUTION OF SERUM SAMPLES FOR METAL ANALYSIS

2mls of the serum samples were diluted with 8mls of distilled water and the mixture filtered using a Whatman filter paper. The diluted samples were analysed for heavy metal concentration using the AAS 240fs varian.

DIGESTION OF P. TUBERREGIUM SAMPLES FOR METAL ANALYSIS

Method according to James (1995)

2g of the samples were weighed into a 100ml conical flask and 30ml of digestion solution known as aquaregia mixture containing 20mls of 70% high purity HNO_3 and 10mls of Hcl were added to it. The mixture was heated to ashing in a thermostatically controlled murfle furnace. A good digestion was achieved at 80^oC for 3 hours. Thereafter, the ash was allowed to cool at room temperature, then transferred to sample bottles and made up to 50ml mark with distilled water. They were anlaysed for the various metals using the AAS 240FS varian.

3.5.6 PHYTOCHEMICAL SCREENING OF P. TUBERREGIUM SAMPLES

The plant extract was screened for the presence of alkaloids, flavonoids, tannins, saponins, glycosides, steroids, carbohydrates, proteins and oils using the procedure outlined by Trease and Evans (1989). In general, the various protocols applied for the detection of the presence or absence of these phytochemical compounds using the above methods involved the addition of an appropriate standard chemical agent to the aqueous extract of the plant in a test tube and shaken vigorously or gently as the case may be. Gentle heat may sometimes be required. The various protocols are described below.

TEST FOR ALKALOIDS

0.5g of the extract was stirred and boiled with 5mls of 1% aqueous hydrochloric acid in a water bath. 1ml of the filtrate was treated with 5drops of Mayer's reagent, Dragendorff's reagent, wagner's reagents and 1% picric acid solution. Turbidity and formation of a precipitate with the reagents was observed for evidence of the presence of alkaliods in the extract.

FROTHING TEST FOR SAPONINS

0.5g of plant extract was shaken vigorously with 3mls of water in a test tube. This was observed for frothing which persisted on warming. To remove false-positive results, a blood haemolysis test was also performed by shaken 0.5g of the extract with 5mls normal saline and the mixture filtered. 5mls of 10% dilution of rat blood in normal saline was placed in two test tubes. 2mls of the filtrate was added to one of the tubes while 2mls of normal saline was added to the other. This, after thorough mixing, was kept for 30 minutes at room temperature and examined under the microscope for complete haemolysis.

Saponins have the ability to produce frothing in ageous solution and to haemolyse red blood cells.

FERRIC CHLORIDE TEST FOR TANNINS

0.5g of pleurotus extract was boiled with 5mls of 45% ethanol for 5minutes, cooled and filtered.1ml of the filtrate was diluted with 5mls of distilled water, ferric chloride reagent was added and the mixture observed for blue –black, blue –green or green precipitate for an evidence of the presence of tannins.

GENERAL HYDROLYSIS TEST FOR GLYCOSIDES

3mls of dilute tetraoxosulphate (iv) acid mixed with 0.5g of *pleurotus tuberregium* extract was placed in a boiling water bath for 15 minutes which was cooled and filtered. The filtrate was neutralised with 20% potassium hydroxide (KOH) and 2mls Fehlings solution (A and B in equal volumes) was added and boiled. The precipitate was filtered off after cooling and 2mls 0.2 N hydrochloric acid was added to the filtrate and the mixture boiled for 30 minutes, cooled and also filtered. The resulting filtrate was neutralised with 20% KOH and 2mls Fehling's solution added , mixture boiled for 5minutes, cooled and observed for visible color change as an evidence of the presence of glycosides

TEST FOR FLAVONOIDS

10mls ethyl acetate mixed with 0.1g of *pleurotus tuberregium* extract was heated in a boiling water bath for 3minutes, filtered and the filtrate was used for the following:

(a) 1ml of dilute ammonia was mixed with 4 mls of filtrate and mixture vigorously shaken
(b) 1ml of 1% Aluminum chloride solution was mixed with 4mls of filtrate and the mixture vigorously shaken
(c) ImI of sodium hydroxide solution was mixed with 4mls of filtrate and hydrochloride solution was added drop wise.

The mixtures were observed for color change as an evidence for the presence of flavonoids.

SALKOWASKI TEST FOR STEROIDS

0.5g of extract of *pleurotus tuberregium* was dissolved in 2mls chloroform and sulphuric acid was added carefully to form a lower layer. A reddish-brown color at the interface was observed which indicates the presence of a steroidal nucleus.

TEST FOR CARBOHYDRATES

500mg of crude Alternanthera brasiliana powder was extracted with 5mls water and heated in a boiling water bath for 3 minutes and filtered. The filtrate was treated with 2mls of

tetraoxosulphate IV acid, boiled and neutralized with 2mls sodium hydroxide. A mixture of equal parts of Fehlings solutions A and B was added drop wise, boiled and observed for color change as an evidence for the presence of polysaccharides.

Keller- killiani specific test for reducing sugars was also carried out by extracting 500mg of crude alternanthera brasliana powder with 5mls of water and heated in a boiling water bath for 3minutes and filtered. The filtrate was treated with 2mls of glacial acetic acid containing a drop of ferric chloride solution. The mixture was transferred to a test tube and 0.5ml of concentrated tetraoxosulphate(iv) acid was laid down the side of the tube.A brown ring at the interface indicates presence of desoxy sugars characteristics of candenolides.

MILLIONS REAGENT TEST FOR PROTEINS

5mls of distilled water was added to 0.1g of crude alternanthera brasiliana powder and was left to stand for 3hours after which it was filtered. O.1ml Millions reagent [15%HgSo4 in 15% H_2So4] was added to 2mls of the filtrate, shaken and observed for color change as an evidence for the presence of proteins.

TEST FOR OILS

0.lg of the powdered extract of *pleurotus tuberregium* was pressed between two filter papers and the papers were observed for translucency as an evidence for the presence of oils.

3.5.7 STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) was used for comparison in all the groups while independent sample T-test and Bonferroni Multiple Comparison tests were used to compare between groups. $P<0.05$ (two-tailed) was considered significant using SPSS version 17.0

CHAPTER FOUR

4.0 RESULTS

Phytochemical screening of the two different samples of pleurotus tuberregium sclerotia (Table 4.1) revealed an abundant presence of Alkaloids, Carbohydrates, Proteins, Saponins, moderate presence of Flavonoids, Glycosides, presence of Tannins, Oils and complete absence of Steroids. Oral administration of 10mg/kg, 100mg/, 1000mg/kg of the crude aqueous extract of the samples in the first investigation phase of the acute toxicity test and 1500mg/kg, 3000mg/kg, 5000mg/kg respectively in the second phase (Table 4.2) could not result in any sign of mortality or mortality in the rats.

A direct analysis of heavy metals on the samples of the pleunotus tuberregium sclerotia (Table 4.3) revealed the presence of Lead, Mercury, Aluminium, Platinium, Cadnium, Arsenic and Nickel in both samples but there was no statistically significant different($p>0.05$) between the concentrations of metals in the samples. Table 4.4 is a table of values showing the serum heavy metal concentrations of the rats as detected in the individual groups after an oral administration of graded doses of the crude aqueous extract of the samples for a period of 14 days. While metals were not detected in the serum of the control rats (Group A) which was fed with rat feed and distilled water only, Nickel was detected in the serum of rats treated with 1500mg/kg (Group B) of the sample extract from Rivers State and in both the serum of rats treated with 1500mg/kg (Group D) and 1000mg/kg (Group E) respectively of the sample extract from Anambra State. Lead and Cadmium were detected only in Group B (rats treated with 1500mg/kg) of the sample extract from Rivers State and Group C (rats treated with 1000mg/kg) of the same sample extract. While Mercury was detected in the serum of rats in Groups B, D and E respectively.

Aluminium was only detected in the serum of rats in Group C. Arsenic was detected in the serum of rats in Groups B and C while Platinium was detected in the serum of rats in Groups D and E. There was no statistically significant differences ($p > 0.05$) in the serum concentrations of metals in test rats treated with same doses of the different sample extracts except for the test Group B in which Cadmium recorded high concentrations of (0.057 ± 0.006) as compared to Group D (0.010 ± 0.010) and test Group E in which Nickel recorded (0.055 ± 0.021) as compared to Group C (0.000 ± 0.000).

4.1 Table 1: Result of the Phytochemical Screening of *Pleurotus Tuberregium* Sclerotium

PHYTOCHEMICAL CONSTITUENT	INFERENCE
Alkaloids	+++
Flavonoids	++
Tannins	+
Saponins	+++
Glycosides	++
Steroids	Absent
Carbohydrates	+++
Proteins	+++
Oils	+
pH	Neutral

Key:

+ = present, ++ = moderately present, +++ = abundantly present.

4.2 Table 2: Result of Acute Toxicity (Lethality Test, LD_{50}) of *Pleurotus Tuberregium* Sclerotia

1st Investigation Phase

Treatment Dose (mg/kg)	No. used/No. dead
10	3/0
100	3/0
1000	3/0

2nd Investigation Phase

Treatment dose (mg/kg)	No. used/No. dead
1500	1/0
3000	1/0
5000	1/0

4.3 Table 3: The Comparison between Mean Concentrations of Direct Metal Analysis of Pleurotus Tuberregium Samples A and B

METAL (mg/L)	CONCENTRATION OF METAL IN SAMPLE A (Mean ± SD)	CONCENTRATION OF METAL IN SAMPLE B (Mean ± SD)	P-VALUE
Nickel	0.157 ± 0.001	0.162 ± 0.002	P> 0.05
Lead	0.293 ± 0.002	0.290 ± 0.001	P> 0.05
Cadmium	0.198 ± 0.001	0.189 ± 0.003	P> 0.05
Mercury	0.268 ± 0.001	0.271 ± 0.001	P> 0.05
Aluminum	0.277 ± 0.002	0.281 ± 0.002	P> 0.05
Arsenic	0.209 ± 0.001	0.212 ± 0.002	P> 0.05
Platinum	0.191 ± 0.002	0.194 ± 0.001	P >0.05

Keys:

Alpha (ي) = 0.05, 1mg/L ≏ 1ppm ≏ 0.001ug/ L

Sample A is *pleurotus tuberregium* sclerotia from Rivers State.

Sample B is *pleurotus tuberregium* sclerotia from Anambra State.

