EFFECTS OF ADMINISTRATION OF A MULTI-HERBAL EXTRACT 'AGBO IBA' ON THE LIVER AND KIDNEY OF PLASMODIUM BERGHEI INDUCED JUVENILE WISTAR RATS

BY

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SUPERVISOR

PROFESSOR GERALD IKECHI EZE

MAY, 2019

DECLARATION

I declare that this research work is an original work. It has not been presented to any examining body.

……..
Odigie Andrew

APPROVAL

This is to certify that this work is approved in fulfillment of the requirement for the award of Masters of Science (M.Sc) degree in Anatomy, in the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city, Edo state, Nigeria.

…….
Date

DEDICATION

To God, the Author and finisher.

ACKNOWLEDGEMENTS

This work was borne out of the need to create awareness of the possible dangers / usefulness of some common local traditional beliefs prevalent in our society today, and to bring quality health care to the door step of the less privileged**.**

I hereby appreciate the following persons; Late Professor Dorothea Baxter Grillo (Professor of Anatomy); who was always there for me, reviewing my work at different stages.

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I want to also thank my lovely wife and children for their support during the course of this work.

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LIST OF ABBREVIATIONS

AOT	-	ACUTE ORAL TOXICITY
ACT	-	ARTEMISININ COMBINATION THERAPY
CAM	-	COMPLIMENTRY & ALTERNATIVE MEDICINE
CDC	-	CENTER FOR DISEASE CONTROL
E,U&CR	-	ELECTROLYTES, UREA &CREATININ
EDTA	-	ETHYLENE DIAMENE TETRA-ACETIC ACID
FECL3	-	FERRIC CHLORIDE
HCL	-	HYDROCHLORIDE
H2O	-	WATER
IAMRT	-	INSTITUTE OF ADVANCED MEDICAL RESEARCH & TRAINING
KFT	-	KIDNEY FUNCTION TEST
KG	-	KILOGRAM
LT	-	LITHIUM HEPARIN
LFT	-	LIVER FUNCTION TEST
LD50	-	MEDIAN LETHAL DOSE
MDR	-	MULTI DRUG RESISTANT
MG	-	MILLIGRAM
NAOH	-	SODIUM HYDROXIDE

PHC - PRIMARY HEALTH CARE

P-BERGHEI - PLASMODIUM BERGHEI

P-CYNOMOLGI- PLASMODIUM CYNOMOLGI

P-CHABAUDI- PLASMODIUM CHABAUDI

P-FALCIPALUM- PLASMODIUM FALCIPALUM.

P-KNOWLESI- PLASMODIUM KNOWLESI

P-MALARIAE- PLASMODIUM MALARIAE

P-OVALE - PLASMODIUM OVALE

P-VINCKEI - PLASMODIUM VINCKEI

P-VIVAX - PLASMODIUM VIVAX

P-YOELII - PLASMODIUM YOELII

STI - SEXUAL TRANSMITTED INFECTION.

SON - STANDARD ORGANIZATION OF NIGERIA

STZ - STREPTOZOTOCIN

UBTH - UNIVERSITY OF BENIN TEACHING HOSPITAL

WHO - WORLD HEALTH ORGANIZATION

ABSTRACT

BACKGROUND – Malaria is still a huge problem at the moment. Given the growing resistance to orthodox drug, herbal extracts have plummeted in Nigeria. Vulnerable groups like under 5's are most affected. Interestingly, end organ damage has also been on the increase. Hence, these concerns prompted this study.

AIM- The study aimed to determine the therapeutic and histo-morphological effects of administration of "Agbo iba" multi-herbal extract.

METHODOLOGY – 42 Juvenile Wistar rats of different sexes, weighing an average of 97.5g, aged between 6-7weeks were assigned into 6 groups of 7 rats each(n=7). Phytochemical analysis was done on the extract as well as estimation of the LD50 prior to the study

Group l was the negative control given only feeds and water, Group 2 (untreated group), 3, 4, 5 and 6 were the treated groups which were induced with Plasmodium berghei by injecting 0.2mls of diluted parasitized red blood cells intraperitoneally to the animals in these groups prior to treatment. Group 3(standard drug group), was treated thereafter with 0.6mls (6.72mg) of the constituted ACT twice daily for 3 days, while Group 4, 5 and 6(low, moderate and high dose groups), were given low dose (0.2ml), moderate dose (0.4ml) and high dose (0.6ml) of the extract respectively for one week. The rats were sacrificed at the end of the experiment and blood serum was obtained for microbiological and biochemical assay. The kidneys and liver were excised, weighed and fixed in 10% formol saline and prepared for light microscopy using the staining method for H & E. Data was presented as figures and tables, and subjected to statistical analysis.

RESULT-The standard drug group had a 90% clearance of parasiteamia compared to the herbal drugs with a clearance ranging from 60 to 85%.

The results showed a decrease in ALT which was statistically significant ($p< 0.05$) in the untreated group compared to the control and treated group .The result also showed a statistically significant

increase ($p < 0.05$) of ALT in the group with higher doses of the extract compared to the control. Similarly, serum AST was significantly decreased in the standard drug and low dose groups and bilirubin was significantly decreased across the six groups, when compared to the control($p<0.05$). Furthermore serum urea was significantly increased in the high dose group. When compared to the control ($p<0.05$).The untreated group had a significantly increased liver weight compared to the control.

Histologically group l (control) had normal findings, group 2(untreated group) showed histo-pathological changes in the liver which revealed marked sinusoidal congestion, peri-portal infiltrates and hemozoin pigments in malaria infection which reduced to different degrees in group 3,4,5 and 6 on treatment with the standard drug and increasing concentrations of the herbal drug extract respectively, though not dose dependent.

CONCLUSION- The 'Agbo iba'marketed in Benin City has some anti-plasmodial activity that was somewhat comparable to the orthodox drug though not dose dependent and not as effective as the orthodox drug. There was however no significant damage to the vital organs with use of the herbal drug.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Malaria origin dates back to a prehistoric period in Africa, before the origin of apes (Poinar *et al*., 2019).The findings by Charles Louis Alphonse Laveran, a French Army Surgeon in Algeria in 1880 resulted in a revolutionary change that earned him the award of the Noble Prize, in 1907(Nye, 2002).

Interestingly scientific alterations in interactions by humans posit that about 6,000 years ago, there was an upsurge in Plasmodium falciparum(P.falciparum) population globally, as well as in Africa, which concurrently occurred with human population growth and travel, eased by agriculture (Joy *et al*., 2003).

Though Africa is a good market for a variety of orthodox drugs, the menace of fake and substandard drugs, wrong information, as well as low socio-economic status, has culminated in the use of herbal medicines (Kloucek *et al*., 2005).

Admittedly majority of Nigerians today consume local herbs like "Agbo" ; which originated from the western part of Nigeria and contain biologically active substances derived from plants or plant parts in the natural or processed form (Oladeji, 2016). Today "Agbo" is prepared across Nigeria in varieties of ways.

Primarily vital organs like the kidney and liver which function to remove waste products and detoxify compounds respectively may invariably be damaged by ingesting harmful substances. Experimental animal models have proved fundamental in appreciating the safety and mechanism of action of drugs in humans, as they represent a crucial step in the study of diseases and treatment modalities (De Mets *et al*., 2010).

1.2 JUSTIFICATION OF THE STUDY

Malaria is still a huge challenge at the moment. This can be seen from both a health and economic perspective. The exponential increase in multi-drug resistance to anti-malarial drugs poses a threat to quality and affordable care, with significant impact on pregnant women and under 5's who are considered as vulnerable groups. The peculiar economy of developing nations even further aggravates the problem. These have increasingly resulted in consumption of local herbs.

Studies revealing herbal toxicity are scanty, although studies carried out by Artimani *et al* (2017) and Alaribe *et al* (2019) showed potential adverse effects.

It is therefore paramount to determine the therapeutic and harmful effects of consumption of "Agbo-Iba", for malaria fever particularly in pregnant women and under 5's.

1.3 STATEMENT OF THE PROBLEM

In 2006 the prevalence of malaria among 3.3 billion people was about 247 million, with a mortality of about a million deaths mostly in under 5's (GBD, 2015;GDHS, 2014). Despite the billions of naira spent in combating malaria, the impact is still very significant in Africa, especially among under 5's (Afroakwah *et al*., 2018). In affected regions like Nigeria, pregnant women and children are most susceptible to malaria attacks, as adults may develop a degree of immunity through continuous exposure (Doolan *et al*., 2009).

Surprisingly in recent times, the use of herbal drugs have risen in Africa, particularly in Nigeria and local vendors of these medicines are seen in different nooks and corners of the country. Nonetheless, herbal medicines in Nigeria are not regulated, so cases of herbal poisoning and multi-drug resistance cannot be ruled out. It is interesting to note that people believe more on information gotten from friends and relatives rather than well researched facts. To this end, wrong information on herbal medicine is usually propagated. The constituents of these drugs are usually not known and the products are not validated or certified by any recognized body.

1.4 AIM AND OBJECTIVES

AIM: To study the antimalarial potential and toxicity profile of a multi-herbal drug (Agbo Iba), on the kidney and liver of plasmodium induced Juvenile Wistar rats.

OBJEC TIVES

1. To determine the therapeutic effects of the herbal extract on plasmodium induced juvenile Wistar rats.
2. To compare the therapeutic effects of the herbal extract with that of lonart anti-malaria therapy.
3. To assess the liver and kidney toxicity using biochemical markers in 'Agbo iba' treated Wistar rats
4. To assess the effect of the herbal extract and increasing doses of the extract on the morphology and microanatomy of the kidney and liver.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. OVERVIEW

2.1.1. MALARIA

Human malaria has been noted for centuries, and the existence of mosquitoes trapped in amber suggested its earlier occurrence (Borkent and Grimaldi, 2004). Malaria is a parasitic disease caused by parasite (plasmodium) and affects vertebrates (Caraballo and King, 2014).

Malaria infection is a major health challenge in endemic areas (Autino *et al.*, 2012). Transmitted by the bites of the female mosquitoes, the infection results from *Plasmodium* parasites with a complex life cycle which entails stages in the definitive and secondary hosts which are manifold (Greenwood *et al.*, 2008*)*; with five species infecting humans ; P. falciparum (most deadly), P. vivax, P.malariae, P.ovale and recently P. knowlensis (Sato, 2021).

2.2. EPIDEMIOLOGY

Malaria is common in the tropical and subtropical areas, where temperature and rainfall promote vector breeding (Greenwood *et al*.,2008;Aly *et al*.,2009).Different parts of the world contributes in varying degrees; Africa 88%, South-east Asia 10% and eastern Mediterranean region 2%.(WHO,2017).

It affects the very poor and vulnerable groups like under 5, pregnant women, HIV/AIDS patients, as well as people with lowered immunity like migrants and travelers from temperate region (Afoakwah *et al*., 2018).

2.3. TYPES OF PLASMODIUM

Malaria is transmitted by the female anopheles mosquito and affects humans, mammals, birds, as well as reptiles (Table 1). It is grouped into human plasmodium, primate plasmodium, avian plasmodium and reptile plasmodium (Sato, 2021).

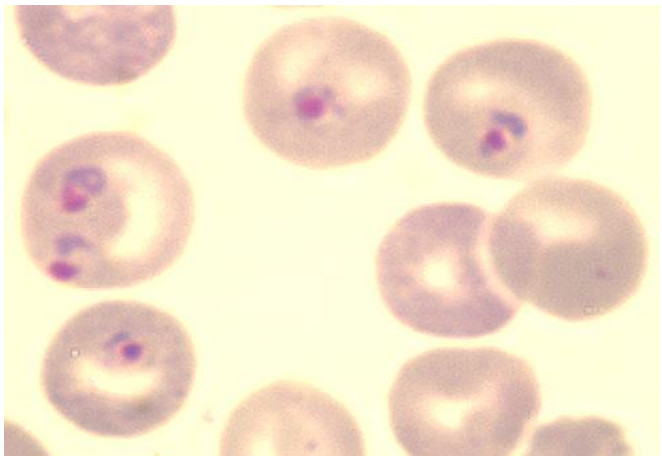

Fig 1: Plasmodium beghei

In human plasmodium, P. falciparum has resulted in the most severe form of malaria. P. ovale, P. vivax, P. malariae and recently P. knowlesi cause less complicated forms, though P. knowlesi can also infect monkeys (Collins, 2012).

Studies on evolution posit that P. falciparum first appeared in avian parasite (Leclerc *et al*., 2004). Similarly other studies suggest a relationship with other parasite such as rodent parasites (Weimin, 2010).

Even though simian malaria parasites such as P. knowlesi or P.cynomolgi are relevant to humans; rodent malaria parasites like P. berghei(fig 1), P. chabaudi, P. vinckei and P. yoelii are more accepted worldwide for research on humans (Dechamps *et al*., 2010).

By contrasting the entire genome of P. falciparum and rodent malaria parasite, Kooij *et al* (2012) discovered an extreme similarity between 85% of the genes from P. falciparum and rodent malaria parasite.

2.4. RODENT PLASMODIUM

Rodent malaria parasite (RMP) is used widely as a prototype of human malaria (Otto *et al*., 2014). It causes malaria in rodents and four rodent plasmodium species that have been described in Africa; P. chabaudi, P. vinckei , P. berghei and P. yoelli. P.yoelli, can be genetically modified and the natural vectors are the anopheles dureni and anopheles stephensi in laboratory situations (Otto *et al*., 2014).

Infection with laboratory mouse strain, used as study model for human malaria and research programs have become popular as this can be used to detect and develop vaccines against malaria (Craig *et al*., 2012). It has been revealed that a number of surface proteins show extreme similarity between rodent and human parasite (Otto *et al*., 2014). The genome of P. berghei has been structurally analyzed and it shows extreme similarity in structure and content with that of humans, and exist in different strains; ANKA, NK65, KSP11, LUKA, SP11and K173 (Craig *et al*., 2012). P. berghei has an organelle that possess a roundish and extra-chromosomal genome (apicoplast genome) about 30kb and a simple pathway that depend on phosphoethanolamine N-methyltransferase and serine decarboxylase activities which redirects host serine to supply more phosphatidyl-choline and phosphatidyl ethanolamine to the parasite just like P. falciparum (Dechamps *et al*., 2010).

About 70-95% of the nucleic acid is similar with the extra-chromosomal genome of P. berghei and 5-25% of P. berghei ANKA parasites are dedicated to sexual development like in humans (Sinha *et al*., 2014).

In contrast to human plasmodium, there is no indication of a dormant hypnozoite stage with P. berghei (Frech and Chen, 2011). The gametocyte morphology of P.berghei and P.falciparum differ, while P. berghei is circular or oval P. falciparum is ruler shaped (Dixon *et al*., 2012). Among the RMP, differences exists as well (Craig *et al*., 2012). This gives the lab mice a specific advantage of being a prototype of human malaria, hence different aspects of human malaria can be investigated (Craig *et al*., 2012).

P.chabaudi is a prototype to study drug resistance-pregnancy associated malaria, cerebral malaria as well as lung pathology; it invades nomocytes and reticulocytes and produce chronic non-lethal infection (Langhorne *et al*., 2008).In contrast, P. berghie infects reticulocyte and produce severe pathology (Craig *et al*., 2012; Lamp *et al*., 2006). P. yoeli studies biology of liver stages (Prudencio *et al*., 2011).

Life Cycle of plasmodium: Plasmodium malaria transmitted by mosquito (definitive host) to the vertebrate (secondary host) during a blood meal appears to be same for all vertebrate malaria (Mace *et al*., 2015). After injection into the dermis, the sporozoite transmitted from the saliva of the mosquito move in any pattern in the dermis until they reach the dermal blood vessels which they infiltrate and , enter into the circulation leaving the inoculation site (*Amino et al., 2006).*Within 8 hours, thousands of sporozoites move quickly to the liver where they multiply in about a week though asexually and transform to tissue schizonts or pre-erythrocytic schizonts and P.ovale and P.vivax produces the dormant hypnozoites. The tissue schizont contains millions of merozoites and when they mature, the infected hepatocytes burst and extrude the merozoites into the blood stream. This then attach to surface receptors on the red blood cell and pierces more red blood cells (Mace *et al*.,, 2015).The merozoites transform to trophozoites within the cell wall and feed on the host red blood cell hemoglobin. The early trophozoite clearly presents as the classic ring forms, seen on light microscopy. The trophozoites expands within the cell membrane to take up most of the red blood cell and after about 1-2 days progress to a second stage of asexual differentiation to form erythrocytic schizont, each containing 12 – 24 merozoites in each infected red blood cell. Uninterrupted asexual differentiation in the blood stream through series of cycles of maturation and rupture of red cells with release of merozoites causes symptomatic infection (fig2).As this occurs, a fraction undergoes sexual differentiation and forms the sexual forms known as gametocytes, which are symptomless but circulate for long period of time. The ingestion of these male and female gametocytes results in sexual reproductive cycle in the mosquito that forms motile sporozoites that transform to a zygote in the mosquito mid-gut (Mace *et al* ., 2015). The zygote differentiates to ookinete, which pierces and encysts in the lining of the intestinal wall of the mosquito. The resulting oocytes expand and burst releasing sporozoites, which then bombards the mosquito's salivary glands to complete the life cycle. During the next blood meal the sporozoites are transmitted back.