4.4 Table 4: Mean ± SD of the Serum Heavy Metal Concentrations of the Individual Groups of the Study Rats

METAL	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
Nickel	0.000 ± 0.000	0.022 ± 0.011	0.000 ± 0.000	0.011 ± 0.001	0.055 ± 0.021
Lead	0.000 ± 0.000	0.021 ± 0.008	0.016 ± 0.008	0.00 ± 0.000	0.00 ± 0.000
Cadmium	0.000 ± 0.000	0.057 ± 0.006	0.015 ± 0.004	0.010 ± 0.010	0.00 ± 0.000
Mercury	0.000 ± 0.000	0.006 ± 0.007	0.000 ± 0.000	0.023 ± 0.024	0.018 ± 0.022
Aluminum	0.000 ± 0.000	0.000 ± 0.000	0.032 ± 0.027	0.00 ± 0.000	0.00 ± 0.000
Arsenic	0.000 ± 0.000	0.024 ± 0.008	0.056 ± 0.076	0.00 ± 0.000	0.00 ± 0.000
Platinum	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.35 ± 0.003	0.054 ± 0.045

4.5 TABLE 5: Comparison of the Haematological Parameters of the Test Groups with that of the Control

Parameter	Group A	Group B	Group C	Group D	Group E	P-VALUE
RBC ($X10^{12}$/L)	6.12±3.77	6.285±0.78	6.555±1.97	5.625±2.59	6.665±2.88	P > 0.05
HGR (g/dL)	11.7±1.7	11.9±1.84	12.85 ± 1.2	11.15±2.19	13.3±0.14	P > 0.05
PCV (%)	35.3±3.39	36.25±3.18	38.9±3.11	32.85±5.87	37.5±1.56	P > 0.05
MCHC (g/L)	32.65±2.19	33.05±1.63	32.95±0.49	33.85±0.64	35.45±1.91	P > 0.05
MCH (pg)	18.8±0.57	19.05±1.34	19.55±0.49	19.8±0.28	19.9±0.14	P > 0.05
MCV (fL)	57.9±2.12	57.75±1.2	59.4±0.57	58.75±1.91	56.4±3.39	P > 0.05
PLT ($x10^9$/L)	517±398.81	557.5±126.57	702±144.25	573.5±72.82	644±56.57	P > 0.05
TWBC ($x10^9$/L)	8.35±0.92	9.00±0.42	9.65±1.63	10.35±3.79	8.56±1.3	P > 0.05
GRAN (%)	21.5±8.06	29.75±1.2	17.95±3.32	13.3±7.92	17.25±10.25	P > 0.05
LYMPH (%)	52.55±6.29	65.7±9.76	60.45±7.0	72.65±13.93	67.0±15.84	P > 0.05

4.6 Table 6: Comparison of the Liver Function Test Values of the Test Groups with that of the Control

PARAMETER	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	P-VALUE
ALP (IU/L)	691.7 ± 64.92	878.3 ± 563.2	406.3 ± 38.32	537.6 ± 483.6	706.2 ± 107.2	P > 0.05
ALT (IU/L)	4.35 ± 2.97	75.05 ± 31.54	18.15 ± 37.76	91.2 ± 20.08	184.7 ± 192.62	P > 0.05
AST (IU/L)	1.6 ± 1.697	2.5 ± 1.4142	1.65 ± 1.2728	2.45 ± 0.4242	5.85 ± 5.232	P > 0.05
Total Bilirubin (mg/dL)	0.405 ± 0.1838	0.6 ± 0.4242	0.615 ± 0.3252	0.575 ± 0.3536	1.27 ± 1.0182	P > 0.05
Direct Bilirubin (mg/dL)	0.14 ± 0.198	0.305 ± 0.099	0.285 ± 0.4102	0.27 ± 0.5092	0.615 ± 0.4384	P > 0.05

4.7 Table 7: Comparison of the Kidney Function Test Values of the Individual Groups with that of the Control

PARAMETER	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	P-VALUE
CREATININE (mg/dL)	0.59 ± 0.02	0.52 ± 0.15	0.54 ± 0.20	0.61 ± 0.11	0.60 ± 0.06	P > 0.05
UREA (mg/dL)	12.90 ± 1.27	13.09 ± 2.67	12.50 ± 2.40	12.70 ± 0.85	12.37 ± 0.65	P > 0.05
SODIUM (Mmol/L)	140 ± 1.41	145 ± 11.31	136 ± 11.31	138 ± 2.83	136.3 ± 2.40	P > 0.05
POTASSIUM (Mmol/L)	3.75 ± 0.64	3.98 ± 1.45	3.68 ± 0.45	3.86 ± 1.05	3.96 ± 1.07	P > 0.05
CHLORIDE (Mmol/L)	89.50 ± 3.54	89.60 ± 12.16	90.35 ± 6.15	89.30 ± 5.37	90.50 ± 12.02	P > 0.05

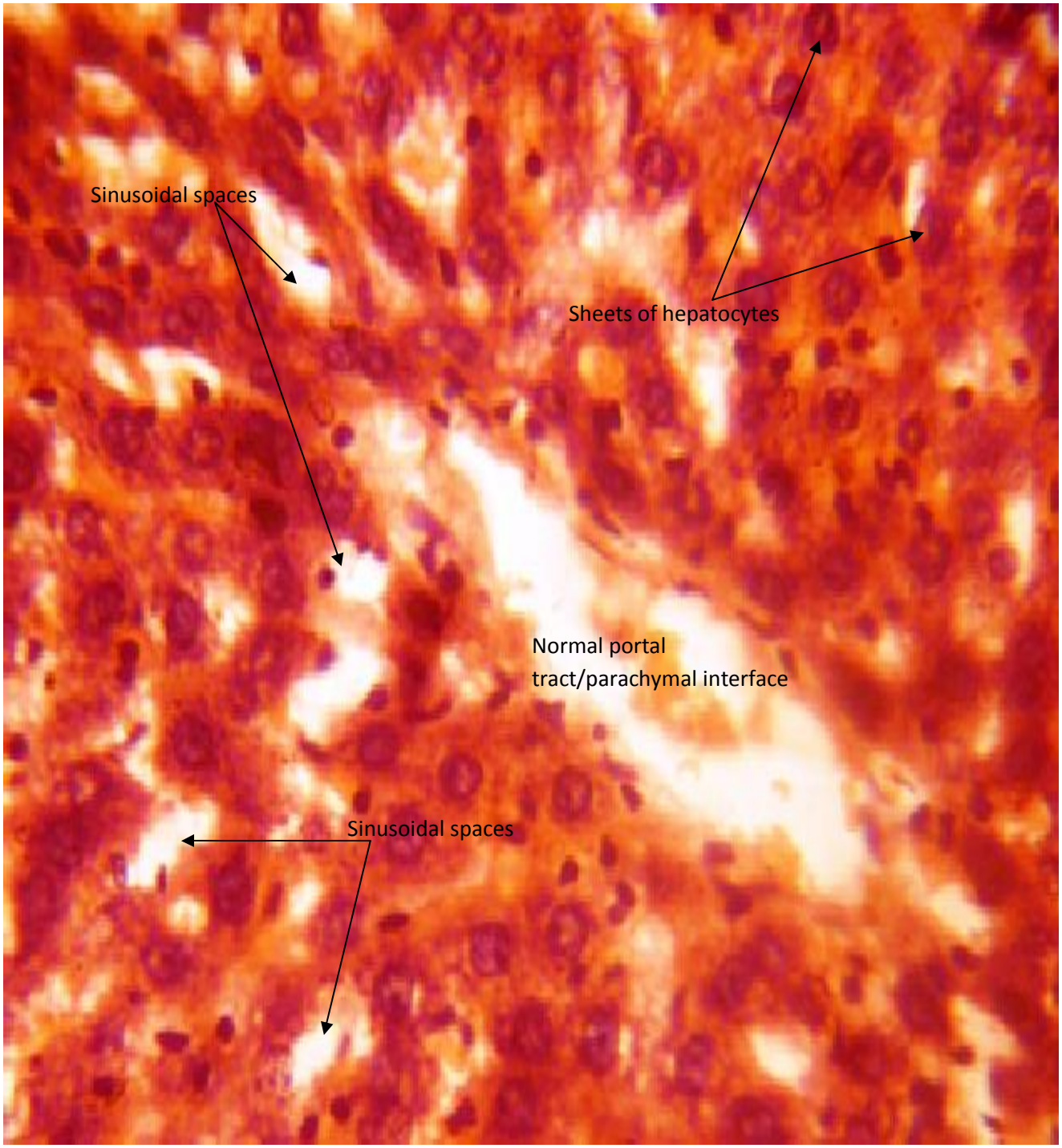

Fig 4.1: REPRESENTATIVE PHOTOMICROGRAPH OF THE LIVER TISSUES OF THE EXPERIMENTAL RATS OF GROUP A

High magnification (x 400) H & E stained section of the liver of rats fed with water and feed only showing intact sheets of hepatocytes, normal portal tract and sinusoidal spaces.

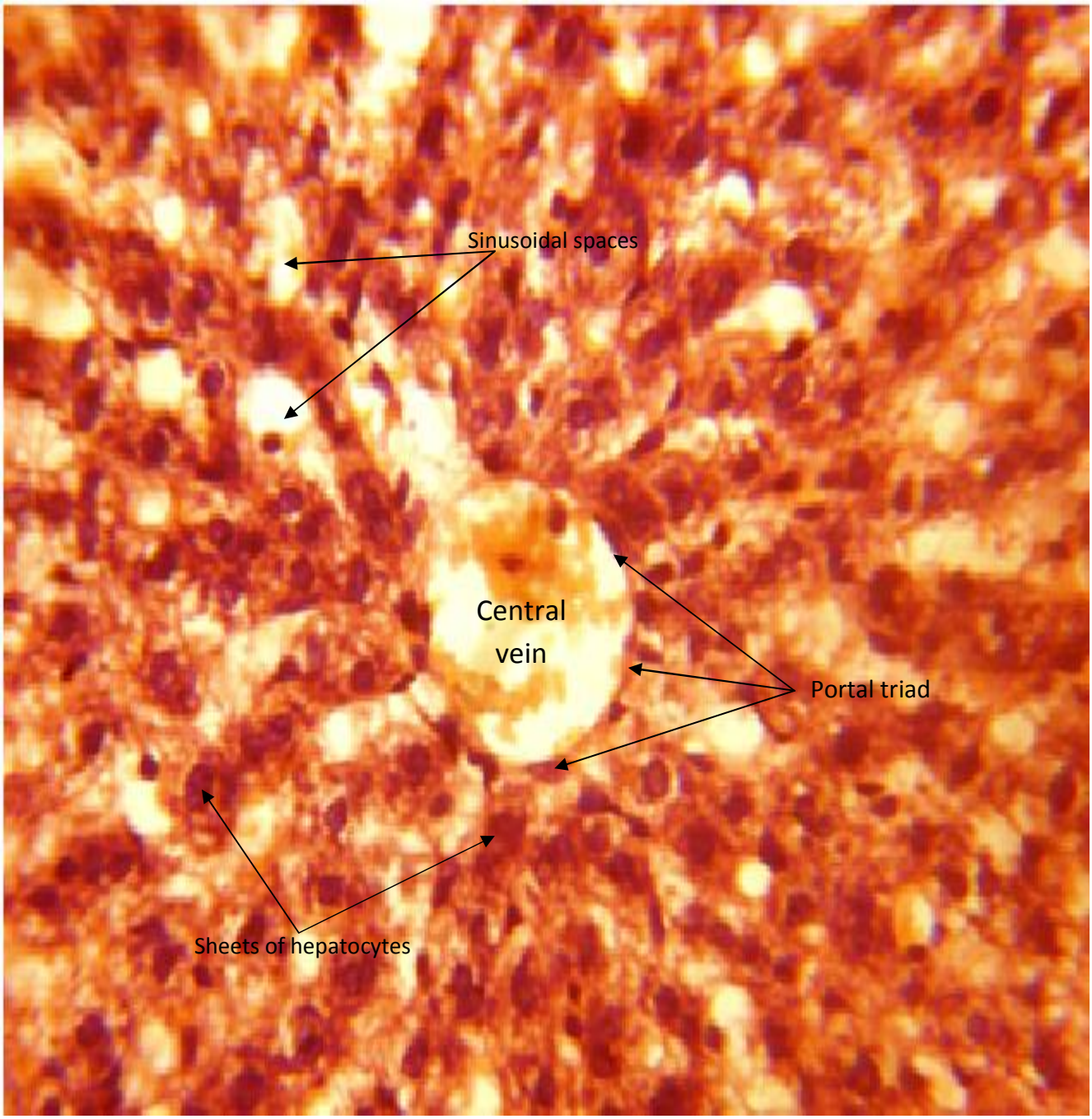

FIG 4.2: REPRESENTATIVE PHOTOMICROGRAPH OF THE LIVER TISSUES OF THE EXPERIMENTAL RATS OF GROUP B

High magnification (x 400) H & E stained section of the liver of rats fed with 1500mg/kg of pleurotus tuberregium sclerotia sample extract obtained from Rivers State showing an organised central vein and portal triad.