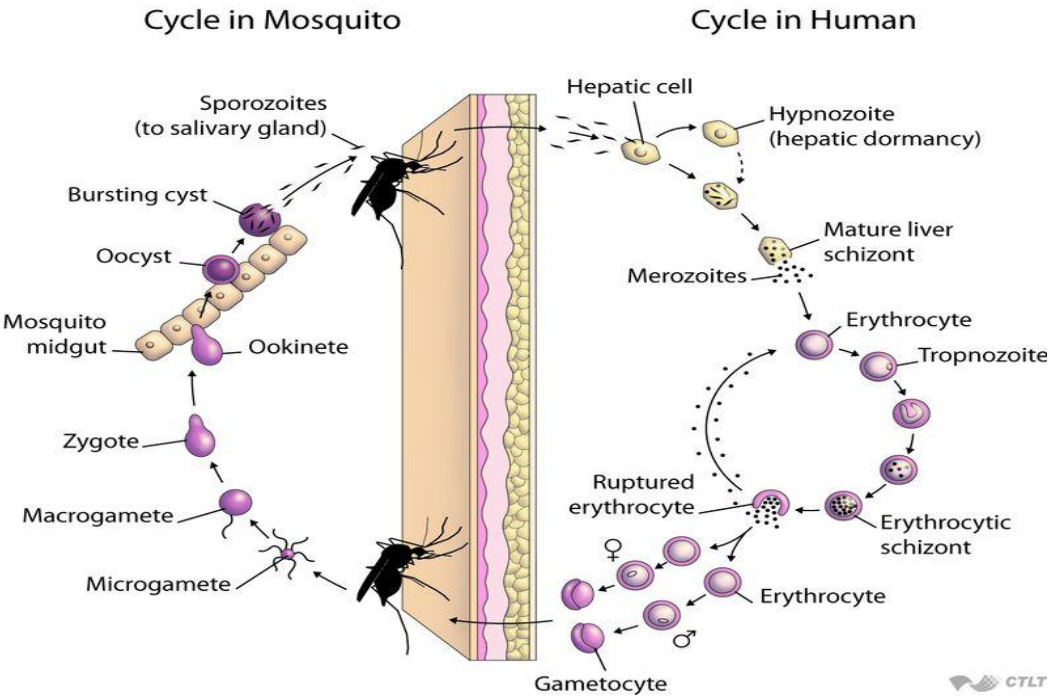

Fig 2: Lifecycle of plasmodium

In P.vivax and P.ovale infections in humans, some of the parasites may remain in the liver as the dormant form or hypnozoite. The dormant period may extend from weeks to months and even years before replication reoccurs. This results in relapses that are typical of P. vivax and P. ovale infections (White, 2011).

Clinical features of malaria fever in humans include; weakness, joint pain, fever, vomiting, headache and when severe jaundice, seizures and coma (Caraballo, 2014; Sabina, 2017).

Complications include anemia, prostration, retinopathy, hypotension, cerebral malaria, haemoglobinuria, respiratory distress, renal failure in black water fever, organomegaly, hypoglyceamia, coagulopaties, hepatopathy, pulmonary edema, acidosis, hyperparasitemia and shock (Sabina, 2017;Okello and Keng, 2019).

Diagnosis is usually by; microscopy of blood film, antigen based rapid diagnostic test or polymerase chain reaction (Krafts *et al*., 2011).

Table 1: Different plasmodium for humans, primates, aves and reptiles.

2.5. TREATMENT OF MALARIA

The major classes of anti-malaria drugs used for treatment include,

4 aminoquinolones: chloroquine,hydroxychloroquine,amodiaquine,pyronaridine.

8 aminoquinolones: primaquine,tafenoquine,bulaquine

Aryl amino alcohols: quinine,quinidine,mefloquine .

Anti-folatecompounds: pyrimethamine,proguanil,chlorproguanil,trimethoprime.

Artemisinincompounds(sesquiterpine-lactones):artheeter,artemeter,artesunate and artemisinin combination therapy(ACTs).

Amino alcohols: halofantrine,lumefantrine

Naphthoquinone : atovaquone and proguanil(malarone).

Antimicrobials: tetracycline,doxycycline and clindamycin.

Iron chelating agents: Desferioxamine(Saifi *et al*., 2013)

Drugs used for prevention include;

Disruptive prophylaxis: monoclonal antibodies

Suppressive prophylaxis: mefloquine, chloroquine, doxycycline and proguanil.

Causal prophylaxis; malarone and primaquine

Relapse commonly occurs between 8 – 24 weeks and is often seen in P.vivax and P.ovale infections (Chu and White, 2016).

Malaria vaccine: the most effective malaria vaccine is the R21/Matrix-M, and it was the first vaccine to meet the World Health Organization goal of malaria vaccine with at least 75% potency (Roxby, 2021).

MALARIA RESISTANCE

ACT's have become the initial and best treatment of uncomplicated malaria in Nigeria (Oguche *et al*., 2014). Though the only group of anti-malaria that is not resistant, nevertheless with the growing abuse of drugs, it may become ineffective in the near future as drug resistance has become an evolving problem in Africa and beyond (Takala-Harrison and Laufet, 2015).

Presently the use of herbs and herbal products have soared, as there is a gradual loss of trust in orthodox drugs as patronage of herbal medicine peddlers have heightened with development of drug resistance (Takala-Harrison and Laufet, 2015).

2.6. HERBAL MEDICINE

Herbal medicine refers to herbal preparation, herbal materials, herbal products, and herbs that carry plant materials or plant parts as active ingredients often dispensed in natural form by herbalists (Oreagba *et al*., 2011). W.H.O referred to herbal medicines as botanical medicines or

phyto medicine which carries plant materials or plants parts as active ingredients (Lack and Rousseau, 2016).

These have become a type of healthcare in Nigeria, as a result of scarcity of orthodox medicine, as well as lack of requisite knowledge and low socioeconomic condition.

Herbal products utilized in Nigeria include: "Holy water" green water leaves, lime water, mango (mangifera indica); cashew (shoot of anacardium occidentale), pawpaw (carica papaya), Potato (solanum erianthum) and Neem tree; azadirachta indica (Onyemekeihia *et al*., 2018). Liquid, pastries, syrups or crushed mixture of different things, such as bark, leaves, stem and roots of particular trees are the various forms in which Agbo can come, and depending on what disease it treats, are so named. In Nigeria, the commonest local herbal mixture is known as 'Agbo', consumed by many people with its name derived from the western part of Nigeria, and is a variety of herbs and concoction, used as an alternative medicine. (Akande *et al*., 2012).It's used to treat myriad of illnesses including; typhoid fever, malaria fever, dysentery, headache, waist pain, rheumatism and sexually transmitted infection (S.T.I).

Literature shows that there are many different substances used in the preparation of Agbo. The Agbo for malaria fever known as 'Agbo Iba' is a transparent yellow solution with a bitter taste and putrid smell, mostly containing: nauclea latifolia (pin cushion tree), mangifera indica(mango),enantia specie(spp) and lime(Odugbemi *et al*., 2007). There are different Agbo mixtures used for treating illnesses which include; Agbo Jedi Jedi, Agbo Atosi, Agbo Aroriro and Agbo iba ponto etc (Akande *et al*., 2012). Agbo-Iba for malaria appears as a transparent yellow solution with a putrid odour and bitter taste, Agbo-Iba ponto for typhoid fever appears as a brick red solution with a bitter taste and putrid odour. Agbo Jedijedi for dysentery looks like dark brown solution with a bitter taste and a malodorous odour (Oreagba *et al*., 2011). Agbo Arariro for body pains appears as a whitish green solution, with a sour taste and sweet smell of mango and very cloudy. Agbo atosi for gonorrhea appears as a brown and dirty looking solution with numerous un-dissolved particles and a fetid smell similar to feaces, as well as a sour taste (Akande *et al*.,

2012). Agbo giri for treatment of convulsion in children consist of "Ocimum gratissimum" and black alum and Agbo narun for treatment of skin rashes consist of Lophira Alata, Ceiba Pentandra and Perug Lariadaemia (Adebayo and Krettlia, 2011).

Today herbal medicine has gained ground (Falodon and Imiejie, 2013).Despite modern technologies and newer drugs, people still prefer natural and primitive therapies. In Minna, Niger State Nigeria, in a study done to show the toxicity and phyto-chemical analysis of five herbal medicinal concoctions sold, showed it consisted of alkaloids, glycosides, anthraquinones, cardiac glycosides, stereodial nucleus, tannins, hydrolysable tannis, saponins, flavonoids, volatile oils and resins (Okunji *et al*., 2012).

In some communities in Nigeria, camphor is used as an ingredient in Agbo (Eweka *et al*., 2008). Similarly studies done to examine the teratogenic effects of camphor solutions, a constituent of some Agbo mixture, on the developing kidneys of Wistar rats, revealed that camphor was toxic to the developing kidney (Eweka *et al*., 2008). Similarly (Allard *et al*., 2013), discovered that most of the herbs studied showed nephrotoxicity.

Nevertheless, a study done in Nigeria to review the concentration of heavy metals from some commercially – packaged beverages consumed in Nigeria, showed that heavy metal concentrations, including iron, mercury, tin, antimony, cadmium, zinc, copper, chromium, lead and manganese seldom exceed the maximum contaminant level recommended by the Standard Organization of Nigeria (SON) and WHO (Orisakwe *et al*.,2017).

Orthodox medicines are regulated; the constituent and adverse effects are usually known in contrast to herbal drug. In a study done to determine the effects of Arteether on the liver, it was found that there were no cyto-architectural changes on histology as stated by the manufacturers, showing that the therapeutic doses of Arteether was well tolerated(Okunlola *et al*., 2013).

A lot of people in this environment consume herbal mixture and know little or nothing about its constituents. In a study done to assess the extent of use and the general knowledge of the benefits and safety of herbal medicines among urban residence in Lagos, Nigeria, it was seen that herbal

medicines was popular among the respondents, but they appear to be ignorant of its potential toxic effects (Oreagba *et al*., 2011).

The kidney and liver are organs for drugs metabolism and waste excretion, so are affected by toxic medications. The kidney is a paired organ located in the posterior abdominal wall whose function includes the removal of waste metabolic products from the blood and regulation of water and electrolytes balance in the body (Obhakhan *et al*., 2014). The liver is a large, reddish-brown organ located on the right upper part of the abdomen, whose function is to digest, absorb, process food and detoxify chemicals and metabolizes drugs (Abdel-Misih and Mark, 2010). As in humans, the majority of drugs administered to animals are eliminated by a combination of hepatic metabolism and renal excretion (Hosey *et al*, 2014).

One of the biggest issues is that there is the general belief, both from the public and regulatory bodies that because herbal supplement have been used for centuries, they must be safe. In a study done to investigate the effects of 'Xylopia Aethiopica' leaves on the histology of the kidney, it was found that the extract was toxic to the kidney and may induce dose- dependent renal damage (Obhakhan *et al*., 2014).A similar study done in Ibadan, to investigate the effect of four extract on the histomorphometry of the kidney, in streptozotocin (STZ) induced diabetics rats, showed renal damage histologically, with the group treated with 'Psidium Guajava' showing better histo-architectural outline of all the four plant extract used (Komolafe *et al*., 2013).This goes to show that some herbs may have some beneficial and protective property.

Furthermore, a study done to determine the effects of a multi herbal extract (Agbo), on plasmodium induced rodents, showed a degree of preventive action against plasmodium induced in mice, with no important adverse effects (Nwabuisi, 2002).

Literature on herbs and the dialysis patient have suggested avoidance of herbs; because they are unregulated and hence may have hepato-toxic potential, especially sassafras (sassafras albidum) because of its safrole content (Asif, 2012). Toxicity can also arise from herb- drug interactions and heavy metals adulterants (Chavez *et al*., 2006).Herbal drugs are not only used in African, as

data shows that over half of the Chinese population of the world use herbal therapy (Xutian *et al*., 2009).Similarly a reasonable number of rural dwellers in the Indian subcontinent depend on indigenous (Ayurvedic and Unani) medical system that use herbs, ash and heavy metals (Pandey and Rastogi, 2013).

The exorbitant cost of malaria treatment vis-à-vis the traditional disconnect inherent in orthodox drugs has left Nigerians' largely dependent on traditional practitioners and medicinal plants for many years in different parts of the world. The first anti-malaria drug used in the occident was derived from the bark of the cinchona (rubiaceae) species; the alkaloid quinine (Achen *et al*., 2011).

Just now, herbal medicine is becoming famous as the most utilized form of alternative medicine by more than 75% of the world's population (Ekor, 2013).

A cause of concern however in all these is that, the use of herbal medicines in this part of the world has no proper regulation. This may lead to poor quality products in the market, as well as increased contamination with heavy metals like nickel, lead e.t.c (Adie and Adedoyin, 2017).

2.7 ORGANS OF STUDY

2.7.1 LIVER

MACROSCOPIC ANATOMY

The liver is the biggest gland in the body and an organ found in vertebrates only (fig 3), having almost similar functions and structure in man and rats (fig 4), it removes various toxic substances, produces proteins and biochemical important in digestion (Abdel-misin and Bloomston, 2010). It's a highly specialized tissue made up mostly of hepatocytes whose role includes metabolism, control of the storage of glycogen, breakdown of red blood cells and manufacture of hormones and bile acid in the gall bladder (Tortora and Derricson, 2010). The liver is divided into the right and left lobes and into 4 parts; left, right, caudate and quadrate lobes. It is connected to hepatic artery, the portal vein, as well as the common hepatic duct.

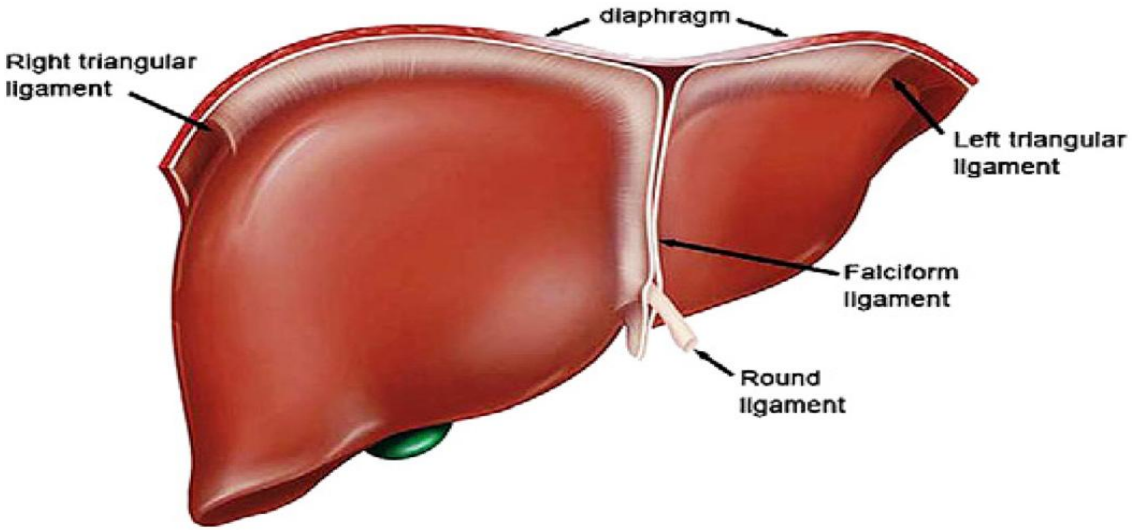

Figure 3: Gross Anatomy of human liver

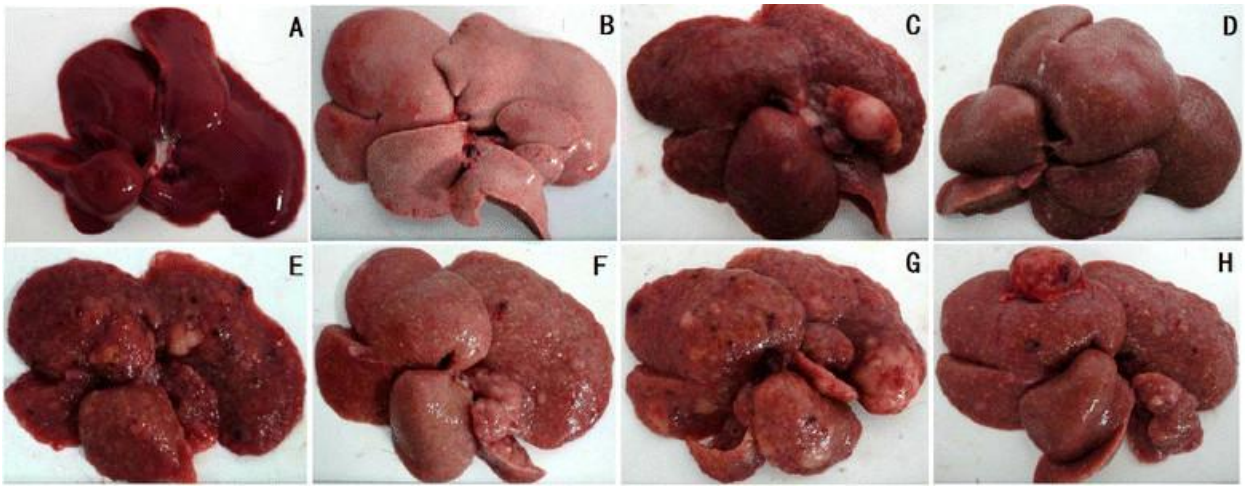

Figure 4: Gross anatomy of rats liver at different stages of development.