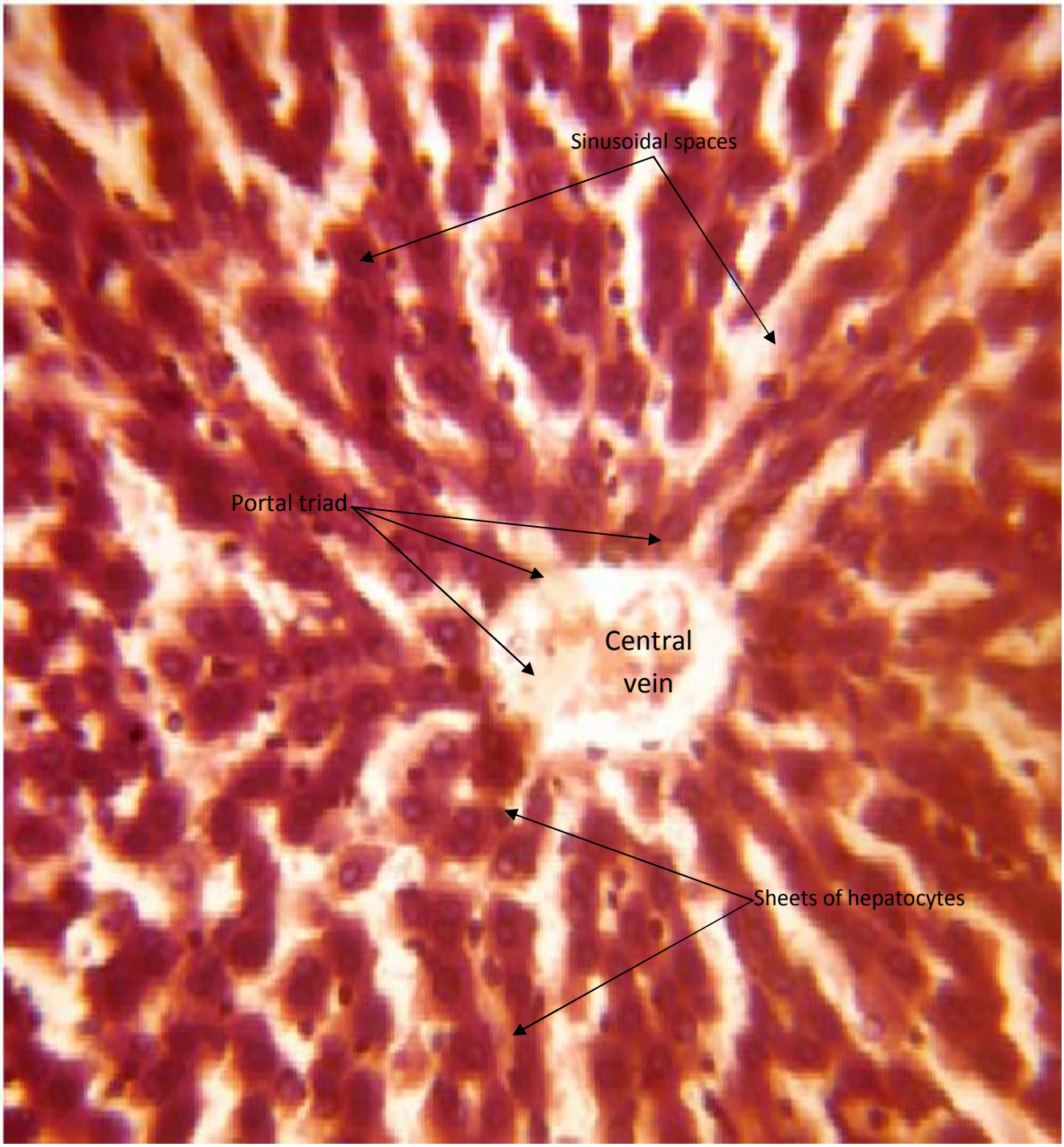

Fig 4.3: REPRESENTATIVE PHOTOMICROGRAPH OF THE LIVER TIISSUES OF THE EXPERIMENTAL RATS OF GROUP C

High magnification (x 400) H & E stained section of the liver of rats fed with 1000mg/kg of pleurotus tuberregium sclerotia sample extract obtained from Rivers State showing regular sheet of hepatocytes, sinusoidal spaces and lobulation of the liver.

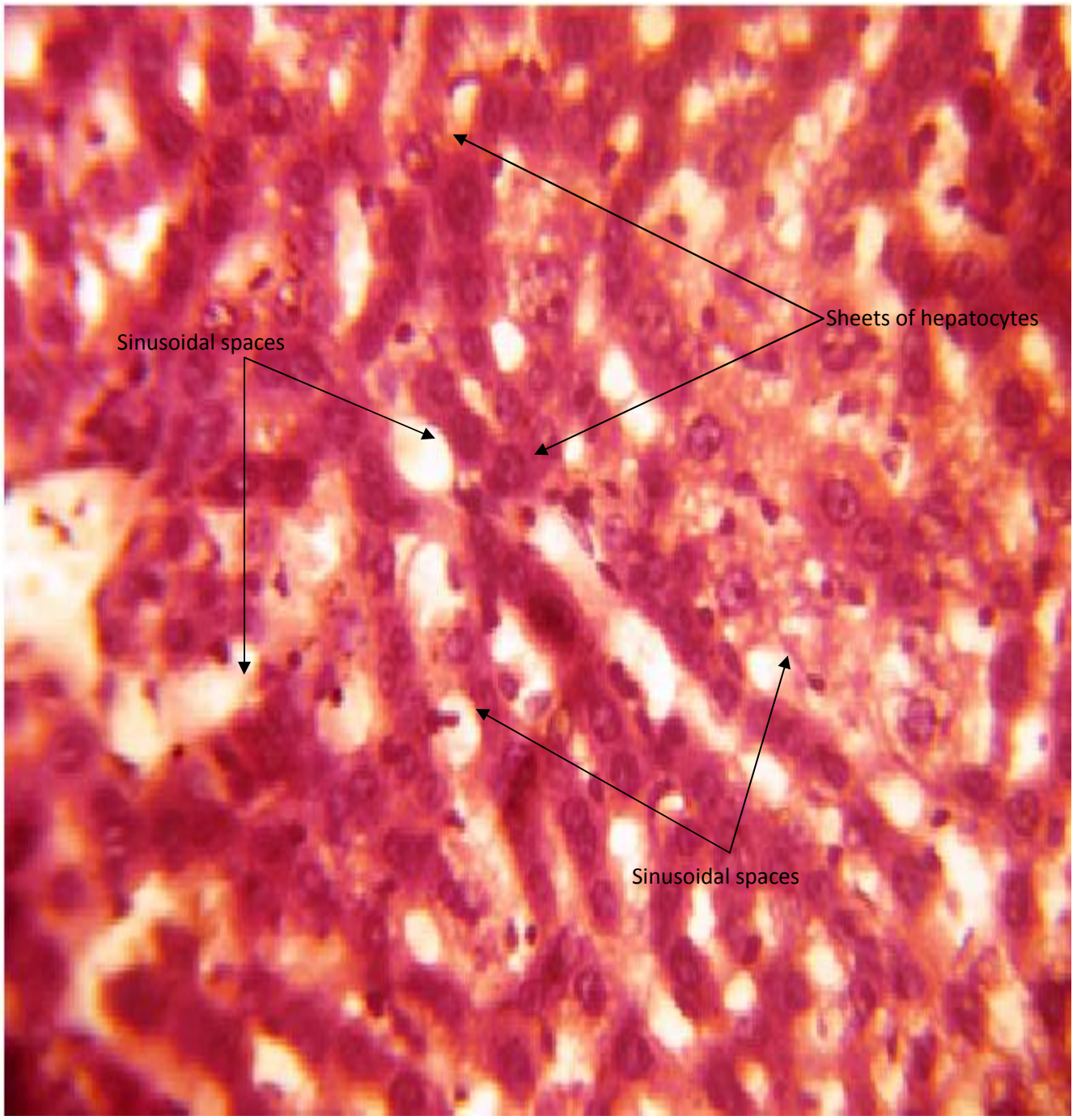

FIG 4.4: REPRESENTATIVE PHOTOMICROGRAPH OF THE LIVER TIISSUES OF THE EXPERIMENTAL RATS OF GROUP D

High magnification (x 400) H & E stained section of the liver of rats fed with 1500mg/kg of pleurotus tuberregium sclerotia sample extracted obtained from Anambra State showing regularity of sinusoidal spaces and intact hepatocytes