MICROSCOPIC ANATOMY

The blood vessel is divided into smaller blood vessels known as sinusoids. This leads to lobules which are the functional unit of the liver, made up of several plates of hepatocytes radiating from a central vein which are the basic metabolic cells (fig 5) with the distinctive component of the lobule being the portal triad held together by the Glisson's capsule(Mescher, 2013,Moore, *et al*., 2018).

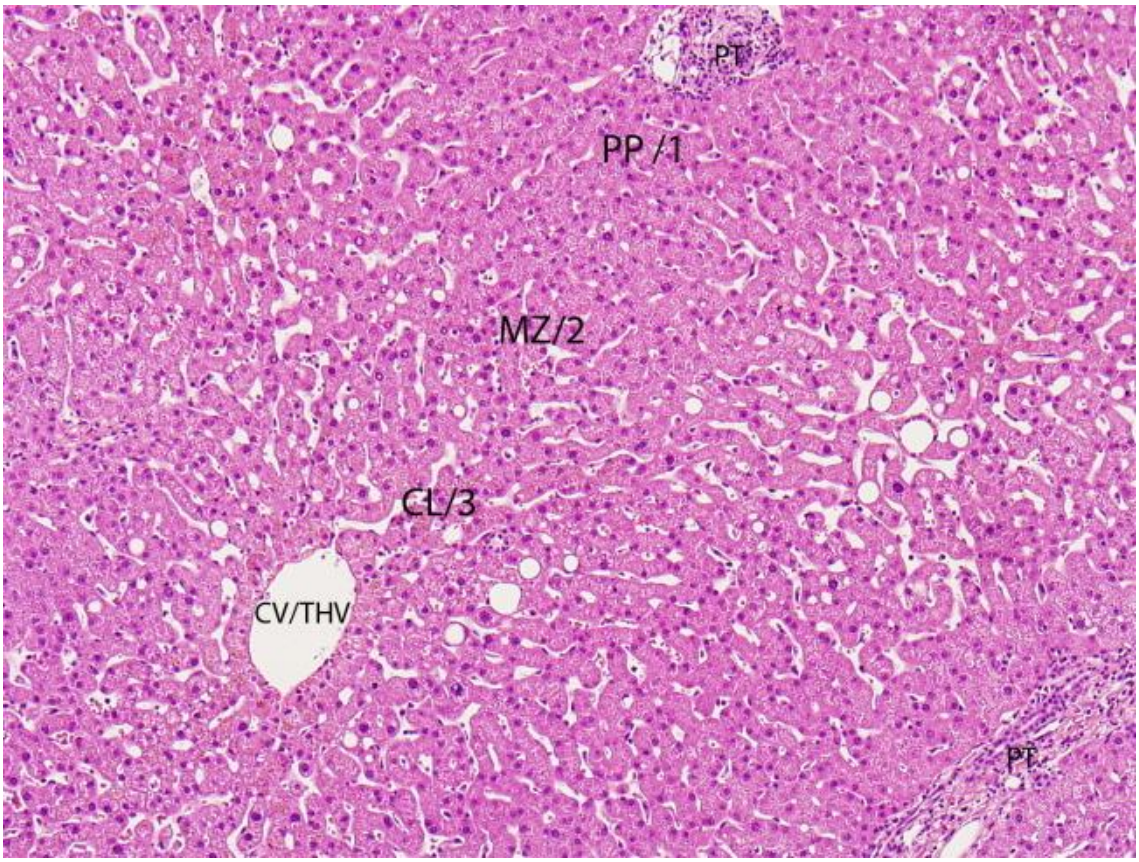

Figure 5: Microanatomy of the liver.

Liver sinusoids lie between hepatocytes and are enlarged capillaries, through which the blood from the hepatic portal vein and artery enters the portal triad and then the central vein. The liver cells include the sinusoidal endothelial cells, phagocytic kupffer cells and hepatic stellate cells found in the perisinusoidal space (Gillian, 2006).

Embrologically, the liver originates from the ventral portion of the foregut endoderm and parts of the adjacent septum tranversum mesenchyme and organogenesis occurs around the 3rd to 8th week and at the tenth week, the liver weighs about 10% of the body weight and 5% at birth (Moore, *et*

al., 2018; Si-Tayeb *et al*., 2010). At 12 weeks, bile is formed by hepatic cells (Berg *et al*., 2010).The link between the liver bud and the foregut (duodenum) gradually thins out forming the bile duct. From the bile duct a ventral offshoot (gall bladder) and (cystic duct) is formed.

As further development occurs, epithelial liver cords mix with the vitelline and umbilical veins to form hepatic sinusoids (Berg *et al*., 2010).Liver cords specialize to form a parenchyma cell which creates the biliary duct lining. The kupffer cells, the hematopoietic cells and the connective tissue cells are derived from mesoderm of the septum transversum (Berg *et al*., 2010). The visceral peritoneum except on its cranial surface is derived from the mesoderm on the surface of the liver. The central tendon of the diaphragm is formed by the portion of the septum which is made up of densely packed mesoderm (Moore, *et al*., 2018).

Molecular Regulation

Foregut endoderm has the capability to express liver genes and to mature into liver tissue (Gordillo, M., 2015).This is stopped by endoderm, non-cardiac mesoderm and notochord. The activity of this inhibitor is stopped in the expected hepatic region by fibroblast growth factor secreted by blood vessel forming endothelial cells near the gut tube at the liver bud outgrowth and cardiac mesoderm(Gordillo, M., 2015).Thus the cardiac mesoderm along with nearby vascular endothelial cells stops an inhibiting factor of this same gene by instructing gut endoderm to express inter-specific genes. Others include bone morphogenetic proteins (BMP) secreted by the septum transversum (Lade and Monga ,2011).Once this ”instruction” is gotten, the liver field cells differentiates into hepatocytes and biliary cell lineages, by a process controlled by hepatocyte nuclear transcription factor; HNF 3 and 4(Lade and Monga,2011).

2.7.2. KIDNEYS

MACRO AND MICROSCOPIC ANATOMY

The kidneys are a pair of bean shaped organ located on the right and left retroperitoneal space. The basic unit of the kidney is the nephron, which is the structural and functional unit of the kidney (Gillian and Richards, 2006). While each human kidney contains about one million nephrons, the mouse kidney contains 12,500 nephrons (Schreuder and Nayta, 2007). The kidneys function in fluid homeostasis in the body, fluid osmolarity, acid-base balance, concentration of various electrolytes, synthesis of hormone like erythropoietin and renin as well as vitamin D production and removal of toxins (Ivy and Bailey, 2014).

The kidney is made up of an outer renal cortex and inner renal medulla. The kidney can be seen as an (8-18) cone shaped renal lobes, which contain renal cortex that surround a portion of medulla called a renal pyramid(Boron,2004). The filtering portion (renal corpuscle) of the nephron is located in the cortex, followed by renal tubule (fig 6). The medullary ray drains into a single collecting duct and the papilla of the pyramid drains urine to the minor calyx, major calyx, the renal pelvis and eventually into the ureter (Boron, 2004).

Histology reveal; kidney glomerulus podocyte, parietal cell, kidney proximal tubules brush border cell, loop of henle thin segment cell, kidney distal tubule cell, thick ascending limb cell, collecting duct principal cell, interstitial kidney cell and collecting duct intercalated cell(McBride, 2016).

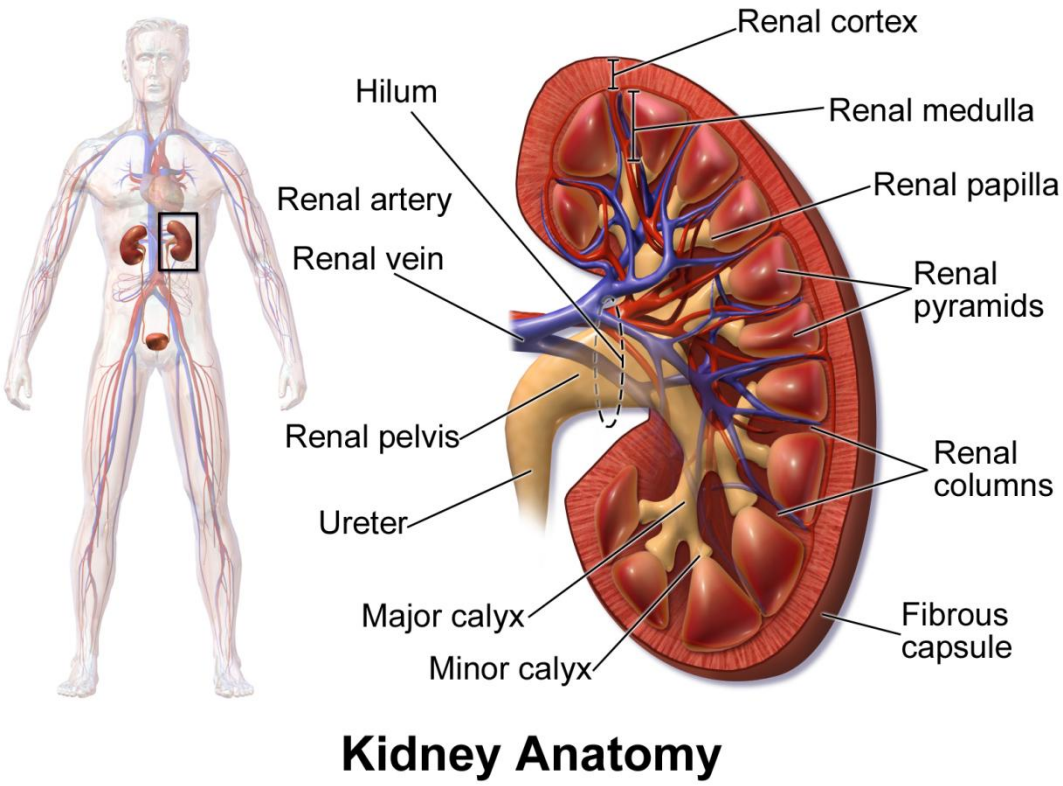

Figure 6: Gross Anatomy of the Kidney

Embrologically, the kidney originates from the intermediate mesoderm and goes through a series of development in 3 stages: pronephros, mesonephros and metanephros(Pietila and Vainio, 2014).This is formed in a head-tail fashion. At the 4th week the pronephros forms the nephrotomes and the mesonephros and mesonephric ducts are derived from intermediate mesoderm (Ludwig and Landmann, 2005). The metanephros forms the permanent kidney. The first excretory tubule appear, forms an S shaped loop and acquire a tuft of capillaries that will produce a glomerulus at their media extremities. The Bowman's capsule is formed in the vicinity of the glomerulus of the tubules. These 2 structures make up the renal corpuscle (Ludwig and Landmann, 2005). The tubule enters the mesonephric or Wolffian duct in a lateral fashion. At the middle of the second month, the mesonephros forms a large ovoid organ on each side of the midline known as the urogenital ridge. Finally at the termination of the second month, a significant part of the tubules has ceased to be visible (Ludwig and Landmann, 2005).

METANEPHROS (DEFINITIVE KIDNEY).

This definite kidney is formed in the 5th week. The differentiation of the duct system contracts from that of the other kidney system and its excretory unit is a specialization of metanephric mesoderm (Bertram *et al* .,2011).

COLLECTING SYSTEM

Collecting duct is formed from the ureteric bud, an offshoot of the mesonephric duct in the vicinity of the opening to the cloaca (Chen *et al*., 2017). The bud goes into the metanephric tissue and expands forming the primitive renal pelvis and divides into cranial and caudal portions. The bud continues to subdivide until twelve or more generations of tubules are created (Chen *et al*., 2017). The second order tubules also expand to contain the 3rd and 4th generation forming the minor calyx of the renal pelvis. As development progresses the 5th and consecutive generation of collecting duct lengthen and meet in the minor calyx and forms the renal pyramid. The ureteric bud forms

the renal pelvis, the ureter, the major and minor calyx and approximately 1 to 3 million collecting tubules (Chen *et al*., 2017).

EXCRETARY SYSTEM

The formed collecting tubules are protected at its caudal end by a metanephic tissue and under the inductive influence of the tubules and capillaries they grow into the pocket at one end of the S and differentiate into glomeruli (Sajithlai, *et al* 2005). These glomeruli together with the tubules form the nephrons or excretory units (Capel *et al.* 1999). The cephalic end of each nephron gives rise to Bowman's capsule which is deeply marked by a glomerulus. The caudal end forms a link with one of the collecting tubules establishing a channel from Bowman's capsule to the collecting unit. Extensions results in the formation of the proximal convulated tubules, loop of Henle and the distal convulated tubules. Urine formation begins early after differentiation of the glomeruli capillary which begins at the 10^{th} week.

MOLECULAR REGULATION

The kidney develops in the ureteric bud epithelium from the mesonephros interacting with metanephric blastema mesenchyme (Ribatti and Marcello, 2014; Tham and Smyth, 2019) .The mesenchyme conveys WT1, a transcription factor that make the tissue able to react to induction by the ureteric bud (Upadhyay and Silverstein, 2014). It also controls production of glial-derived neurotropic factor (GDNF) and hepatic growth factor (HGF or scatter factor) by the mesenchyme and this proteins stimulates branching and growth of the ureteric bud. The tyrogene kinase receptor for GDNF and MET are produced by the epithelium of the ureteric bud creating signaling pathway between the two.PAX2 propagates condensation of the mesenchyme in readiness to tubule formation and WNT4 results in condensed mesenchyme to epitheliarize and produce tubules(Chai *et al* 2013).

CHAPTER THREE

MATERIALS AND METHOD

3. 1 PREAMBLE

This study protocol was approved by the ethical committee{number : CMS/REC/2019/060}.The study was also in line with the internationally accepted standards for laboratory animal use and care as found in the US National Research Council guidelines (NRC, 2011).

INSTRUMENTS/EQUIPMENT

Weighing scale (both digital and manual), syringes, needles, oro-gastric tube, protocol-note, calculator, camera, writing pad, biro, forceps, scissors, ethylenediamine tetra-acetic acid(EDTA)bottles, lithium heparin bottles(LH) and slides .

CHEMICALS/REAGENTS

Chloroform, buffered formal saline, giemsa stain, haematoxylin and eosin stain.

OTHERS

Wistar rats, cages, plates, water bowls, bags of grower mash feeds, 'Agbo-Iba' aqueous extract and pack of 'Artemeter-Lumenfantrin' tablet, as well as cotton wool and tap water.

3.2 STUDY SETTING

The study was carried out in the Anatomy department of the University of Benin, Benin City, Edo state. It is located along Ugbowo-Lagos road, in the outskirts of the city and it has a teaching hospital, with standard laboratory services.

3.3 EXPERIMENTAL ANIMALS

Fourty-two(42) juvenile Wistar rats of both sexes, aged between 6-7 weeks with an average weight of 97.5g were procured from the animal house of the Anatomy Department, University of Benin. They were maintained with grower mash and water liberally. The animals were housed in plastic cages (polypropylene cages) with wire mesh measuring 45×35×28 cm. The animal house was well

ventilated at room temperature (37 degrees centigrade) with a 12 hourly day/night cycle and the animals were allowed to gain acclimatization for 2 weeks.

3.4 STUDY DESIGN

This study was an experimental animal study.

3.5 EXPERIMENTAL DESIGN PROCEDURE.

The study was carried out in two phases. **Phase 1** was the acute oral toxicity, which was done with 30 rats prior to the start of the main study, to check for the median lethal dose (LD50) of the herbal extract by a method described by Lorke (1983).

The animals were fasted for twelve (12) hours (over- night) prior to the experiment. The animals were assigned into six (6) groups of five (5) rats each, placed in separate plastic cages and were administered with increasing doses of the herbal extract; 0.1ml, 0.2ml, 0.4ml, 0.8ml, and 1.2mls of the extract, with the dose calculated using an average human dose of the extract as reference.

The animals were observed for mortality up to fourty-eight (48) hours. There was however no mortality at the highest dose of 1.2mls. So LD50 was taken to be greater than or equal to 5000milligram/kilogram (mg/kg) body weight in this study, which meant that the desirable therapeutic dose was safer than the toxicity level.

Phase 2 was the main study; forty-two (42) rats were used which were randomly assigned into six (6) groups of seven (7) rats each. These were grouped according to the sex of the Wistar rats, with males and females in separate cages.

GROUPING OF THE ANIMALS

The rats were separated into six (6) cages, representing six (6) groups.

Group 1, the control, given only feeds and water.

Group 2, the untreated group, given feeds and water and infected with Plasmodium berghei but not treated.

Group 3, the standard drug group, given feeds and water, infected with plasmodium, and treated with the standard drug,

Group 4, low dose group (0.2mls), given feeds and water, infected with plasmodium and treated with low dose of the herbal extract.

Group 5 moderate dose group (0.4mls), given feeds and water, infected with plasmodium and treated with moderate dose of the extract.

Group 6 high dose group (0.6mls), given feeds and water, infected with plasmodium and treated with high dose of the extract.

3.6 PROCUREMENT OF THE FEEDS AND DRUGS

They were fed on grower mash (gotten from Top Feeds, Sapele, Nigeria).

The **orthodox drug** Lonart tablet (Artemeter/Lumefantrin 20/120mg) was manufactured by Bliss GVS PHARMA LTD, Nigeria. The animals were given water liberally (*ad libitum*).

For the **herbal drug "AGBO IBA",** a pilot survey on the source of the constituents, was carried out at the start of the study around the month of November, 2018, at three (3) major markets in Benin City. Local vendors were interviewed, to identify the constituent, the source of the raw materials and the mode of preparation. It was found that the raw materials were purchased from the local market and consisted of mango leaves, mango back, lemon grass, dogoyaro leaves, pea leaves, pea back and 'Alabokun'(caffeine and acetylsalicylic acid).

About (one) 1liter of the aqueous extract was then procured from a local vendor around Oba market in the heart of the town and used for this study. The extract was stored in a refrigerator during the course of the study.

3.7 PHYTOCHEMICAL ANALYSIS

Phytochemical analysis was done at the Pharmacognosy Department of the University of Benin (UNIBEN) using standard procedure by Sofowora and Evans (Sofowora,1993; Trease and Evans,2002).

DETERMINATION OF CARBOHYDRATE, STEROID, FLAVONOID, TANNINS, ALKALOID, PHENOLIC ACID, and SAPONIN were done.

1. TEST FOR CABOHYDRATE

2 drops of the aqueous extract of the sample and 2 drops of 10% alcoholic solution of alpha napthol($C_{10}H_7OH$) was shaken and then 2 drops of concentrated H_2S04(sulphoric acid) was then added in the test tube. Formation of a brown ring at the interphase between the acid and extract indicated the presence of carbohydrate.

2. TEST FOR STEROID

2 drops of the aqueous extract of the sample and 2 drops of chloroform as well as 2 drops of concentrated H_2S0_4(sulphoric acid)was carefully poured down the side of the text tube. Formation of a reddish brown ring at the interphase between the acid and the chloroform layer indicated the presence of steroidial nucleous.

3. TEST FOR FLAVONOID

2 drops of the aqueous extract and I drop of NaoH(sodium hydroxide) and 1 drop of dilute HCL(hydrogen chloride) was added together. The change of the yellow colored solution to a colorless solution indicated the presence of flavonoid.

4. TEST FOR TANNIN

2 drops of the aqueous extract and 5mls of ferric ammonium citrate ($C_6H_8FeNO_7$) and 0.8g sodium acetate (CH3COONa) were added and boiled. Formation of a blackish bulky precipitate insoluble in hot water indicated the presence of tannin.

5. TEST FOR PHENOLIC ACID

2 drops of the aqueous extract and 1 drop of ferric chloride (FeCL3) were added. Formation of a black blue color indicated the presence of phenolic compound.

6. TEST FOR SAPONIN

2 drops of the aqueous filtrate and 4mls of water (H_2O) were vigorously shaken. The absence of persistent frothing indicated the absence of saponin.

7. TEST FOR ALKALOID

2 drops of the aqueous extract were tested with alkalodial reagent, mayor reagent, dragendoff and picric acid respectively; formation of a cream precipitate, yellow precipitate and brown precipitate indicated the presence of alkaloid.

3.8 PARASITE AND INFECTION.

Plasmodium berghei (NK 65 strain) obtained from the Institute of Advanced Medical Research and Training (IAMRAT), University College Hospital, Ibadan, Nigeria, was used for this study. Passage of the organism was done on 5 mice, from a donor mouse in the laboratory at IAMRAT, as the parasite had been maintained in the lab by serial passage. The five mice were used as carriers to transport the parasite. This was transported in a plastic cage in a vehicle to the animal house of the Anatomy department of the University of Benin same day.

3.9 INOCULATION PROCEDURE

A pre-patent period of 72 hours was given, after which parasitaemia was determined by collecting a small drop of blood from the tail of each infected mouse by venesection onto a clean grease free slide. Thin and thick blood film was prepared and analyzed at the microbiology laboratory of UBTH.

After determining plasmodium specie and parasitaemia, the mice were sacrificed and about 2mls of blood were collected directly from the heart into an EDTA bottle and diluted with phosphate buffered saline to$1x10^6$ parasitized erythrocytes/ml.

The Wistar rats were weighed, and then inoculated intraperitoneally by injection using a one (1) milliliter syringe with the prepared infected blood (Basir et al., 2012).This was done using 0.2mls of the diluted infected blood to inoculate the rats by a method described by Kabiru et al (2012).A pre-patent period of another 72hours was given, and parasitaemia was determined by the same method, used in the mice. The slides used for smearing and the Wistar rat were coded, using lead pencil and gentian violet respectively, for identification later.

3.10 BLOOD CYTOLOGY EVALUATION

At the Micro biology laboratory of UBTH, the blood film was stained with giemsa stain and allowed to air-dry and viewed with oil immersion objectives (Akin-osanaiya, *et al.*, 2013). A parasitaemia of (+) was taken as significant using the 'plus system' for thick film for parasite density (Tangpukdee, *et al*., 2009).

3.11 ADMINISTRATION OF ANTIMALARIAL DRUG AND CONCOCTION

Prior to the start of the treatment, the weight of the rats in the different groups were measured with a manual scale. Rats with parasitaemia, of at least (+) were either treated with a standard anti-malaria drug (Artemeter/Lumenfantrin) or the herbal concoction for 3 days and 7 days respectively. The rats were observed daily for any changes.

PROCEDURE

The rat was held at the back of the head with a piece of cloth rapt round it and through a syringe and tube (oro-gastric tube), the drug was given orally by forced feeding (garvage).

DRUG DOSE CALCULATION

Standard drug

It was prepared using the standard dosage regimen in mg per kg body weight for the drug, using the dose of an average 70kg man as reference and 0.6mls of the constituted drug (Artemeter 20mg/Lumenfantrine 120mg) was administered.

Since each tablet of Lonart contained 20mg Artemeter and 120mg Lumenfantrine, the whole tablet contained 140mg of both drugs.

SPECIMEN COLLECTION

On the last day (DAY 7) at the end of the experiment, the observations were made on the general physical activity, mobility and agility of the rats in each of the groups. The rats were fasted for over twelve hours (12) weighed and sacrificed after anaesthetization using cotton wool soaked in chloroform. The abdominal cavity was immediately dissected open, with a pair of forceps and scissors and blood sample was collected directly from the heart (cardiac puncture). This was

collected into EDTA and LH bottles for estimation of malaria parasitemia, and electrolyte,urea,creatinine(E,U&Cr)/liver function test(LFT)respectively. This was analyzed in the microbiology and chemical pathology lab of UBTH respectively.

The animal's organs (liver and kidneys) were removed, grossly examined and weighed immediately with a digital weighing scale after removing blood clots and excess tissues. The organs were immediately placed in buffered formal saline (10%).The specimens were analyzed in the histopathology laboratory of UBTH.

Interpretation of the slides was done thereafter.

3.12 BIOCHEMICAL ASSAY

Biochemical markers associated with liver functions were analyzed using plasma obtained from the Wistar rats upon necropsy.

DETERMINATION OF ASPERTATE TRANSAMINASE

The analysis was done using randox automated machine at UBTH. It was used for in vitro determination of Aspartate aminotransferase(AST) in serum (cat no AS l0l,Rl,Buffer,l x l00ml) 2x200t,R2-2,4 dinitrophenyl-hydrazine,l xl00ml).

DETERMINATION OF ALANINE AMINOTRANSFERASE

The analysis was done using randox automated machine, for the quantitative in-vitro determination of alanine aminotransferase(ALT) in serum.(cat no AL 100.R1 Buffer 2x100ml.R 2,2,4 dinitrophenylhydrazine x100).

DETERMINATION OF ALKALINE PHOSPHATASE (ALP)

This was done using an automated device (Teco Diagnostics l268 N.lakeview Ave.Anaheim CA 92807. l -800 -222-9880)

PRINCIPLE

The alkaline phosphatase acts upon the amp-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically.

TOTAL PROTEIN

PRINCIPLE

Cuprous ions in an alkaline medium interact with protein peptide bonds resulting in the formation of a colored complex

ALBUMIN

PRINCIPLE

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

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

KIDNEY FUNCTION TEST (KFT)

DETERMINATION OF CREATININE

Creatinine was assayed by the modified jaffes method (Vaishya et al., 2010)

PRINCIPLE

Creatinine reacts with picric acid in an alkaline medium to form picrates which is yellow colored and read at 50nm colorimetrically.

3.13 MICROBIOLOGICAL ASSAY

MALARIA PARASITE TEST

Giemsa Stain

Giemsa solution is composed of eosin and methylene blue (azure).The eosin component stains the parasite nucleus red, while the methylene blue component stains the cytoplasm blue. Methanol was used to fix the thin film and dehaemoglobinization and staining of the thick film took place simultaneously. The PH was 7.2.

METHOD - The slow method (3% stain working solution) was employed.

MATERIALS - Giemsa stain, a small container for giemsa working stain, absolute methanol acetone free, a pasteur pipette with a rubber teat, a small container or beaker for methanol, staining

troughs, a timer, slide, drying rack, protective latex gloves powder free ,a laboratory coat, disposable and distilled or deionized water, buffered to PH of 7.2.

PROCEDURE.

Thin film- 3% giemsa working solution was prepared and placed in a small container, using a pasteur, a thin film was prepared and fixed by dropping methanol on it, and this was then dried on a drying rack. The stain was poured slowly until the blood film was covered on the slide and the timer was set to 45-60mins and clean water was poured into the tray to float off the iridescent scum. The left over stain was discarded and washed with clean water and the slides were carefully carried away to dry.

Thick film

A thick film was made with a pipette. The slides was dried, then rinsed with water at PH of 7.2.The stain was applied within 5 minutes and was left to dry and read under the microscope(x l00 oil for immersion).

3.14 HISTOLOGICAL EXAMINATION

When the period of the experiment had elapsed, the Wistar rats were sacrificed by fasting them overnight and anaesthetizing with chloroform by inhalation. The abdominal region of the Wistar rats was dissected and the liver and kidney were excised, examined and weighed. The liver was then fixed immediately in 10% neutral buffered formalin prior to histological preparation.

TISSUE PROCESSING

The tissues were moved to an automatic processor where they underwent a process of dehydration. This was done with alcohol in ascending graded manner (ethanol) 70%, 90%, 96% (2 changes each) and absolute alcohol (3 changes each). The tissues were then cleared in xylene (2 changes each) and impregnated in paraffin wax (3 changes each). After embedding and blocking out, serial sections of 5 micron thick were obtained using a rotary microtone (Microm HM 340E Walldorf, Germany). The tissue sections was deparaffinised, hydrated and stained using the routine haematoxylin and eosin staining method: H&E (Feldman and Wolfe, 2014).

Principle

H & E is a "salt dye" and the mechanism of staining is as follows: Positively charged hematoxylin will stain negatively charged tissue components, and negatively charged eosin will stain positively charged tissue components. The H & E is an effective stain for demonstrating the major histological structures, particularly nuclei which are most important structures in the viewing of histological sections for pathological changes.

Procedure

The tissue sections were deparaffinised, hydrated and stained using the routine H&E staining method as follows:

- Sections were taken to water:
- Stained in hematoxylin for 7 minutes.
- Rinsed in running tap water for 20 seconds.
- Dipped quickly in 1% acid alcohol.
- Rinsed in running tap water for 30 seconds.
- Blued in 1% ammonia water, dipped for 5 seconds.
- Rinsed in running tap water for 2 minutes.
- Dipped in 80% alcohol for 15 seconds.
- Stained in eosin working solution for 3 minutes.
- Dipped in two changes of 95% alcohol, for 15 seconds each.
- Dipped in 3 changes of absolute alcohol, for 30 seconds each.
- Cleared in 2 changes of xylene, 2 minutes each.
- Mounted with synthetic resin, DPX.

Coverslipping prepared slide: 1-2 drops of cyto-seal was applied to tissue, to avoid air bubbles and coverslip was placed at the edge of the slide then the capillary action of the cover glass was allowed against the slide to cover the tissue.

Result: Nuclei stained blue, while cytoplasm stained red.

Photomicrography

After staining tissues for histological studies using H&E staining method, sections were viewed and examined under an optical photomicroscope(Leica MC170 HD,Leica Biosystems,Germany) and photomicrographs were taken at x40 and x100 magnification using an attached Eakins I4MP digital microscope camera and results were then reported . This was done at the Anatomy Department, UNIBEN.

3.15 DATA ANALYSIS

Data was subjected to statistical analysis using IBM SPSS (statistical product and service solutions) statistic software (version 25). Data were presented as mean ± SEM. Comparison was done between the mean values of the treated group and the controls, using the one way Analysis of Variance (ANOVA). The statistical values were presented as tables and charts.

All differences was considered to be statistically significant at $P<0.05$. This was done with the assistance of a statistician.

3.16 ETHICAL CONSIDERATION

Ethical approval was obtained from the ethical committee of the University of Benin (Ethical approval no: CMS/REC/2019/060).

CHAPTER FOUR

4.0. RESULT

4. 1. PHYTOCHEMICAL ANALYSIS

From table 2, the phytochemical analysis done showed that 'Agbo Iba' herbal extract contained alkaloids,steroidal compounds,flavonoids,tannins,phenolic acids and carbohydrate.

TABLE 2: RESULTS OF PHYTOCHEMICAL ANALYSIS OF AGBO - IBA

S/N	TESTS	REAGENTS	RESULT
1	Alkaloids	Mayor reagent Dragendoff reagent Picric acid	+
2	Steroidal compounds	H_2 So_4 Chloroform	++
3	Flavonoids	NaOH HCL	+
4	Tannins	Ferric-ammonium citrate Sodium acetate	+
5	Phenolic acid	$FeCL^3$	+
6	Saponin	H_20	-
7	Carbohydrate	Alpha napthol	+

Present: +, Absent: -

4.2 EFFECTS OF THE DRUGS ON BIOCHEMICAL PARAMETERS

LIVER

From table 3, the level of the ALT was significantly lower in the untreated group and moderate dose group when compared to the control, and significantly higher in the high dose group when compared to the control group ($P< 0.05$).