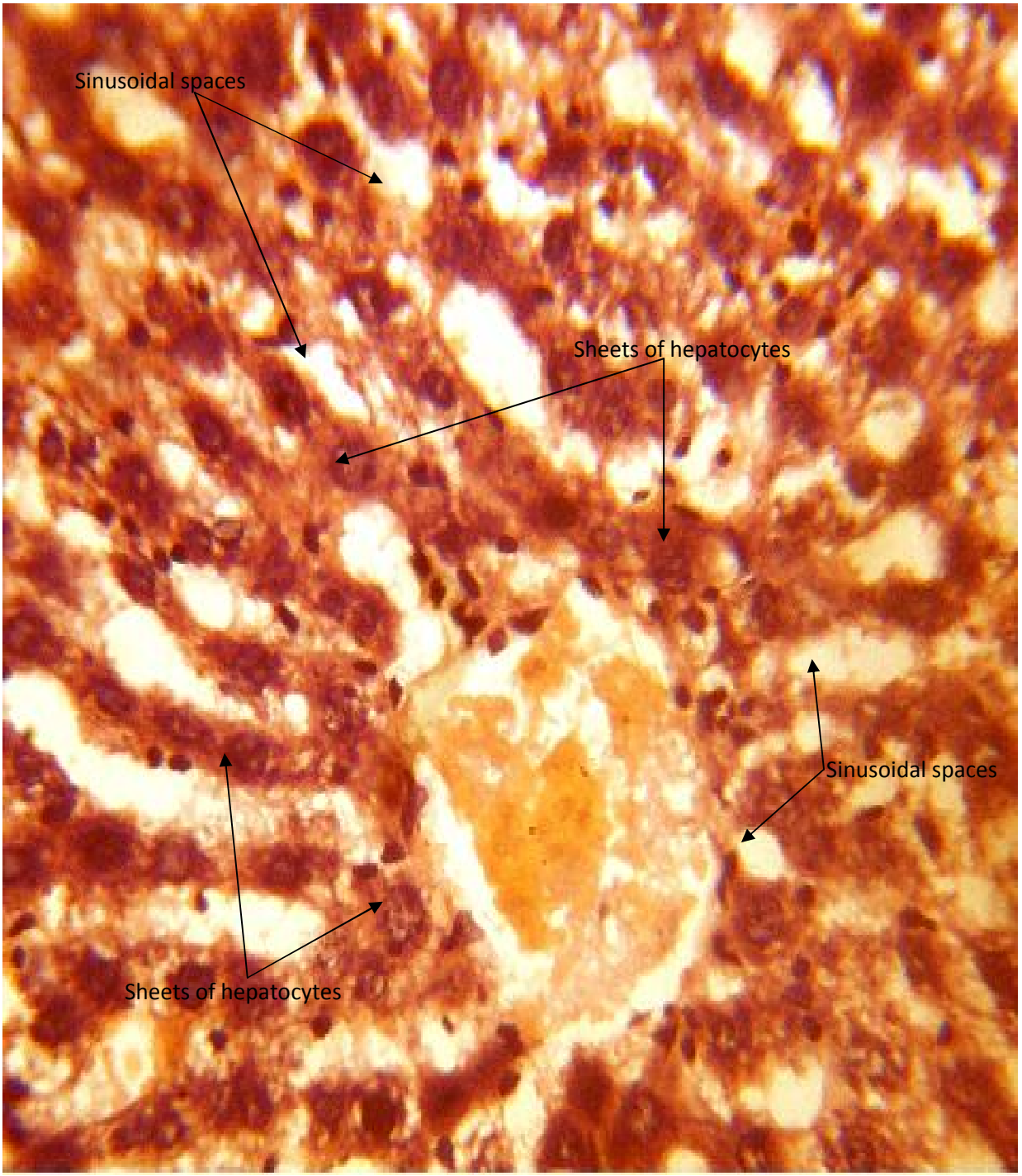

FIG. 4.5: REPRESENTATIVE PHOTOMICROGRAPH OF THE LIVER TISSUES OF THE EXPERIMENTAL RATS OF GROUP E

High magnification (x 400) H & E stained section of the liver of rats fed with 1000mg/kg of pleurotus tuberregium sclerotia sample extract obtained from Anambra State showing uniform sinusoidal spaces and intact hepatocytes.

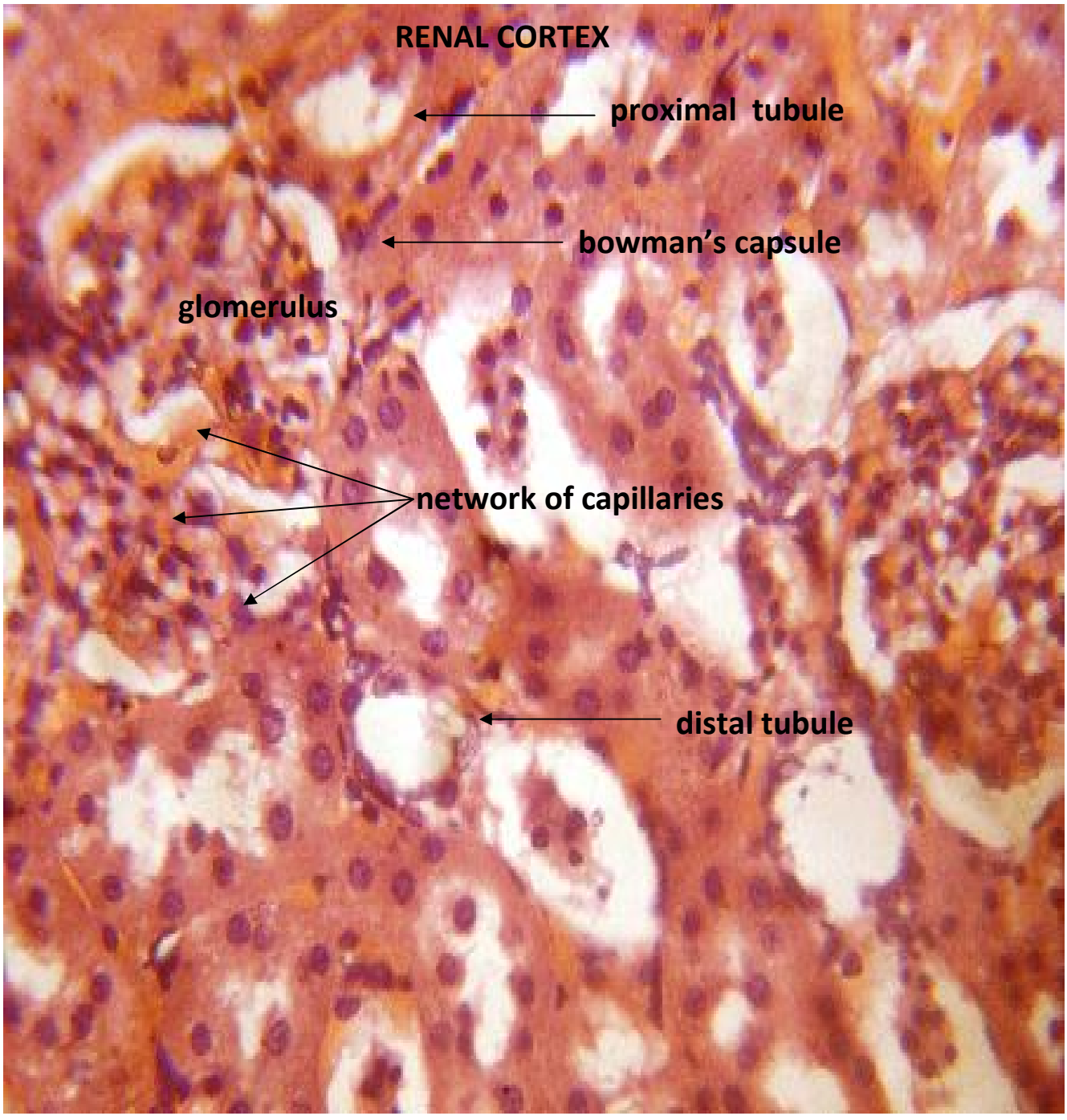

FIG 4.6: REPRESENTATIVE PHOTOMICROGRAPH OF THE KIDNEY TISSUES OF THE EXPERIMENTAL RATS OF GROUP A

High magnification (x 400) H & E stained section through the Renal cortex of rats fed with water and feed only showing organised glomeruli lobules, epithelium lining the capsule and tubules with a relatively regular distinct lumen.

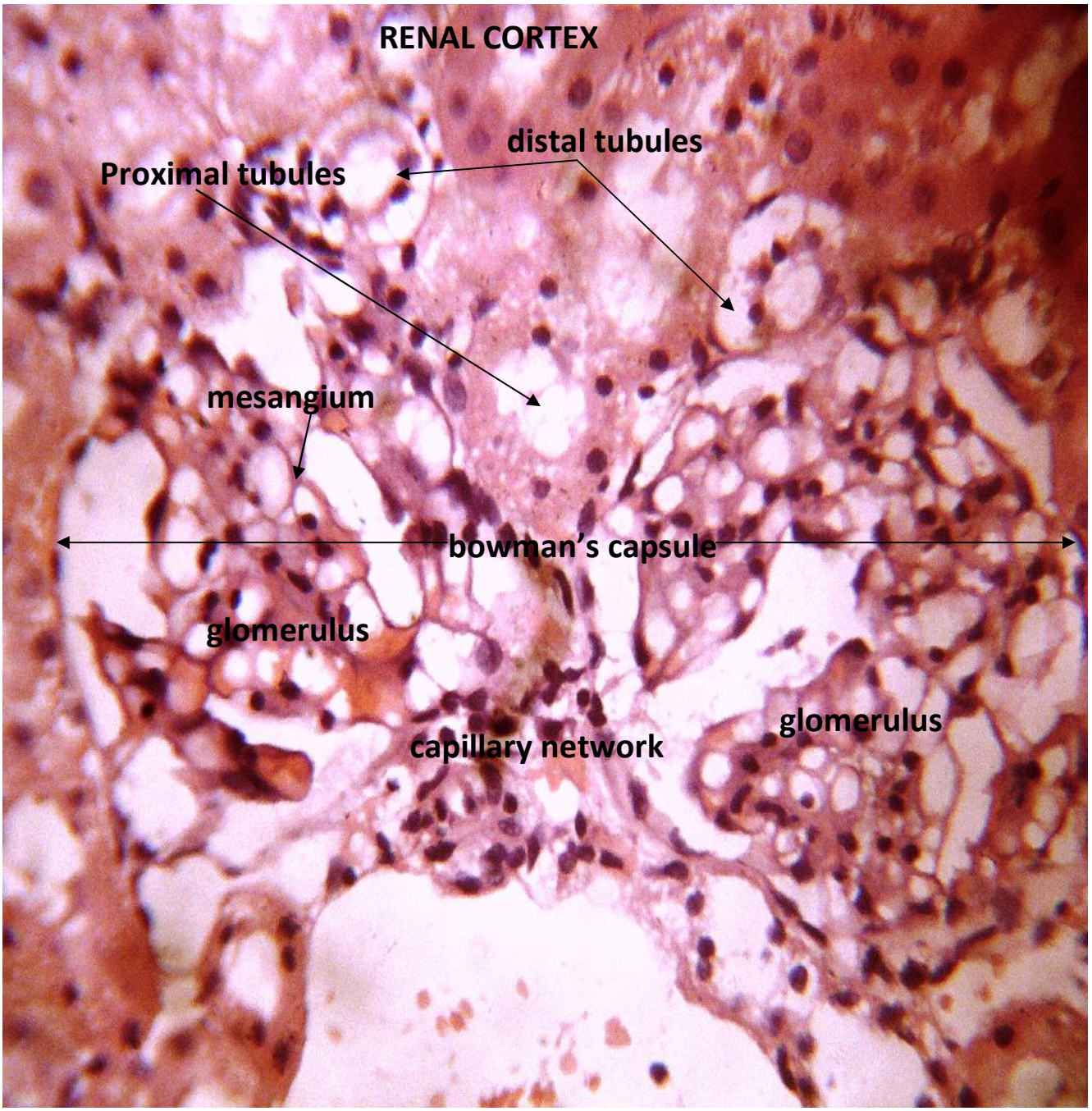

Fig 4.7: REPRESENTATIVE PHOTOMICROGRAPH OF THE KIDNEY TISSUES OF THE EXPERIMENTAL RATS OF GROUP B

High magnification (x 400) H & E stained section through the Renal cortex of rats fed with 1500mg/kg of pleurotus tuberregium sclerotia sample extract obtained from Rivers State showing an organised glomeruli apparatus, intact proximal and distal tubules with regular distinct lumen.