The level of AST was significantly lower in the untreated group and the low dose group when compared to the control group ($P< 0.05$).Differences in mean serum ALP levels were not significant different across all the groups($P> 0.05$)

The conjugated bilirubin in the untreated group was significantly lower when compared to the control group. The value of total bilirubin in the entire group was significantly lower when compared to the control ($P< 0.05$).

KIDNEYS

From table 4 below, the level of urea in the high dose group was significantly higher ($P<0.05$) when compared to the control. The sodium in the low dose, moderate dose and untreated group was significantly lower when compared to the control ($P<0.05$). The differences in mean serum potassium and bicarbonate levels in all the groups were not statistically different ($P<0.05$).The chloride level in the untreated group, low dose and moderate dose group was statistically lower than the control ($P<0.05$). The mean serum level of creatinine across the groups were not statistically different ($P<0.05$).

4. 3 WEIGHT MEASURES

BODY WEIGHT

From figure 7, the average body weights of the rats in all the groups were statistically analyzed. Although there was progressive weight gain in the different groups, there were no significant differences ($P>0.05$) across all the groups when the initial body weights were compared with the final weights.

HEPATIC WEIGHT

From figure 8, the average liver weight across the group was statistically analyzed. There was significant increase ($P<0.05$) in the weight of the liver of the untreated group (Gp2), when compared to the control group.

KIDNEY WEIGHT

From figure 9, the average kidney weights in all the groups were statistically analyzed. There was no significant difference ($P>0.05$) in the weight of the kidneys, when compared to the control group.

HEPATOSOMATIC INDEX

From figure 10, the average liver-body weight ratio was statistically analyzed across the groups. There was no significant difference ($P>0.05$) in the hepatosomatic index, when other groups were compared to the control group.

RENOSOMATIC INDEX

From figure 11, the average kidney-body weight ratio was statistically analyzed across the groups. There was no significant difference ($P>0.05$) in the renosomatic index, when the other groups were compared to the control.

4.4 THERAPEUTIC EFFECT

Figure 12 shows, the therapeutic effect of the antimalarial drug and concoction assessed in percentages. From the bar chart there was absence of infection with plasmodium berghei in the control group, while in the untreated group there was100% evidence of infection with no form of clearance of plasmodium berghei .While the standard drug group had about 90% significant clearance of the plasmodium berghei, the low dose, moderate dose and high dose groups had 80%,85% and 60% significant clearance of plasmodium berghei respectively.

4.5 HISTOLOGY OF THE LIVER

Plate 1 is a photomicrograph of the rat's liver of the normal control. The micrograph shows a view of an area of liver tissue. It demonstrates the portal triad (A), the central vein (B) and the radiating plates of hepatocytes(C).

Plate 2 is a photomicrograph of malaria (plasmodium berghei) infected rat liver (Gp2).The micrograph shows a view of an area of liver tissue. It demonstrates sinusoidal congestion (A), hemozoin pigments (B), periportal infiltrates of inflammatory cells (C).

Plate 3 is a photomicrograph of a rat's liver infected with malaria parasite and treated with the standard drug. It demonstrates: portal triad (A), radiating plates of hepatocytes (B) and central vein(C). We notice a marked reduction in sinusoidal congestion as well as absence of peri-portal infiltrates or hemozoin pigments.

Plate 4 is a photomicrograph of a rat's liver tissue inoculated with malaria parasite and treated with low dose herbal extract. It demonstrates: central vein (A), scanty hemozoin pigment (B), mild active congestion(C), hemozoin pigment (D).

Plate 5 is a photomicrograph of a malarial infected liver tissue treated with moderate dose herbal extract. It demonstrates: central vein (A), mild peri-portal infiltrates of inflammatory cells (B), radiating plates of hepatocyte(C), few hemozoin pigments (D).

Plate 6 is a photomicrograph of a malaria infected liver tissue treated with high dose herbal extract. It shows presence of: periportal infiltrates (A), hemozoin pigments(B) and portal vein(C)

TABLE 3: EFFECT OF THE DRUGS ON LIVER FUNCTION PARAMETERS

	Control	**Untreated**	**Standard drug + P. berghei**	**Low dose + P. berghei**	**Moderate dose + P. berghei**	**High dose + P. berghei**	**F**	**P**
ALT (µ/L)	111.75±4.75[b]	92.25±4.55[a]	98.50±6.55	110.00±7.88	90.75±7.89[a]	136.75±4.61[ab]	7.61	0.001
AST (µ/L)	32.75±2.02[b]	22.25±1.89[a]	28.00±1.87[b]	14.25±0.75[a]	25.00±5.43	26.25±4.13	3.61	0.020
ALP (µ/L)	53.75±10.02	38.75±3.77	50.50±7.93	46.7±53.30	51.50±7.05	44.75±4.23	0.696	0.633
Conjugated Bilirubin	0.30±0.09[b]	0.15±0.03[a]	0.28±0.05	0.20±0.00	0.18±0.03	0.18±0.05	1.54	0.227
Total Bilirubin	0.50±0.06[b]	0.30±0.00[a]	0.30±0.00[a]	0.30±0.00[a]	0.33±0.05[a]	0.25±0.05[a]	5.62	0.003

[a] = significantly different from the control group, ($P<0.05$)
[b] = significantly different from the untreated group, ($P<0.05$)

TABLE 4: SHOWING EFFECTS OF THE DRUGS ON RENAL FUNCTIONS PARAMETERS.

	Control	**Untreated**	**Standard drug + P. berghei**	**Low dose + P. berghei**	**Moderate dose + P. berghei**	**High dose + P. berghei**	**F**	**P**
Urea	21.75±0.75	29.00±0.41	19.75±1.65[b]	24.25±1.65	29.75±3.35	38.25±5.45[a]	5.75	0.002
Sodium	140.25±1.80[b]	125.00±3.24[a]	134.75±3.20[b]	125.75±1.49[a]	130.50±4.44[a]	135.75±2.14[b]	4.28	0.010
Potassium	26.55±1.71	36.65±4.32	24.55±1.57	33.15±3.15	30.05±2.85	27.70±3.46	2.24	0.095
Bicarbonate	19.75±0.48	19.75±0.48	20.75±0.75	19.75±0.75	20.50±1.19	20.75±0.75	0.44	0.816
Chloride	109.25±1.80	97.00±1.91[a]	104.00±2.45[b]	96.50±1.50[a]	101.50±3.30[a]	104.00±1.41[b]	4.96	0.005
Creatinine	0.73±0.05	0.70±0.04	0.70±0.04	0.68±0.06	0.88±0.23	1.08±0.09	2.12	0.110

[a] = significantly different from the control group, ($P<0.05$)

[b] = significantly different from the untreated group, ($P<0.05$)

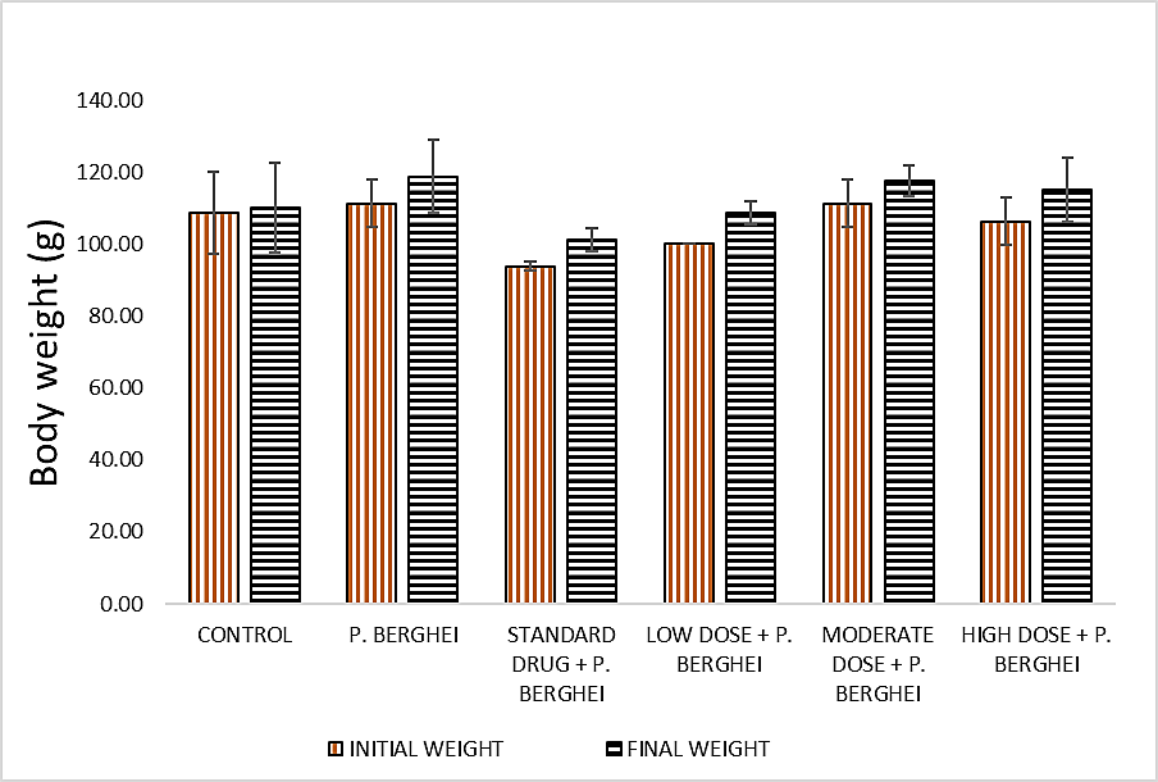

FIGURE 7: Chart showing body weight across the experimental groups

There were no significant differences (P>0.05) across all the groups when the initial body weights were compared with the final weights

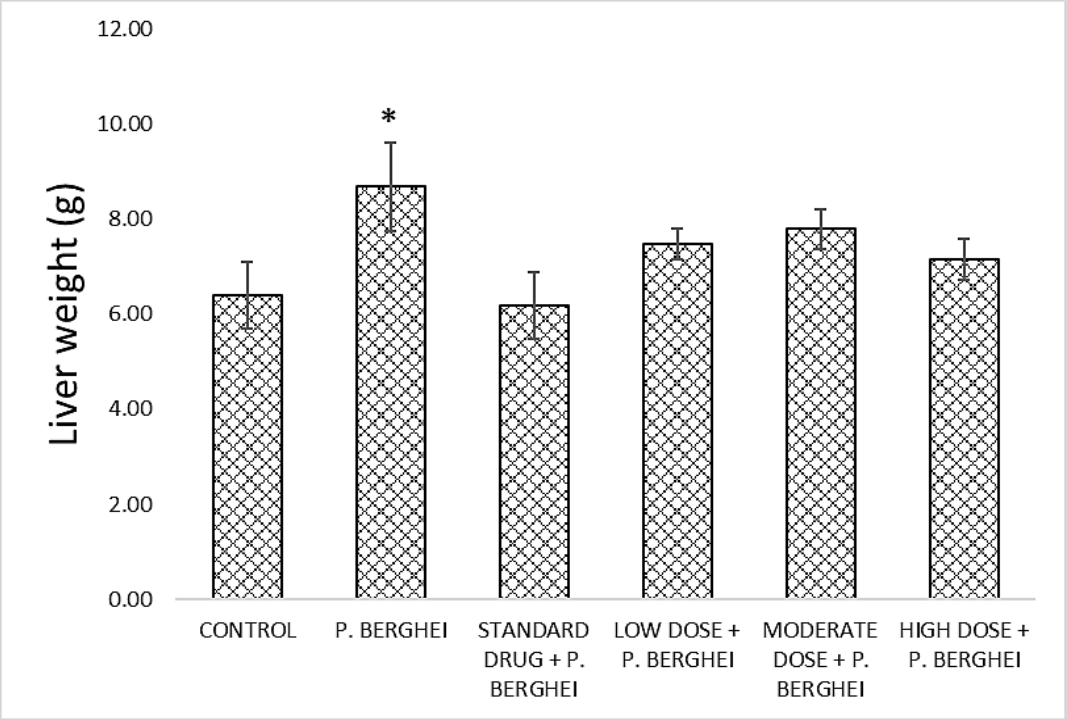

FIGURE 8: Chart showing the weight of the liver across the experimental groups. There was a significant difference in the weight of the untreated group ($P<0.05$) compared with the control.

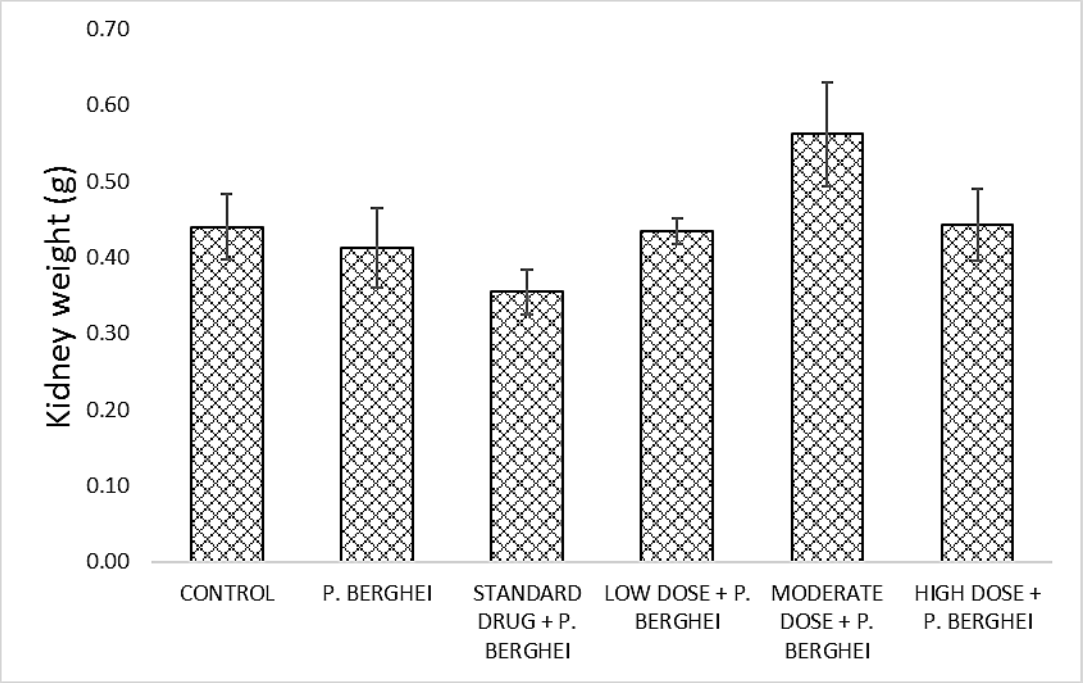

FIGURE 9: Chart showing the weight of the kidney across the experimental groups

There was no significant difference (P>0.05) in the weight of the kidneys across all groups.

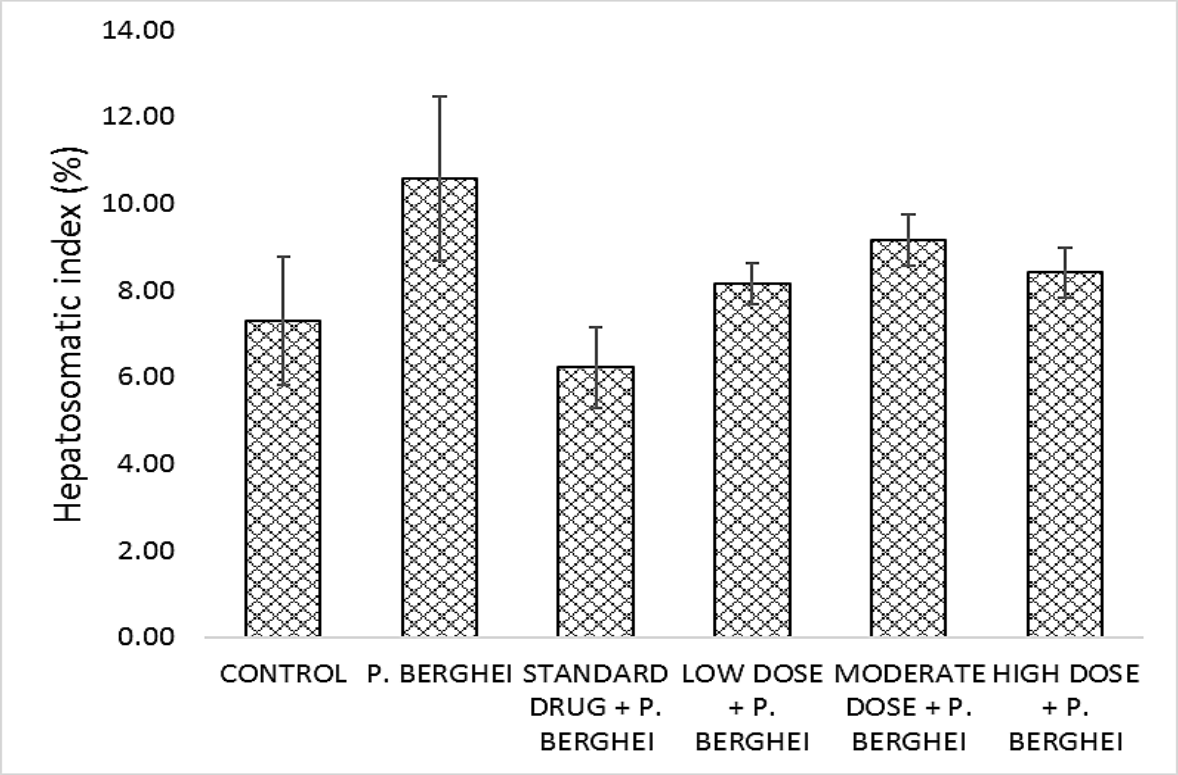

FIGURE 10: Chart showing the hepatosomatic index across the experimental groups.

There was no significant difference (P>0.05) in the hepatosomatic index across all groups.

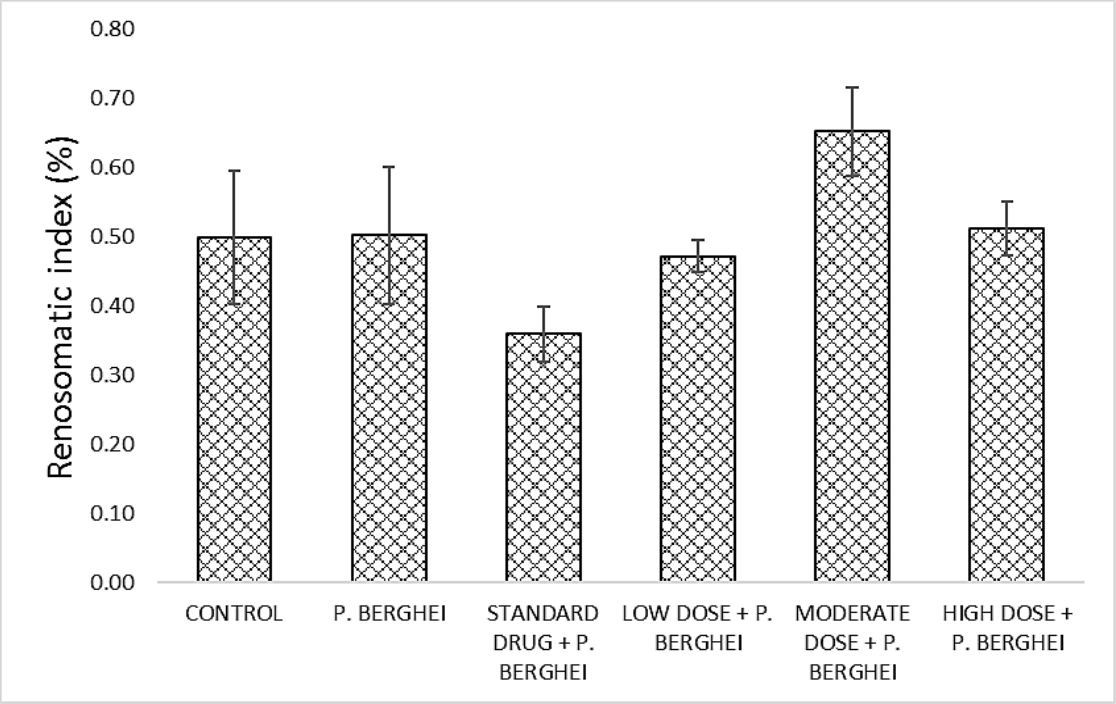

FIGURE 11: Chart showing the renosomatic index across the experimental groups.

There was no significant difference (P>0.05) in the renosomatic index across the groups.

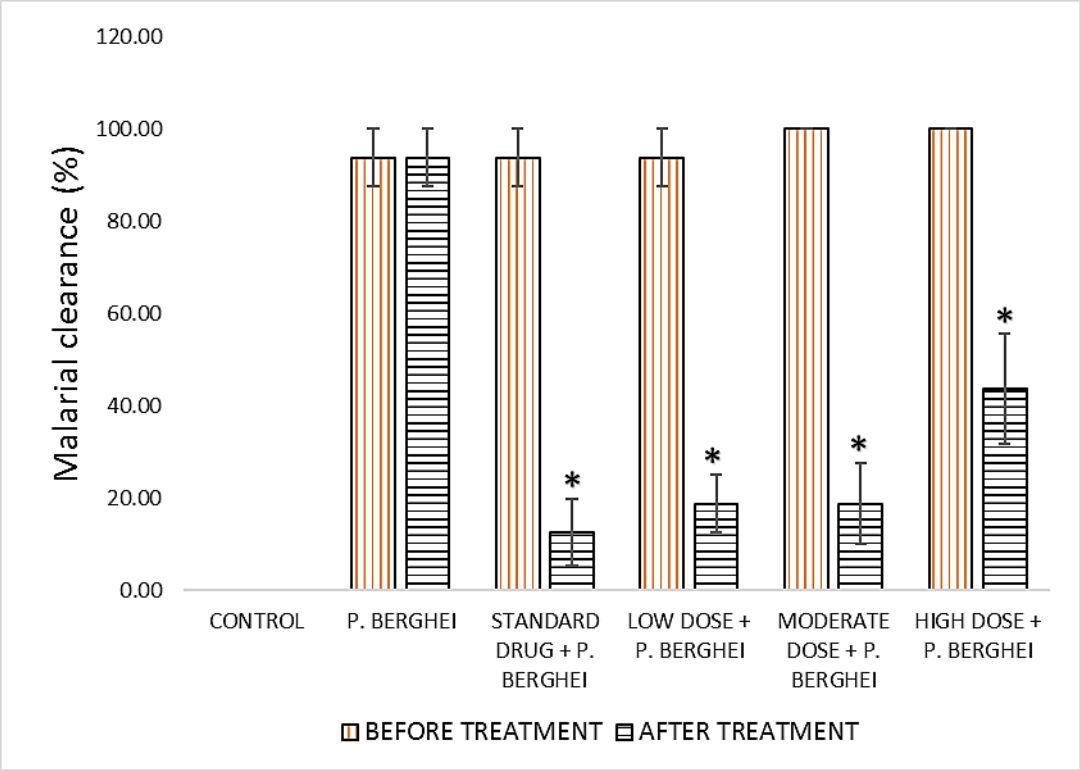

FIGURE 12: Chart showing the percentage of malarial clearance across the experimental groups.

There were significant decreases ($P<0.05$ compared with pretreatment group) of malarial clearance levels in groups C, D, E and F when the pretreatment and post treatment results were compared.

PLATES

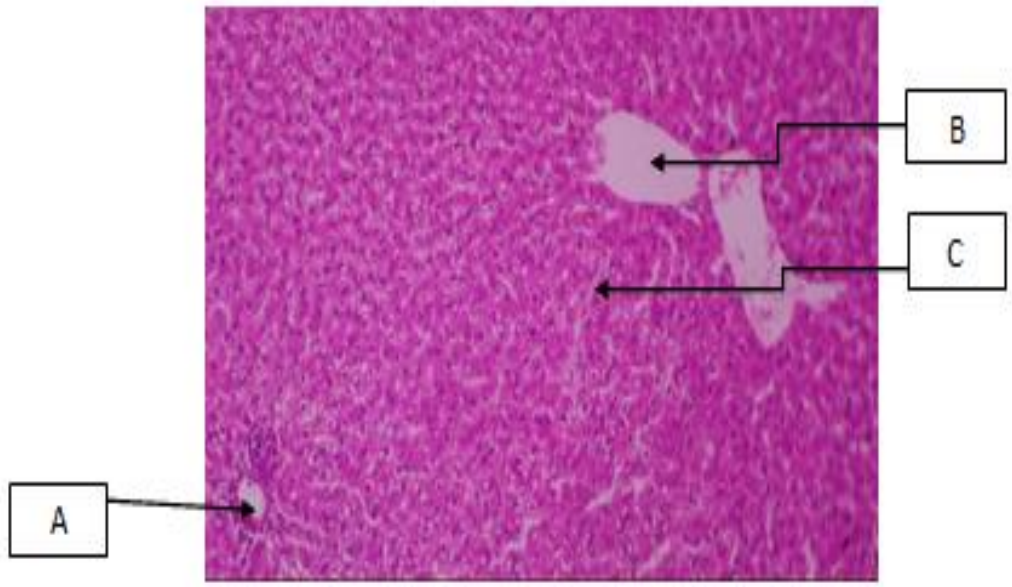

Plate1, showing a normal liver tissue (x40). H&E

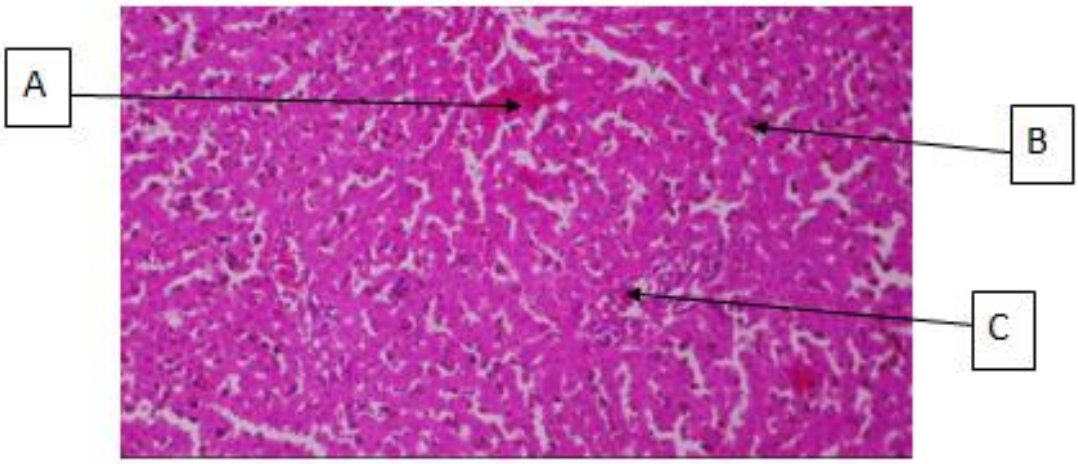

Plate 2 showing rat liver infected with malaria parasite (H&E x 100)

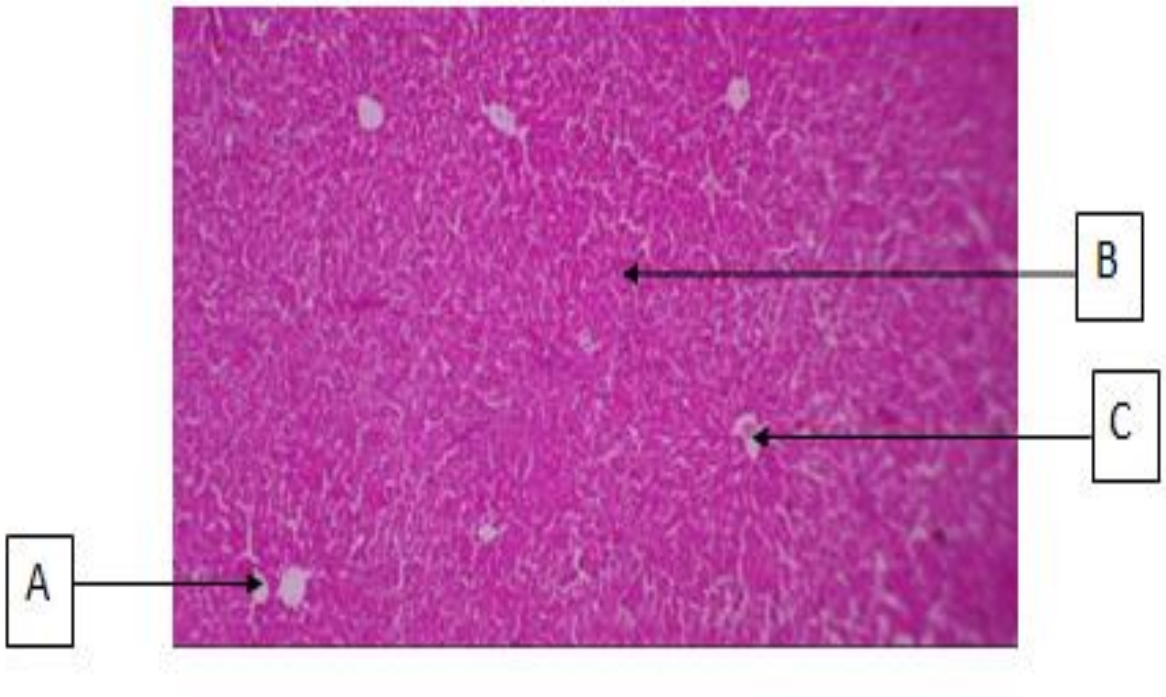

Plate 3 shows infected rat's liver treated with standard drug(H&E x40)

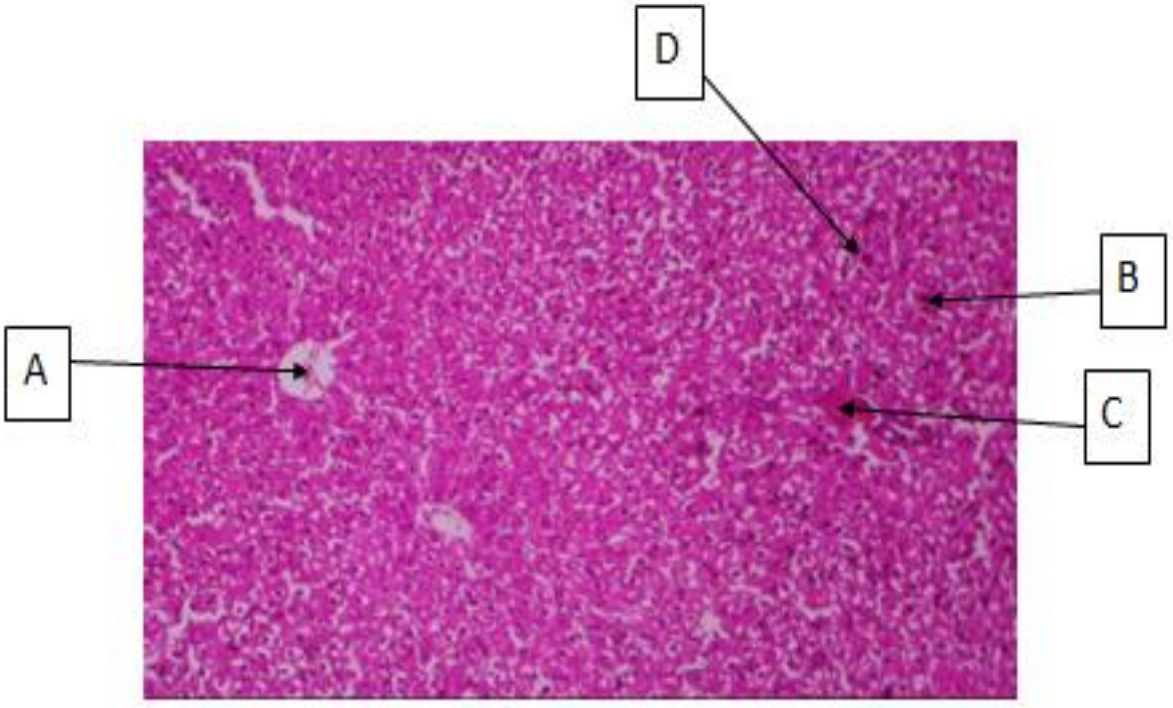

Plate 4 shows infected rat's liver treated with low dose extract(H&E x 100)