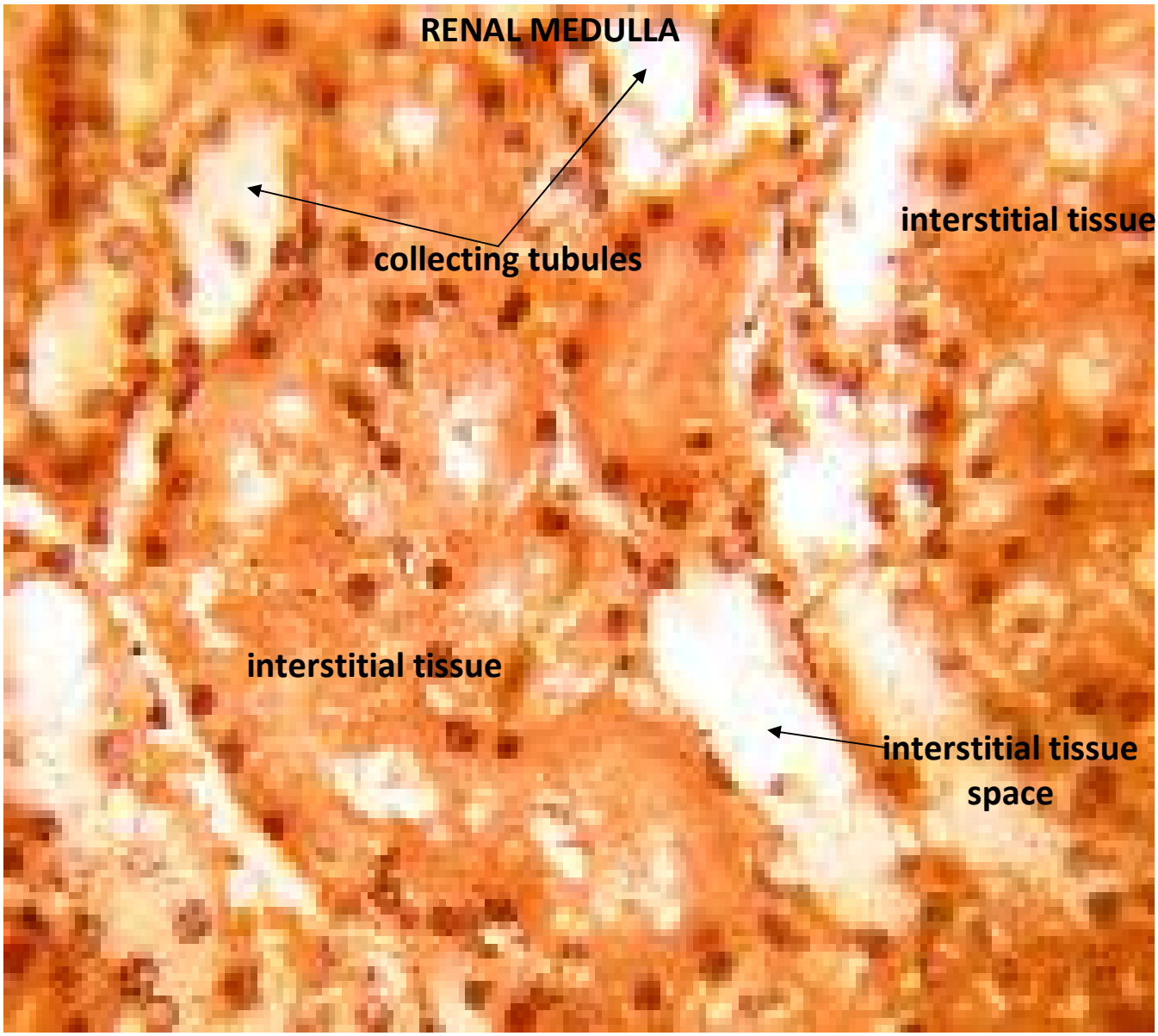

FIG 4.8: REPRESENTATIVE PHOTOMICROGRAPH OF THE KIDNEY TISSUES OF THE EXPERIMENTAL RATS OF GROUP C

High magnification (x 400) H & E stained section through the Renal medulla of rats fed with 1000mg/kg of pleurotus tuberregium sclerotia sample extract obtained from Rivers State showing intact collecting tubules lined with relatively simple cubic epithelium and a small amount of interstitial tissues.

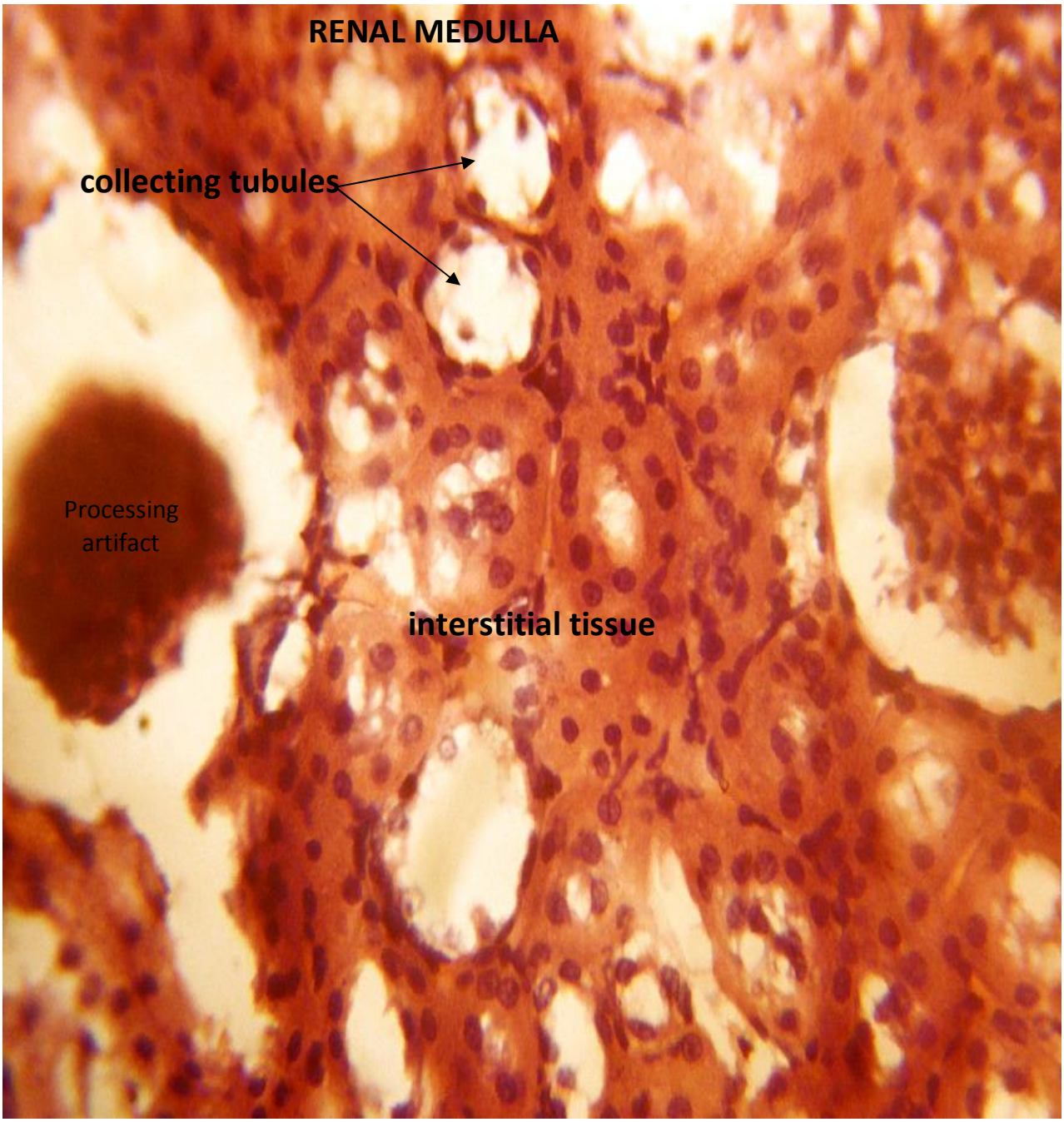

FIG 4.9: REPRESENTATIVE PHOTOMICROGRAPH OF THE KIDNEY TISSUES OF THE EXPERIMENTAL RATS OF GROUP D

High magnification (x 400) H & E stained section through the Renal Medulla of rats fed with 1500mg/kg of pleurotus tuberregium sclerotia sample extract obtained from Anambra State showing organised collecting ducts, capillary network lined by relatively simple cubic epithelium.

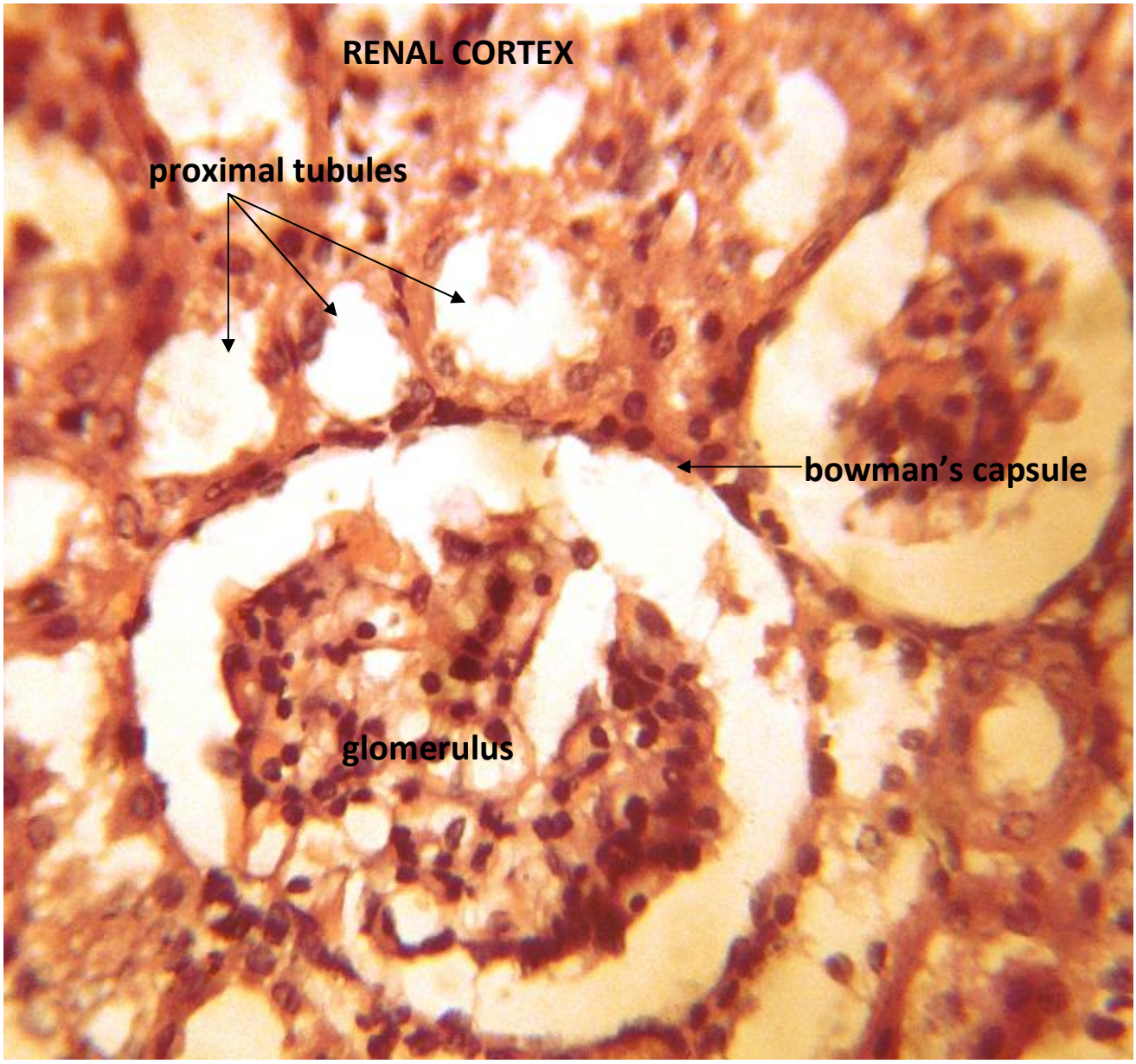

FIG 4.10: REPRESENTATIVE PHOTOMICROGRAPH OF THE KIDNEY TISSUES OF THE EXPERIMENTAL RATS OF GROUP E

High magnification (x 400) H & E stained section through the renal medulla of rats fed with 1000mg/kg of pleurotus tuberregium sclerotia sample extract obtained from Anambra State showing organised glomeruli lobules, epithelium lining the capsules and tubules with a relatively regular distinct lumen.