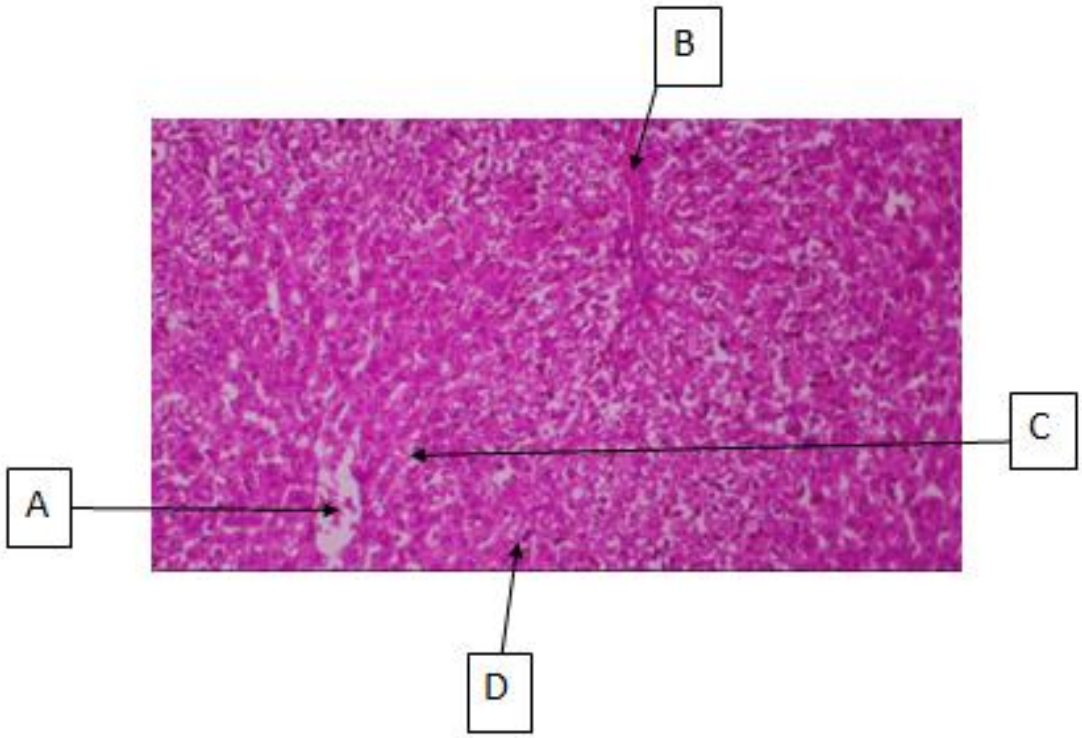

Plate 5 showing rat liver tissue treated with moderate dose of the herbal drug (H&E x 100)

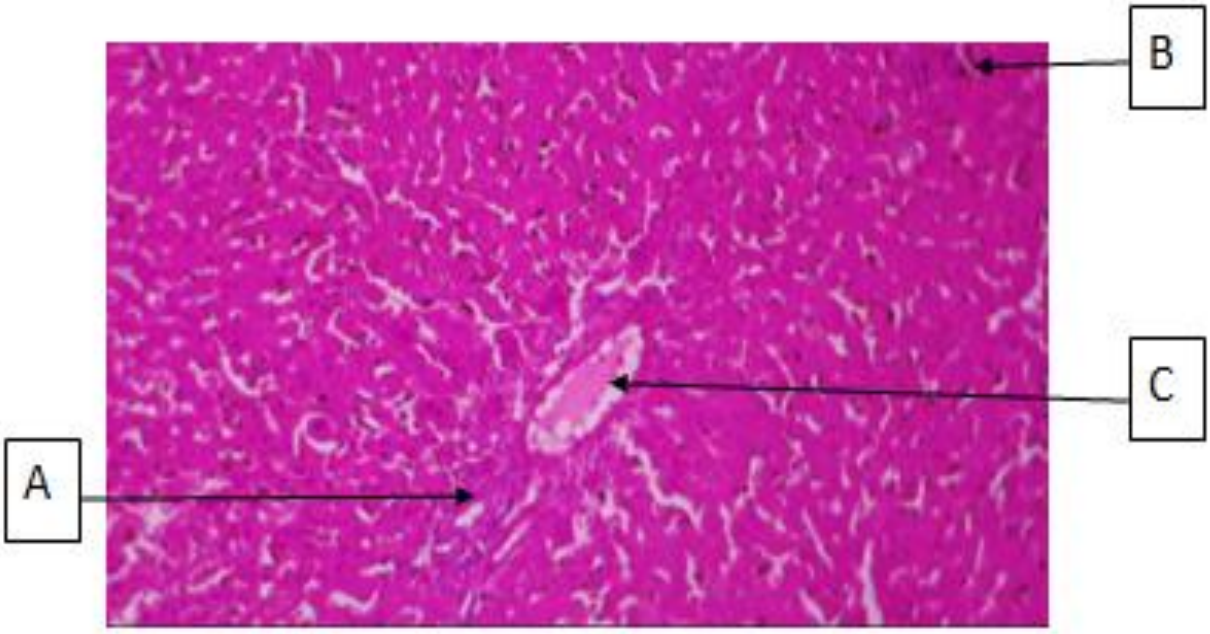

Plate 6 showing rat’s liver treated with high dose herbal drug(H&E)

CHAPTER FIVE

5.0. DISCUSSION

5. 1. BACKGROUND

Apart from the therapeutic effect of a drug in disease condition, safety is also paramount. Moreover, this is important when herbal drugs are administered, as they do not go through any clinical trial before they are released on to the market.

'Agbo Iba' used for malaria treatment in this research has been in existence for some years in Nigeria and just now; there seem to be an increased usage. Although few literatures have reported toxicity, general safety as well as good therapeutic value cannot be assumed.

This study was done in two phases. Phase 1 was the calculation of the LD_{50} and phases 2 was the main experiment. This pattern of phases was similar to other animal studies (Nwodo and Joshua, 2015). The first phase was necessary to assess the safety of the concoction prior to the experiment.

To avoid gender related disease vulnerability, males and female rats were used in this study, although the animals were placed in separate cages, as was the case in some other animal studies (Zhang, 2004). This may be important in experiment of this nature where pregnancy is undesirable as this may affect the findings of the study.

Usually, the therapeutic value of medicinal plants may be assessed through determination of the biological activity of their phytochemical constituents.

Phytochemical analysis done showed that 'Agbo iba' contained alkaloids, carbohydrates, steroids, flavonoids, tannins and phenolic acids. These was similar to some other studies on malaria which showed alkaloids, flavonoids,steroids and tannins content (Ubulom *et al*., 2011). This was also similar to some other studies in which herbal plants were used to treat malaria (Bankole *et al*., 2016).

Studies shows that phenols, anthracenosides, phytosterols or flavonoids all have anti-malarial activity(Hilar *et al*.,2006).Some of this chemicals are constituents of the 'Agbo Iba' used in this

study, especially alkaloids and flavonoids which have been found to have antiplasmodial effect(Ettebong *et al.*,2005)as these secondary metabolites elicits bioactivity independently or synergistically with other plants (Shigemori *et al.*,2003) .This may explain the seemingly anti-plasmodial activity of 'Agbo-Iba'.

5.2 BIOCHEMICAL EFFECTS

5.2.1 Biochemical Effects on the Liver

Elevated levels of AST and ALT are indicative of injury to liver tissue (Mc Gill, 2016). However ALT is a more specific enzyme marker for liver cell damage. ALP may signify blockage or obstructive liver disease. If an herbal decoction is safe and therapeutically effective then it is good for consumption.

According to this study, there was significant increase in ALT in the high dose treated group when compared to the control. This was at variance with another study in Nigeria, where artesunate, amodiaquine and selenium were co-administered to treat plasmodium berghei infected rats. The findings revealed that ALT experienced no significant alterations. (Adebayo *et al*., 2018). These differences may be due to the effect of combining different drugs which may be less deleterious to the liver.

The study also showed that, AST was significantly lower in the untreated and low dose group when compared to the control and ALP had no significant differences. This was similar to the study on '*Ocimum gratissimum'* herbal extract to treat malaria, which showed an insignificant decrease in ALP and AST (Ebeye *et al*., 2014). This may signify that the phytochemicals present in both herbal extracts were relatively safe. Similarly in another study in which 'Creptolepis' herbal extract was used to treat malaria induced rats, it was found that the liver enzymes were within normal range (Ansah and Gooderhem, 2002).

Untreated malaria parasite may also cause derangement in liver enzymes. In a study to assess the effects of Plasmodium falciparum on the liver enzyme in humans, it was found to cause elevation in serum bilirubin, ALP, ALT and AST significantly compared to the control (Oyewole *et al*., 2010).

This was at variance with this study, as the untreated group did not have any significant liver enzyme elevation. This may be due to differences in human and rodent plasmodium or their response to the drugs. Moreover the liver produces bilirubin from the breakdown of heamoglobin, so elevation of bilirubin in the human experiment may be a sign of hemolysis of red blood cell rather than liver derangement.

From this study, the value of bilirubin in all the treated groups was significantly lower when compared to the control. This was corroborated by other studies, which showed reduction in bilirubin level (Somasak *et al*., 2015; Thiengsusuk *et al*., 2013).This may be due to hepato-protective effects of some of the phytochemical and drugs used in treatment.

5.2.2 Biochemistry of the Kidneys

From the study, the urea level in the high dose treated group was significantly increased when compared to the control. This was at variance with other studies using herbal decoctions to treat malaria, in which the urea, creatinine and uric acid was significantly reduced in the treated groups in a dose-dependent fashion compared to the control (Anigboro, 2018; Ajayi *et al*., 2017). The reason may be that in high doses there may be injury to the kidneys from a particular constituent of the herbal decoction which may not have been present in the other herbal decoctions or the increase in urea may have been from diet and dehydration worsened by the high dose of the herbal decoction.

According to the study, creatinine in all the groups, were not statistically different when compared to the control. The findings was at variance with a study, in which neem leaves used as an herbal anti-malaria, caused dose dependent reduction in creatinine level (Somasak *et al*.,2015). In another study using orthodox drug (Atesunate) on the effect of varying doses on the kidneys, it was found that there was a surge in the creatinine level of the treated group when compared to the control(Campos et al., 2001).The reason may be that the herbal decoction used in this study may not have any significant reno-protective or deleterious effect when used in normal doses.

5.3 BODY WEIGHT MEASURE

The changes in total body weight and the organ weight may be a sign of toxicity of the herbal decoction used, as increased organ weight (either absolute or relative) has been noted to be a measure of organ toxicity (Piao *et al*.,2013).

From this study there were no statistically significant differences in the body weight across all the groups when the initial body weight was compared with the final weight. This finding was similar to the study done using 'Khaya Senegalensis' to treat malaria infected Wistar rats in which the body weight was not significantly altered (Olunishe *et al*.,2003),but this was at variance with the study using 'Xylopia ethiopica', where there was decrease in the total body weight of the treated rats compared to the control (Ozoko *et al*.,2015).This may be because the herbal extract may have contained constituents which had some lipid lowering effects or toxic substance which may not have been present in this study. This was however not in agreement with the study by Akhigbe *et al* (2002), which revealed progressive increase in the body weight with 'Spondress Mombin extract' of the treated group.

5.4 THERAPEUTIC EFFECT OF AGBO IBA HERBAL EXTRACTS

Sub-Saharan Africa is one of the major regions where malaria is endemic, with mortality, especially among pregnant women and children below 5 years (WHO, 2015).This justifies the use of juvenile rats in this study. Resistance is on the increase, though ACT's is still the hope amidst resistance for treating malaria just now (Lin *et al*., 2010). Alternatives like 'Agbo Iba' usually made up of; hippocratic indica, nauclea latifolia, enantia species, lime and the back of mangifera indica (mango) amongst other things has been consumed by a significant number of Nigerians, for treating malaria fever, as well as various illnesses today.

According to the results of this study, "Agbo iba" extract showed significant parasitemia clearance in the low, moderate and high dose groups, comparable to the orthodox ACT (lonart). This was

similar to a study in which a multi- herbal extract "Am 207" for treating malaria fever exhibited good anti-plasmodial activity comparable to chloroquine and nibima (Martey *et al.*,2013).Similarly, another investigation done by Nwabuisi (2002), to determine the prophylactic effect of a multi-herbal extract(Cajanus Cagan leaf, Euphorhia lateriflora leaf, Magnifera indica leaf, stem cassia alata leaf, cymbopgon giganteas leaf and Uvaria chamae back) gave noteworthy anti-malarial activity with no apparent significant side effect.

The parasitemia clearance recorded in this study was not dose dependent, as the group that had the high dose of the extract experienced less clearance. This correlated with a study where "Am 207" herbal extract was used to treat malaria fever, as it was found that the clearance was not dose dependent, as the suppression was highest in the low dose group (Martey *et al.*,2013). This finding may be due to the other constituents in the herbal drug which may not have anti-plasmodial activity in high doses and may reduce the effect of the other constituents with anti-plasmodial activity.

5.5 HISTOMORPHOLOGY

5.5.1 Histomorphology of the Liver

From this study there was a significant increase in the liver weight of the untreated group when compared to the control. This was in agreement with other studies which showed statistically significant increase in liver weight in the untreated group (Basir *et al*., 2012). These findings may have been as a result of the apparent effects of malaria parasite on the liver.

From this study histologically, there were presence of sinusoidal congestion, periportal infiltrates of inflammatory cells and hemozoin pigments which reduced on treatment.

This was in agreement with a study done to assess the histomorphological findings of the liver of plasmodium-infected albino mice following administration of aqueous leaf extract of 'Mangifera indica' which showed a dose dependent ameliorative change in the organization of histoachitecture such as reduction in collagen fibres, reticular fibres and hemozoin (Olayode *et al*.,2015).

Similarly, Ebeye *et al* (2007) found peri-portal inflammatory cell infiltrate and central vein congestion with similar herbal extract. But this was at variance with the study using artesunate,

amodiaquine and selenium in which there was evidence of degenerative changes in the morphology of the hepatocytes (Adebayo *et al*.,2018). The ameliorative changes with the use of some herbal drug is probably due to the effects of some of the phytochemical constituents.

CONCLUSION

In conclusion, the increased consumption of herbal drug in Nigeria may be a reason to begin local production and proper regulation of these products. The use of herbs requires good knowledge regarding the toxicity, dosage, purity, suitable solvent for extraction and adverse effects.

Based on the results of this study, the use of ‘Agbo Iba’ herbal extract may result in some degree of parasitemia clearance which invariably may lead to malaria resistance and in higher doses may be harmful; consequently this cannot be used as an alternative to orthodox medicine unless improved upon.

RECOMMENDATION

1. Government should as a matter of priority, promote the development and regulation of herbal medicines in Nigeria.
2. The constituents used in making all herbal preparation must be clearly stated on the body of the product.

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APPENDIX 1

GLOSSARY

DEFINITION OF TERMS

MALARIA is a mosquito-borne infection affecting humans and other animals caused by plasmodium parasite; a single celled microorganism (Dahalan et al., 2019).

PARASITISM derived from parasites, is a relationship between two organism where one organism, the parasite lives on or in another organism, the host, causing it some harm and is adapted structurally to this way of life (Poulin, 2007).

PLASMODIUM, is a genus of unicellular eukaryotes that are obligate parasite of vertebrates and insects (CDC, 2015).

RE-CRUDESCENCE is the return of symptoms after a symptom-free period, caused by parasites surviving in the blood as a result of inadequate or ineffective treatment occurring within 2 weeks of treatment vis-à-vis re-infection which occurs after 2 weeks (Mace, *et al*., 2018; Kim, *et al*., 2019; Popovici, *et al*., 2019).

MULTI-DRUG RESISTANCE (MDR) is antimicrobial resistance shown by plasmodium to multiple antimicrobials (Magiorakos *et al*., 2011)

COMBINATION THERAPY as used in this study is a therapy that uses more than one medication to treat a single disease (Collier, 2012).

PASSAGE as used in this study is an in-vitro procedure, which involves transfer of microorganisms into and from laboratory apparatus such as test tubes and petri dishes usually in a laboratory (Chapuis *et al*.,2011).

RODENTS as used in this study are mammals of the order rodentia, which are made up of a single pair of continuously growing incisors in each of the upper and lower jaws (Single *et al*., 2001).

WISTAR RATS as used in this study are laboratory rats of the species *rattus novegicus domesticus* which are bred albino rats and kept for scientific research (Vandenbergh, 2000). It was developed at the Wistar Institute in 1906 for use in biological and medical research.

TRADITIONAL MEDICINE is the gathering of knowledge, skills and practices based on theories, beliefs and experiences indigenous to different cultures that are used to promote health, as well, as to prevent, diagnose, improve or treat physical and mental illness (Abdullahi, 2011).