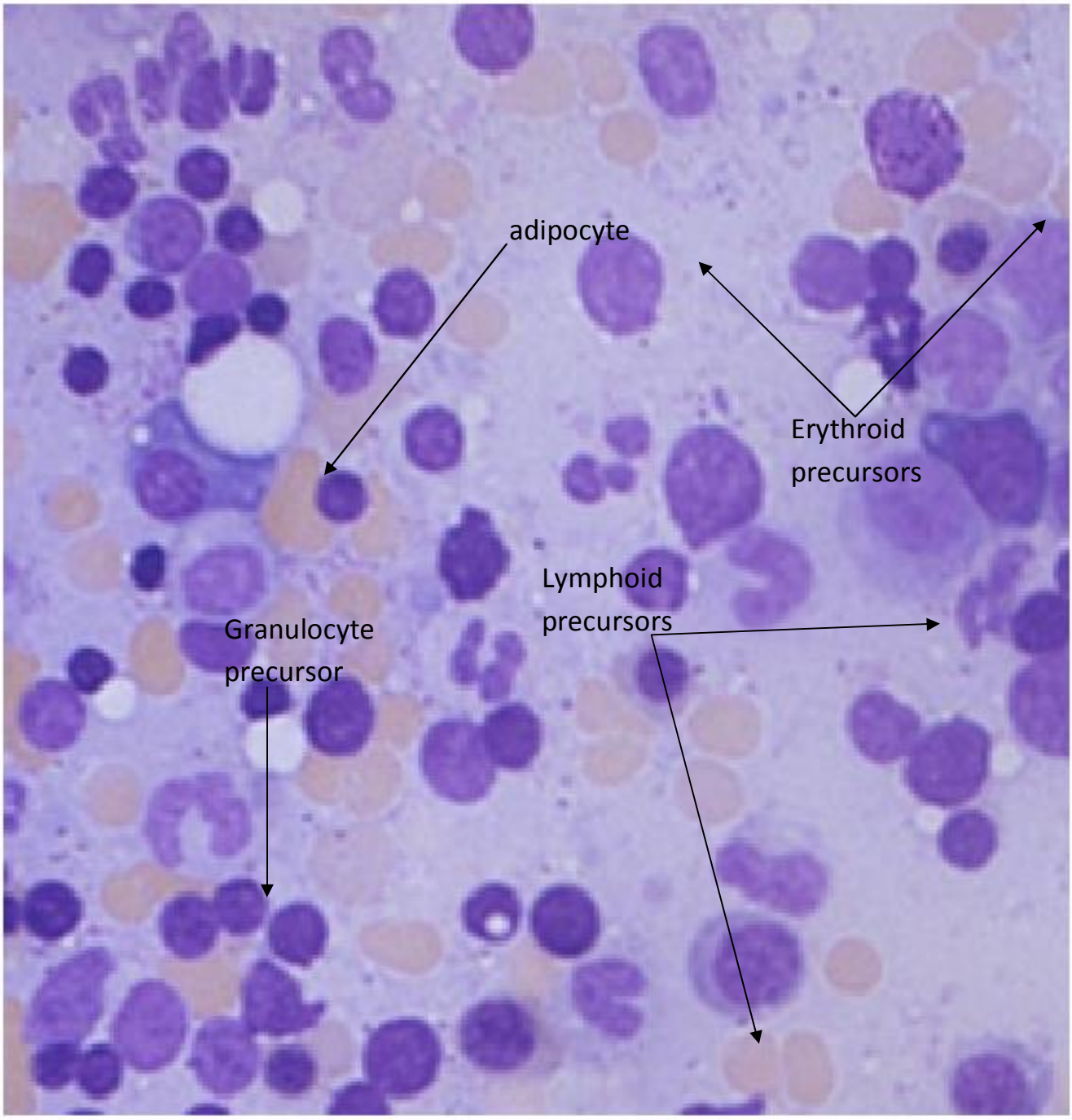

FIG 4.11: REPRESENTATIVE BONE MARROW MICROGRAPH OF THE EXPERIMENTAL RATS IN GROUP A

High magnification (x 400) Lieshman stained bone marrow of rats fed with water and feed only showing even distribution of hematopoietic cells admixed with adipocytes and little marrow islands.

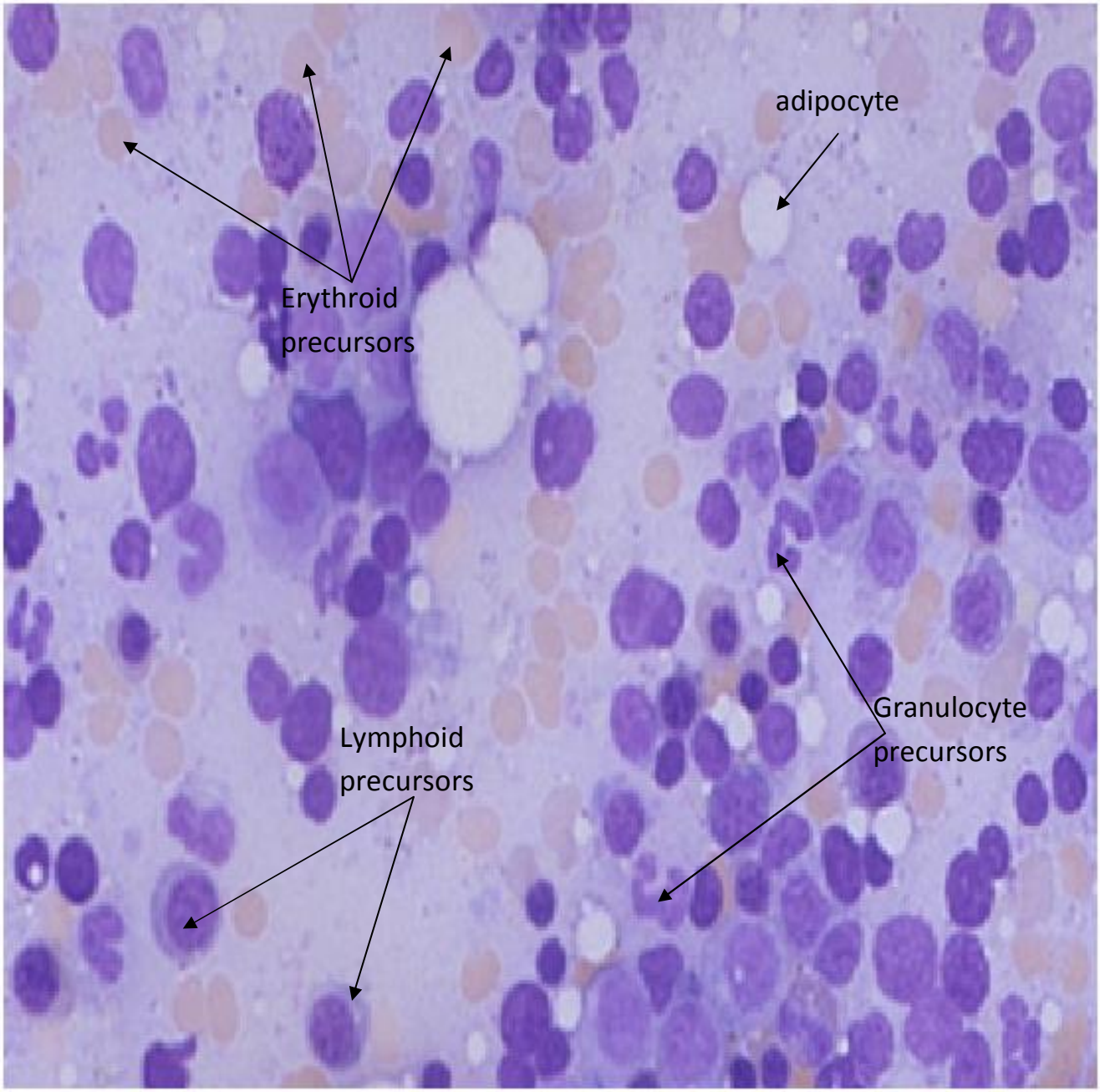

FIG 4.12: REPRESENTATIVE BONE MARROW MICROGRAPH OF THE EXPERIMENTAL RATS IN GROUP B

High magnification (x 400) Lieshman stained bone marrow of rats fed with 1500mg/kg of pleurotus tuberregium sclerotia sample extract obtained from Rivers State showing regular dispersal of hematopietic cells with distinct cell lineages and little islands admized with adipocytes.

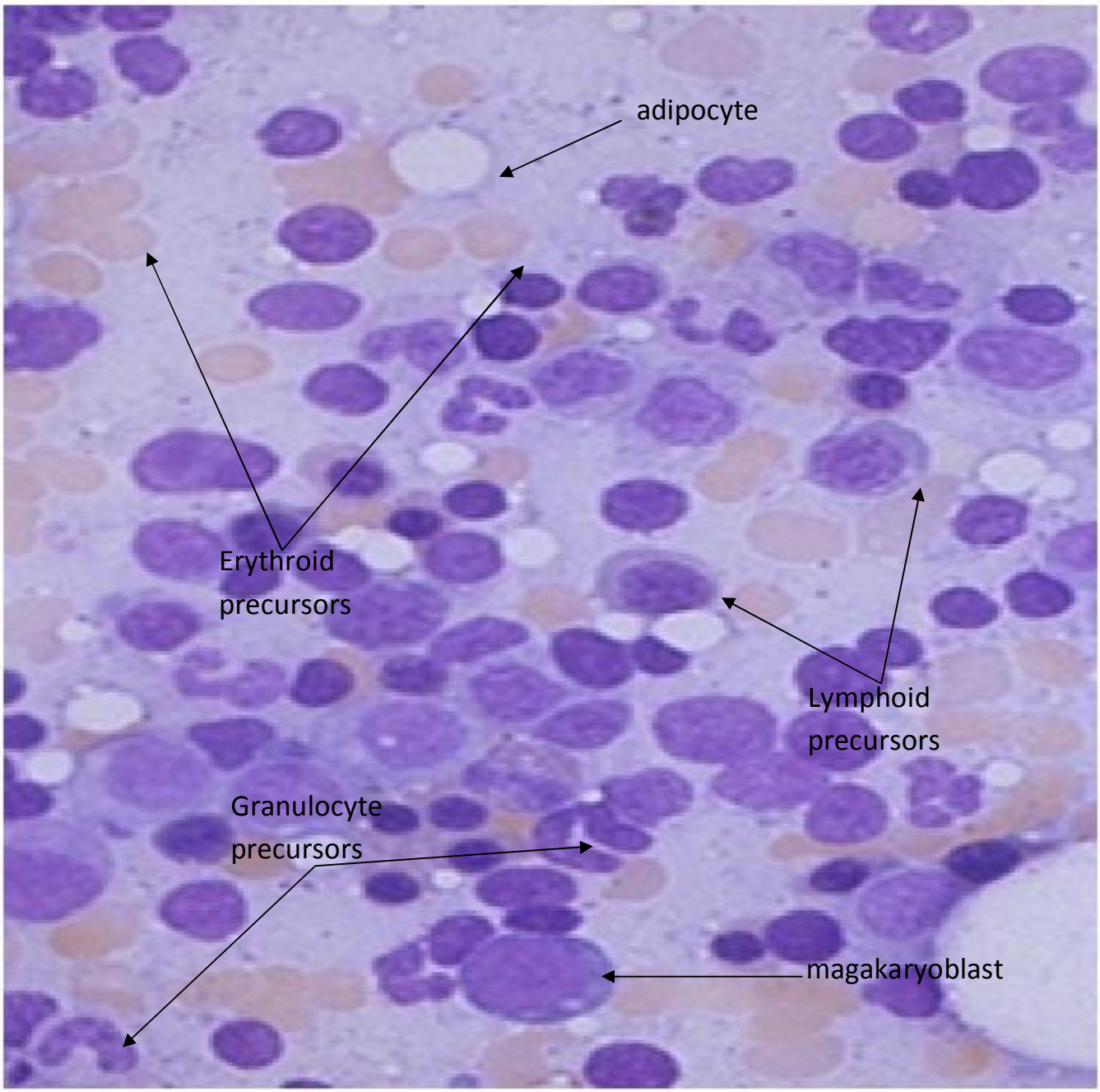

FIG 4.13: REPRESENTATIVE BONE MARROW MICROGRAPH OF THE EXPERIMENTAL RATS IN GROUP C

High magnification (x 400) Lieshman stained bone marrow of rats fed with 1000mg/kg of pleurotus tuberregium sclerotia sample extract obtained from Rivers State showing regular dispersal of hematopietic cells with distinct cell lineages and little islands admized with adipocytes.

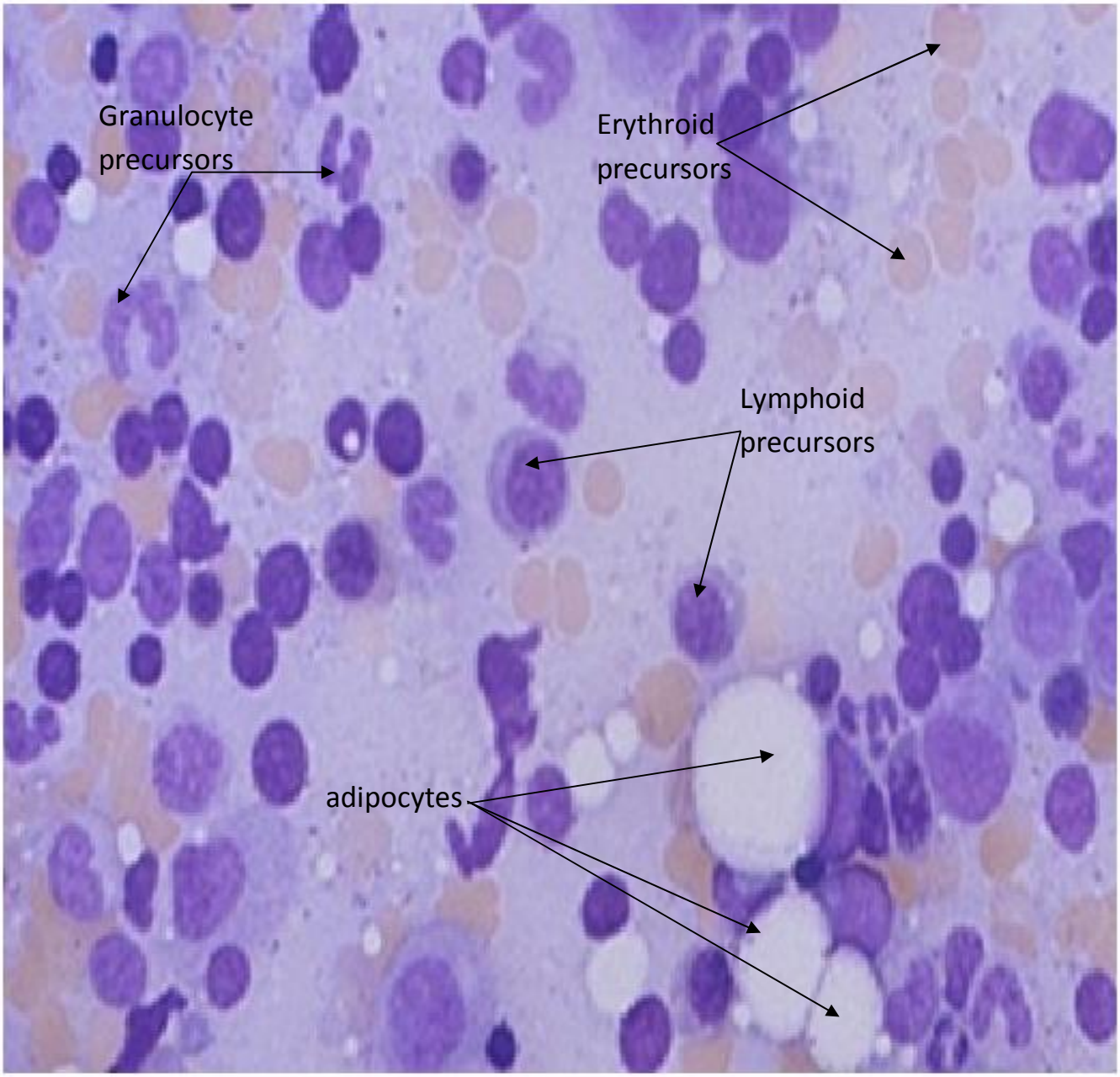

FIG 4.14: REPRESENTATIVE BONE MARROW MICROGRAPH OF THE EXPERIMENTAL RATS IN GROUP D

High magnification (x 400) Lieshman stained bone marrow of rats fed with 1500mg/kg of pleurotus tuberregium sclerotia sample extract obtained from Anambra State showing uniform distribution of hematopoietic cells admized with adipocytes and little marrow islands.

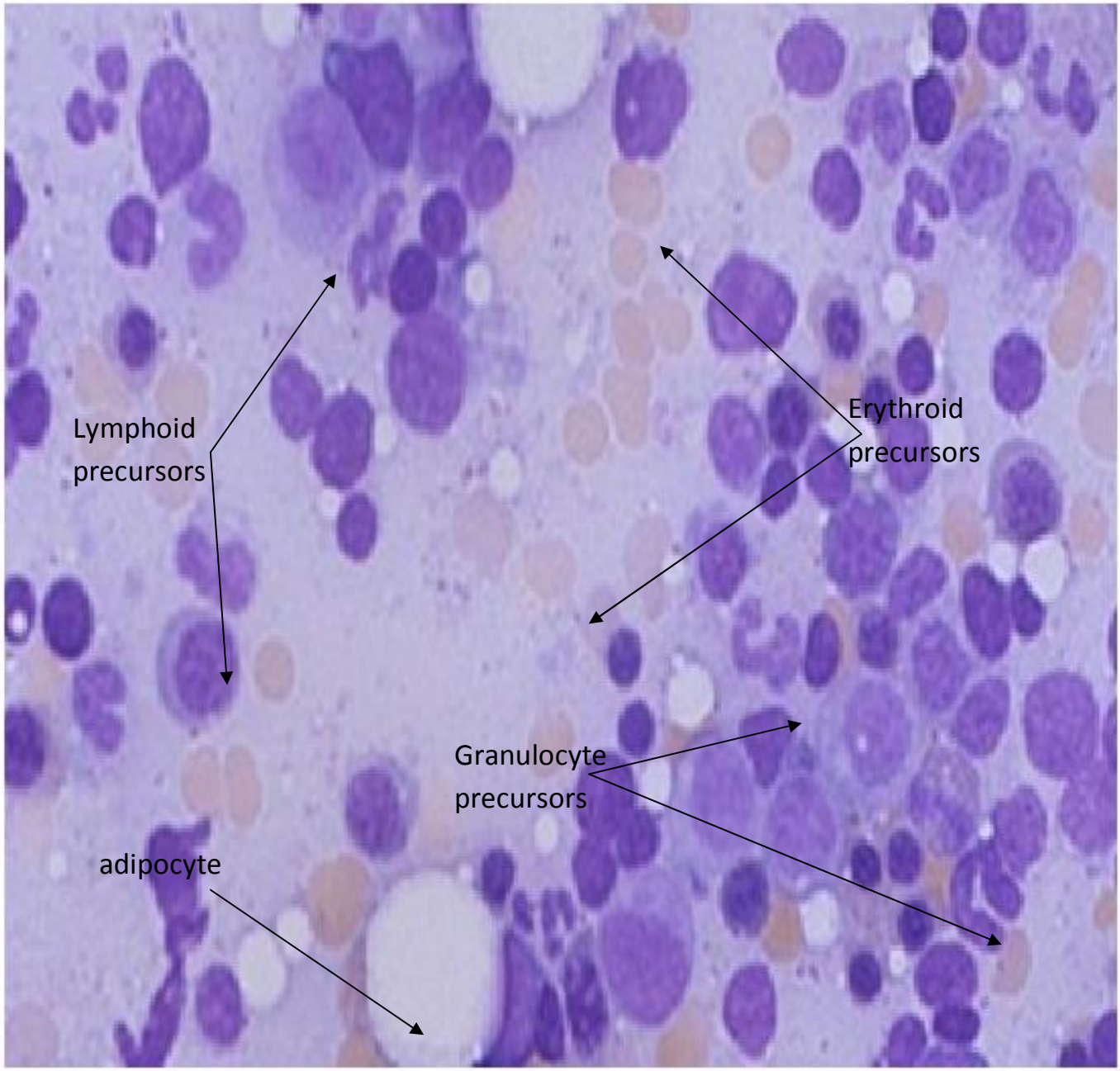

FIG4.15: REPRESENTATIVE BONE MARROW MICROGRAPH OF THE EXPERIMENTAL RATS IN GROUP D

High magnification (x 400) Lieshman stained bone marrow of rats fed with 1000mg/kg of pleurotus tuberregium sclerotia sample extract obtained from Anambra State showing uniform distribution of hematopoietic cells admized with adipocytes and little marrow islands.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.0 DISCUSSION

The major parts of dietary nutrients in the tropics are derived from plant products (Ihekoronye and Ngoddy, 1985). Analysis of the fresh sclerotium of *P. tuberregium* samples of this study revealed low presence of oils, tannins, moderate presence of glycosides, flavonoids and an abundance of proteins, alkaloids and saponnins. This suggests that if consumed in sufficient quantity, the sclerotium of *P. tuberregium* could greatly contribute towards meeting human nutritional requirements for normal growth and provide good protection against diseases arising from malnutrition.