COMPLIMENTARY AND ALTERNATIVE MEDICINE (CAM) is a class of medicine that includes a variety of treatment options that are not within the realms of orthodox medicine (Shuval and Averbuch, 2012).

HERBS are small, seed producing plant without a woody stem in which all parts above ground level die back to the ground at the end of each growing season (Small, 2006).

PHYTOCHEMICAL: Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that

work with nutrients and fibres to act as a defense system against diseases or more accurately, to protect against disease(Ekwueme *et al*., 2015).

ACUTE TOXICITY are unwanted effects that occur either immediately or at a short time interval after a single or multiple administration of such substance (Enegide *et al* ., 2013).

UNWANTED (OR ADVERSE) EFFECTS are effects that produce functional impact in organs and/or biochemical lesions, which could alter the functioning of the organism in general or individual organs (Wittes *et al*., 2010).

LIVER FUNCTION TESTS (LFT) is a group of clinical biochemistry laboratory blood investigations designed to give insights about the state of the liver (Lee, 2009).

Finally**, LETHAL DOSE 50 (LD50)** is the median lethal dose of a drug which kills 50% of the test animal when used in measuring acute toxicity (Erhirhie *et al*., 2018).

APPENDIX 2

CALCULATIONS AND PROCEDURES

METHOD USED TO CALCULATE THE DOSE OF THE DRUG AND HERBAL EXTRACT.

Now the total daily adult dose for Lonart is 4 tablets twice daily, which amounts to 8 tablets a day. Since 1 tablet contains 140mg of the drug, 8 tablets will contain (8 x 140) which is 1120mg.Since an average man weighs 70kg, then

1120mg = 70kg

Since 1kg = 1000g, so 70kg = 70,000g

Then 1120mg = 70,000 g

Now the average weight of the rats in the study was calculated from the individual rat's weight at the commencement of the study as 97.5g.

To find the dose for the rats will then be

1120mg = 70,000 g

? mg = 97.5g, if we cross multiply we have, ? mg = 1.56mg, which is the dose for one rat in a day. If we dissolve one tablet which is 140mg in 100 milliliters (ml) of water, then the equivalent in mls will be

140mg = 100mls ,then 1.56mg = ?(mls),so if we cross multiply, we will get 1.1ml per day and since it is taken twice a day in divided doses, then 1.1ml divided by 2,will give us 0.55ml,which was approximated to 0.6mls.

This was given to each rat in the standard drug group twice daily for 3 days.

Herbal extract.

According to the local vendors; an average man normally consumes about 50centillitre (cl) of the aqueous extract per day, which is taken twice daily in divided doses.

Since 1 centiliter = 10mls

Then 50 centilitre = 500mls, so an average man consumes about 500mls a day in 2 divided doses, which is 250 mls twice daily, so 250mls/dose

250 mls – 70kg

Since 1kg = 1000g, then, 70kg = 70,000g

250mls – 70,000g

Therefore for the rats

250mls = 70,000g

? ml = 97.5g

Cross multiply and we have 0.35mls, which was approximated to 0.4mls per dose

So for the low,moderate and high dose,we gave,0.2mls as low dose

0.4mls as moderate dose and 0.6mls as high dose.

BIOCHEMICAL ANALYSIS.

DETERMINATION OF ASPARTATE AMINOTRANSFERASE

PRINCIPLE

GOT

Alpha-oxaloglutarate + L – aspartate ---------- L-glutamate + oxaloacetate

Aspartate aminotransferase measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4 – dinitro phenylhydrazine.

Sample – serum

Reagent composition

Contents	Initial concentration of solution
Rl .Buffer	
Phosphate buffer	100 mMol .ph 7.4
L-aspartate	100 mMol/l
Alpha – oxoglutarate	20mMol/l
R2. 2,4 – dinitrophenyl hydrallazine	20mMol/l

PROCEDURE

Wavelength Hg 546cm

Cuvette l cmg light path 37^{o} c.

For measurement against reagent blank

0.5mls of reagent was pipette into the tubes, and then 0.lmls of distilled water was pipetted into the tubes labeled blank, while 0. l ml of the sample was pipetted into the tubes labeled test. This was mixed thoroughly and incubated for exactly 30mins at 37^{0} c. After incubation, 0.5mls of 2,4 dinitrophenyl hydrazine was added to both the blank and test, again mixed thoroughly and allowed to stand for exactly 20mins at 20-25^{o}c.Again 5mls of sodium hydroxide was added to both the blank and the sample. This was mixed and the absorbance of the sample was read against the reagent blank after 5 minutes.

For measurement against the sample blank

0.5mls of reagent l was pipetted into test tube; 0.5mls of the sample was also pipetted into the tube. This was mixed and incubated for exactly 30mins at 37^{0} c.

0.5 mls of reagent 2, was pipetted into test tube of which 0.l mls of the sample was also pipetted into the tube labeled blank. Then 0.5mls of the sample was pipetted into the tubes with reagent. They were mixed thoroughly and allowed to stand for exactly 20mins at 20-25^{0} c. Then 5mls of sodium hydroxide were then added to both tubes. This was mixed and then the absorbance of the sample was read against the sample blank after 8 minutes.

DETERMINATION OF ALANINE AMINOTRANSFERASE (ALT)

PRINCIPLE

GPT

Alpha-oxoglutarate + L alanine ---------- L-glutamate + pyruvate.

Alanine aminotransferase was measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenyl hydrazine.

Sample - serum

Reagent composition

R l .Buffer

Phosphate buffer	100mMol/L,ph 7.4
L-alanine	200mMol/l
Alpha oxoglutarate	2.0 mMol/L
R 2 – 2,4- dinitrophenylhydrazine	2.0mMol/l

Two test tubes were arranged and labeled as test and blank.0.5mls of reagent was pipetted into the tubes.0.lml of distilled water was pipette into the tube labeled as blank, while 0.lml of sample was pipetted into the tubes labeled as test and mixed thoroughly and incubated for 30mins at 37^0 c. Then 0.5mls of reagent 2 was added into the 2 tubes and mixed thoroughly for exactly 20mins, at 20-25^0 c, and then 5mls of sodium hydroxide was added to the two tubes and mixed. The absorbance of sample was read against reagent blank after 8 minutes.

DETERMINATION OF ALKALINE PHOSPHATATE

PRINCIPLE

Procedure for estimation

Wavelength	hg 546cm
Cuvette	1cm light path
Incubation temp	37^0 c.

Reagent composition

Sodium thymolphthalein monophosphate in 0-2 M2-amino-2-methyl—propanol buffer, inactive ingredients,alkaline phosphatase substrate 3-6mM wetting agent,magnesium chloride 10mM,preservatives as well as ph of 10.2 and 0.l.

Specimen collection

This is cooled and assayed immediately. Each laboratory should possess a timed pattern for sample collection and analysis because ALP levels may rise significantly whenever stored at 2-8^0 c.

PROCEDURE

l. For each sample, dispense 0.5mls of alkaline phosphatase substrate into labeled test tubes and equilibrate to 37^0 c for 3 minutes.

2. At timed intervals, 0.05ml of each standard and control were added to its respective test tubes. This was mixed gently; de-ionized water was used as sample for reagent blank.

3. It was then incubated for exactly 10minutes at 37^0 c.

4. Then 2.5mls of alkaline phosphatase colour developer was added at timed interval and mixed well.

5. The wavelength of the spectrophotometer was set at 590nm

6. Then the absorbance of the sample was read and recorded

DETERMINATION OF PROTEIN

PRINCIPLE

Total protein

Reagent composition

Contents	concentration of solution
R l Biuret reagent	
Sodium hydroxide	100 mMol/l
Na –K-tartrate	16mMol/l
Potassium iodide	155 mMol/l

Cupric sulphate	6mMol/l
R 2 Blank reagent	
Sodium hydroxide	100mMol/l
Na-K-tartrate	16mMol/l
CAL ,Standard	
Protein	
Wavelength	Hg 546 nm(530-570)
Cuvette	l cm light path
Temperature	20-25 ^{0} c.

Measurement against reagent blank

PROCEDURE

Three test tubes were arranged and labeled as blank, standard and test.2000ul of biuret reagent was pipetted into the tubes, 20ul of protein standard was pipette into the tubes labeled as test. The mixture was incubated for 10 minutes at room temperature and the absorbance of the sample was read against the blank.

DETERMINATION OF ALBUMIN

PROCEDURE

Labeled as test, standard and blank,3000ul of bromocresol green reagent was pipetted into the tubes the mixtures was read immediately by reading the absorbance of sample against blank.

Reagent COMPOSITION

CONTENTS

R 1,BCG concentrates

Succinate buffer 75mMol;ph4.2

Bromocresol green 1.7mMol

Brij 35 Preservation

KIDNEY FUNCTION TEST (KFT)

DETERMINATION OF CREATININE

PROCEDURE

100ul of test and standard was added to the 2ml of working reagent consisting of l ml of alkaline solution and l ml of picric acid. This was then mixed and incubated at room temperature for 10mins and read at 510nm.The creatinine value was calculated using the reagent absorbance.

HISTOLOGY

H & E Staining

Preparation

Formular:

Haematoxylin-- - - - - - - -	2.5g
Absolute alcohol- - - - - - -	50ml
Ammonium or Potassium alum- - - - - -	50g
Distilled water- - - - - - - -	500ml
Mercury oxide- - - - - - - -	1.5g
Glacial acetic acid- - - - - - - -	20ml

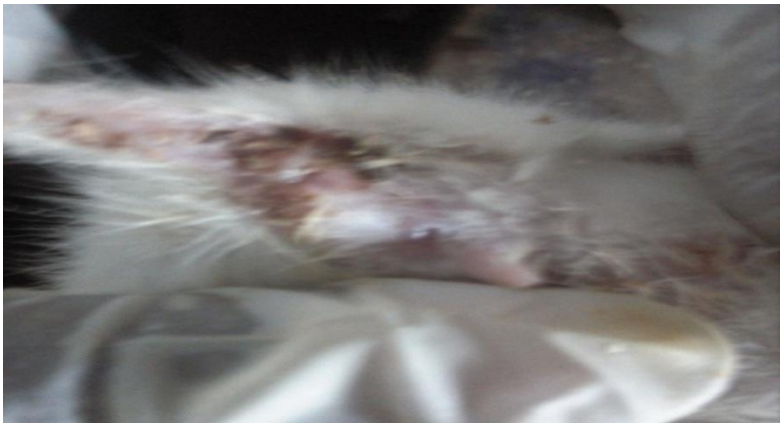
The haematoxylin was dissolved in the absolute alcohol, and the alum in the water using heat if necessary and the two solutions were mixed together. The mixture was heated to boiling point and mercuric oxide was added cooled rapidly by plunging the flask into cold water. The solution was then ready for staining as soon as it was cooled. Although optional, it is preferable to add the glacial acetic acid before use as this gives more precise and selective staining.

APPENDIX 3

FIELD WORK

Passage done in(IAMRAT).

Weighed organ with digital scale.

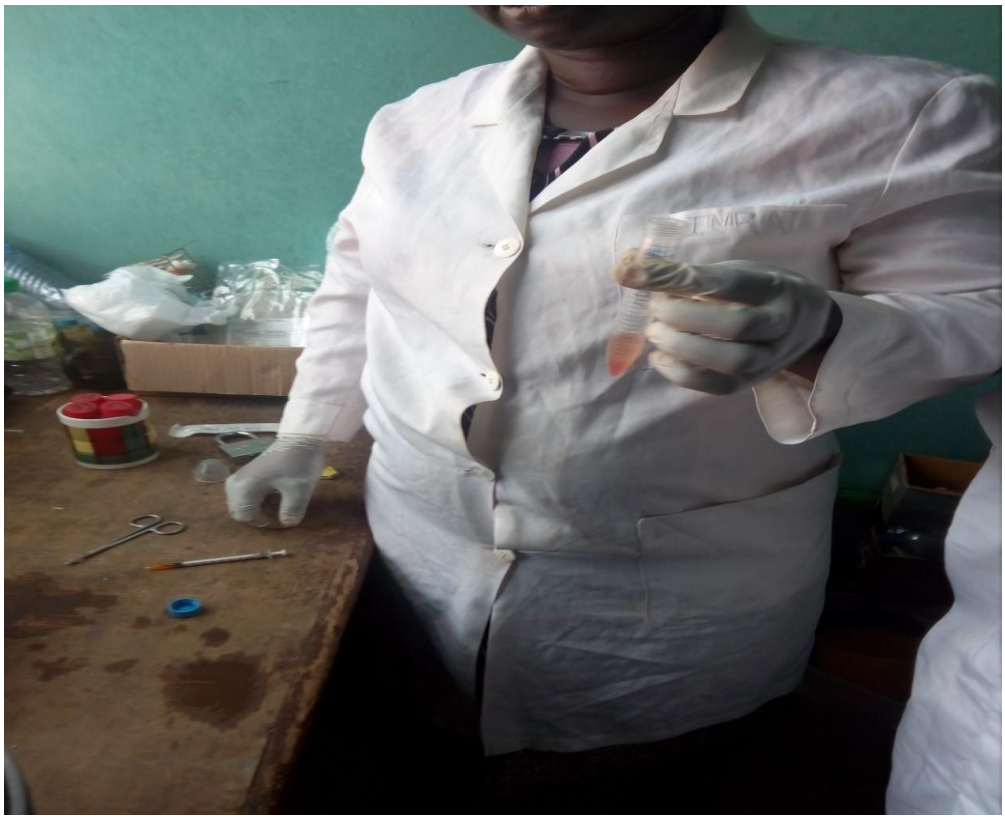


Sacrificed wistar rats

Cardiac puncture during passage.

Manual scale used.

Sacrifised mouse



Dilution of parasitized red cells

Rats during acclimatization

Automated analyzing machine for biochemical tests.

APPENDIX 4

ETHICAL CLEARANCE

RESEARCH ETHICS COMMITTEE

COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

Chairman: Prof. E.S. Idogun
MB. ChB, MWCP, MSc, FMCpath
MPH, D.Th.
08023185483

P.M.B 1154, BENIN CITY

Email: researchethics.cms@gmail.com

Our Ref: CMS/REC/01/VOL.2/060

Date: 22nd March, 2019.

Re: "A study of the histomophological effects of administration of a multi herbal drug extract (Agbo-Iba) on the liver and kidney of malaria induced wistar rat".

Name of Principal Investigator: Andrew Odigie
Department of Anatomy,
School of Basic Medical Sciences,
College of Medicine,
University of Benin,
Benin City.

REC Approval No: CMS/REC/2019/060

This is to inform you that the research described in the submitted proposal, the Informed Consent Forms and other participant information materials have been reviewed and approved by the College Research Ethics Committee, University of Benin.

This approval dates from 22nd March, 2019 to 21st March, 2020. In multi-year research, endeavour to submit your annual report to the REC early in order to obtain renewal of your approval and avoid disruption of your research.

The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the code including ensuring that all adverse events are reported promptly to the REC. No, changes are permitted in the research without prior approval by REC except in circumstances outlined in the code. REC reserves the right to conduct compliance visit to your research site without prior notice.

Thank you.

PROF. E.S. IDOGUN
Chairman, REC

20/3/19

APPLICATION FOR PLASMODIUM BERGHEI

Mr Andrew Odigie.

Department of Anatomy

University of Benin,Benin City. Edo state.

Professor Ademowo George Olu-Segun

Head of Department,

Institute for Advanced Medical Research and Training(IAMRAT),

College of Medicine,UCH,Ibadan,Oyo State.

Dear sir,

PURCHASE OF PLASMODIUM BERGHEI INFECTED MOUSE.

I wish to request for your assistance to enable me purchase plasmodium berghei infected mice from your research institute for use in my research work.

I am a post graduate student of the University of Benin,in the department of Anatomy,School of Basic Medical Science.As part of my requirement for the award of MSc in Anatomy,I will be carrying out a research on "A STUDY OF THE HISTOMOPHOLOGICAL EFFECTS OF ADMINISTRATION OF A MULTI HERBAL DRUG EXTRACT(AGBO IBA)ON THE LIVER AND KIDNEY OF MALARIA INDUCED WISTAR RAT".

My supervisor is Professor Dorothea Baxter Grillo;Professor of Anatomy,at the University of Benin.

I have included my Ethical Approval to this request. I will be very grateful if my request is granted and thanks for your anticipated co-operation.

Yours Faithfully

Andrew Odigie.

.....

Supervisor

PROF. BAXTER-GRILLO

.....

D.Baxter Grillo