The LD_{50} of *pleurotus tuberregium* sclerotia samples from this study was established to be within the safe limit as described by Dubors and Gelling (1959). It was observed that increasing the doses of the aqueous extract up to 5000mg/kg orally was not lethal as none of the test animals showed any sign of mortality an indication that the extract is safe. According to Osagie (1998), a major limiting factor to the use of many tropical plants as food is the ubiquitous occurrence in them of a wide range of substances capable of precipitating deleterious effects in man and animals. Jakimska et al (2011) also reported that it is the diet of an animal that dictates its accumulation of metals in its tissues.

A direct heavy metal analysis on the two different wild samples of *P. tuberregium* sclerotia of this study; one obtained from Anambra State, a part of Nigeria relatively free from oil mining activities and the other from Rivers State, a part of Nigeria rich in oil and oil mining activities revealed the presence of Lead, Mercury, Aluminum, Platinum, Cadminum, Arsenic and Nickel in each of the samples but there was no statistically significant differences ($p > 0.05$) between the concentration of metals present in the two samples. This is in agreement with the findings of Stigter et al (2000) who showed that although the presence of heavy metals in some environments has been attributed to petroleum prospecting and mining as well as oil spills, their concentrations in crude oil are generally lower than reported.

This study further detected the presence of these metals in the serum of the test rats due to the consumption of aqueous extracts of the samples. In this analysis, metals which were earlier detected directly on the samples were also detected in the serum of some of the test rats. This result seems to be in accordance with a previous study by Shannon et al

(2008) who observed that single nucleotide polymorphisms (SNPs) affects individuals risk with exposure to heavy metals and their accumulation in the body. Thus, those free from polymorphisms has been found to detoxify metals easily and to be less genetically susceptible to their accumulation and toxicological effects, though this in the case of our present study requires actual genetic investigation to substantiate such a claim.

Plumlee(2004) stated that metals are stored primarily in soft tissues (brain, spleen, lung, liver and kidney), bone and teeth after their ingestion and absorption showing that SNPs could affect their rate of absorption into these tissues and thus possible detection in serum. The presence of these metals in the serum of the test rats after steady oral intake of samples of *P. tuberregium* sclerotia necessitates public awareness. In food, the tolerable amount of heavy metals vary from country to country but based on the WHO recommendations and the local policies. According to the 2007 Indian pharmacopoeia guidelines for permissible limits of heavy metals, the permissible limits of Arsenic, Cadmium, Lead and Mercury are 0.02, 0.06, 0.1 and 0.01mg/L respectively (Reena et al, 2011). In our present study, the respective mean concentrations of the metals as detected in the serum samples of our test rats are 0.04, 0.03, 0.02 and 0.02mg/L. These are critical values when compared against this approved limits suggesting that a life long consumption of *P. tuberregium* may pose a toxicological risk due to the ubiquitous occurrence of metals in our environment, non biodegradable nature of metals and their bioacummulative potentials and considering its use as a common vegetable soup thickener in our daily diets.

There was no statistically significant difference recorded between the serum levels of metals in test rats treated with the same dose of the different samples except for Group B in which Cadmium recorded higher concentrations of (0.057 ± 0.006) as compared to Group D (0.010 ± 0.010) and test Group E in which Nickel recorded (0.055 ± 0.021) as compared to Group C ((0.000 ± 0.000). Since these were not observed in the previous direct metal analysis on the samples, these differences could again be attributed to the effect of genetic differences in individual's risks of exposure to heavy metals as has earlier observed by Shannon et al (2008).

Accurate determination of haematological parameters provides a sensitive and reliable physiological indices on the assessment of toxicological effect of any substance to the blood. The plant extracts from River State and Anambra States were compared in order to observe if their constituents of heavy metals would influence the haematological parameters of our study rats. The results of this comparison which showed no significant differences ($p>0.05$) reveals that contrary to popularly held belief, environmental

influences especially oil drilling may have no effect on the haematological parameters of the plant, *pleurotus tuberregium*. This maybe as a result of the similarity in the phytochemical constituents of the two extracts. Also from the results of our analysis, even though there was a general increase in the haematological parameters there was no statistically significant difference in the mean concentrations of parameters in all the study groups. This may be due to short duration of our study or doses of the extracts administered.

According to Lund (2000), the bone marrow is an important potential target organ of chemical exposure. While the haematological parameters provides information regarding possible toxic effects elicited in the peripheral blood, morphological evaluation of bone marrow provides information about haematological features that otherwise would have been missed by peripheral blood parameters alone. The results of the haematological parameters of this study were therefore supported by a bone marrow film evaluation which revealed an even dispersal of hematopoietic cells admized with adipocytes characteristics of a normal marrow micrograph in all the study groups. This is an indication that the extract could not produce significant toxicological effects on the rats within this period and doses.

According to Moslen (1996), the liver is the first major organ to be exposed to ingested toxins, due to its portal blood supply. Toxins maybe as well partially removed from the circulation during the first pass through the liver, providing protection to other visceral organs such as the kidney while increasing the likelihood of liver injury. The kidneys on the other are major excretory organ, as a result healthy functioning of the kidneys provides an assessment of the health status of an organism. The results of our biochemical analysis which showed no statistically significant differences ($P > 0.05$) in ALP, ALT, AST, Total Bilirubin, Direct Bilirubin, Creatinine, Urea, Sodium, Potassium and Chloride in all the study groups is indicative that the extract could not establish a toxicological effect on the liver and kidney of the rats. This was supported by the histological study of the liver and kidney which showed normal histoarchitectures both in the control and test rats; the liver cells having a distinct cell outline with the cells radiating from a central vein. The nuclei stained deeply basophilia while the cells cytoplasm stained deeply eosinophilia and hepatic sinusoids running between two sheets of liver cells. The kidney of the rats maintained normal tissue parenchyma, normal framework of glomeruli, urinary spaces, tubules and vacuoles; characteristics of their normal histoarchitecture. Both hepatotoxicity and nephrotoxicity are known to occur in persons with exposure to heavy

metals. Muna and Aticka (2009) demonstrated necrosis, vacuolation, fatty degeneration, congestion within central veins, hemorrhage between hepatic cords and infiltration of inflammatory cells in the liver of albino mice exposed to metal toxicity. They also detected necrosis of tubules, hemorrhage in the interstitial tissue with enlargement of epithelial layer lining tubules and focal inflammatory cells infiltration in the kidneys of the mice.

5.1 CONCLUSION

The results obtained within this duration of study and doses administered are in accordance with the safety claims on the use of *p. tuberregium* scelerotia as food or medicine.

5.2 RECOMMENDATIONS

1. That further studies is needed to ascertain whether a chronic consumption of *P. tuberregium sclerotia* is associated with toxicity and/or illness in rats.

2. That molecular diagnostic techniques such as PCR is needed to detect some genetic susceptibility factors such as the presence of SNPs in rat fed with *p. tuberregium sclerotia* and it's effect on heavy metal bioaccumulation in their serum.

3. That samples of *p. tuberregium sclerotia* should be removed off heavy metals prior to human consumption as food or medicine.

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ANALYSIS OF HEAVY METALS IN PLEUROTUS TUBERREGIUM SCLEROTIA, TOXICITY IN BLOOD, BONEMARROW AND SOME SELECTED ORGANS OF ALBINO RATS

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DEDICATION

This work is dedicated to the loving memories of late Pa Ogbuabor Alphonsus.

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Ogbuabor Alphonsus Ogbonna

ABSTRACT

Pleurotus tuberregium is a common mushroom which is used as food or medicine, more commonly as a vegetable soup thickener. This study investigated the presence of heavy metals in wild samples of *pleurotus tuberregium* sclerotia consumed within our localities, compared the degree of heavy metal contamination of the samples, investigated the presence of heavy metals in the serum of albino rats due to its consumption and the effects of its consumption on blood, bone marrow, liver and kidney of the rats. Two wild samples of *pleurotus tuberregium* sclerotia were collected from Bomu village in Gokana Local Government Area of Rivers State a part of Nigeria rich in oil and mining activities and Agbudu in Orumba South Local Government Area of Anambra State a part of Nigeria relatively low in oil and oil mining activities. The samples were authenticated at the Department of Plants and biotechnological Sciences, University of Nigeria, Nsukka. A phytochemical screening of the samples was carried out as described by Trease and Evans. Samples were analyzed for the presence of heavy metals using an Atomic Absorption Spectrophotometer (AAS 240FS Varian) at the Springboard Research Laboratories, Awka, Anambra State. A crude aqueous extract of the samples was prepared. A total of 37 male albino rats 6-7 weeks old weighing 200g $\pm$ 20g were used for a protocol involving the oral acute toxicity and sub-chronic toxicity of the crude aqueous extract of the samples. The oral acute toxicity of the extract was determined using 12 of the rats according to the procedure for the two phases testing as described by Lorke. A repeated 14-days dosing oral toxicity was then applied for the sub-chronic study. The remaining 25 out of the 37 rats were divided into five groups (A – E) of five rats each. After a previous 14days period of acclimatization, Group A were fed with water and feed only and served as the control, Group B received 1500mg/kg of the sample extract from Rivers State, Group C received 1000mg/kg of the same sample extract while Groups D and E received 1500mg/kg and 1000mg/kg respectively of sample extract from Anambra State. Whole blood samples were analyzed for haematological profile using the fully automated analyser Mind Ray, Japan while serum was analyzed for biochemical parameters involving the liver and kidney indices using Boehringer knoll chemistry semi-automated analyzer 4010 system. The liver and kidney tissues were examined for histological changes and the bone marrow aspirates examined for effects of metal toxicity as well. Data was analyzed using SPSS version 17.0 and results expressed as mean $\pm$ standard error of the mean. Results were considered significant at $P<0.05$. Phytochemical screening of the samples revealed low presence of oils, tannins, moderate presence of glycosides, flavonoids, an abundance of proteins, alkaloids and saponnins. The crude aqueous extract was found to be non-toxic up to 5000mg/kg. Lead, mercury, aluminum, platinium, cadmium, arsenic and nickel were detected in both samples but there were no statistically significant differences ($P > 0.05$) between concentrations of metals. These metals were also detected in the serum of some of the test rats. There were no statistically significant difference ($P > 0.05$) in the serum concentrations of metals in test rats treated with the same doses of the different sample extracts except for the test Group B in which cadmium recorded high concentrations of (0.057 ± 0.006) as compared to Group D (0.010 ± 0.010) and test Group E in which Nickel recorded (0.055 ± 0.021) as compared to Group C (0.000 ± 0.000). There were no statistically significance differences ($p>0.05$) in the haematological parameters in all the groups and as compared to the controls . There were no statistically significant differences ($p>0.05$) in the biochemical parameters of the liver and kidneys in all the groups and as compared to the controls. The bone marrow aspirate revealed normal micrograph in all the groups while the liver and kidneys maintained their normal histoarchitectures. These result patterns are in accordance with the safety claims on the use of *p. tuberregium* sclerotia as food or medicine.

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