# TOXICITY STUDIES OF THE ETHANOLIC STEM BARK EXTRACT OF *RANDIA NILOTICA* STAPF. IN RATS

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NIGERIA

NOVEMBER, 2015

## DECLARATION

I declare that the work in this thesis entitled **"Toxicity Studies of the Ethanolic Stem Bark Extract of *Randia nilotica* Stapf. in Rats"** has been performed by me in the Department of Pharmacology and Therapeutics under the supervision of Dr. N.M. Danjuma, Dr. J.I. Ejiofor and Prof. S.B. Oladele. The information derived from literature has duly been acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any other university.

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|---|---|---|
| Name of Student | Signature | Date |

## CERTIFICATION

This Dissertation entitled "TOXICITY STUDIES Of THE ETHANOLIC STEM BARK EXTRACT OF *RANDIA NILOTICA* STAPF. IN RATS" by Micah TIMOTHY meets the regulation governing the award of the degree of Master of Science in Pharmacology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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## DEDICATION

This Dissertation is dedicated to my Parents, Mr. and late Mrs. Timothy Tambiyi. You saw the "fire" in me and helped fan it to this conflagration.

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ABSTRACT

The use of medicinal plant extracts to maintain health, prevent or cure various diseases is known since ancient times. However, most times, there is little or no knowledge of the associated possible toxic injuries on vital organs morphology or functions from the use of such plants in whole or parts. *Randia nilotica* had long been known and used traditionally for various diseases, with no information on its toxicity profile. This study aims at evaluating the ethanolic stem bark extract of the plant for acute, sub-chronic and chronic toxicities. Three groups of animals were treated with extract (250, 500 and 1000 mg/kg daily) and a fourth one with distilled water for 28 and 90 days. Blood samples were collected at the end of treatment periods and used for biochemical and haematological analysis. The brain, heart, kidney, liver and spleen of all the rats in each group were also isolated and weighed prior to fixing in 10% buffered formalin and Bouin's fluid (brain only) for histopathological examinations. The extract was found to contain saponins, carbohydrates, cardiac glycosides, triterpenes, flavonoids and tannins, while alkaloids, steroids, cardenolides and anthraquinones were absent. The $LD_{50}$ was greater than 5000 mg/kg body weight, since no mortality was found at this dose level. 1000 mg/kg extract significantly ($p \leq 0.05$) decreased the PCV level following 28 days oral administration, while for the 90 days daily oral administration, both the 500 and 1000 mg/kg doses caused significant ($p \leq 0.05$) decrease in PCV, Hb and RBC counts. The mean cell volume (MCV) was also significantly ($p \leq 0.05$) decreased, but only at 1000 mg/kg following 90 days daily oral administration. The 28

days oral administration caused no alteration in blood urea nitrogen, creatinine and calcium levels at all dose levels, but the 90 days treatment caused significant ($p \leq 0.05$) increase in blood urea nitrogen. No significant ($p \leq 0.05$) increases and decreases were observed in the liver enzymes, total protein, albumin, total cholesterol, triglycerides and high density lipoprotein for the 28 days treatment, but the 90 days administration showed significant ($p \leq 0.05$) increase of high density lipoprotein (HDL) level only at the 250 mg/kg dose. Significant ($p \leq 0.05$) decrease in brain weight was observed following 90 days daily oral administration. The histopathology of the organs showed both dose and duration- dependent alterations. The 28 day oral administration showed neuronal degeneration and congested blood vessels of the brain, the heart showed myocardial congestion, infiltration of inflammatory cells and myocardial haemorrhage. The kidney showed atrophy of glomeruli, haemorrhage, tubular necrosis, cellular infiltration, hyper cellular glomeruli and adhesion of the parietal surface with Bowman's capsule. The alterations on the spleen were congestion of blood vessels and depletion of lymphocytes while the liver had congestion of blood vessels, perivascular infiltration of inflammatory cells and necrosis of hepatocytes. The 90 days treatment further revealed cerebral congestion in the brain, fragmentation of the heart's muscle fibres and oedema. Effects on the kidney were vacuolation of glomeruli, infiltration of inflammatory cells and congestion of glomeruli. The alterations on the spleen were congestion of blood vessels and depletion of lymphocytes, while the liver had congestion of blood vessels, perivascular infiltration of inflammatory cells and necrosis of hepatocytes. The major inference that could be drawn from the above work is in the fact that the stem bark extract of *Randia nilotica* is toxic to the organs at the doses tested, over prolonged period. It is therefore concluded that, while it could have pharmacological utility, *Randia nilotica* merits further attention with respect to toxicity

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ABBREVIATIONS

The following abbreviations and symbols were used throughout this project report:

%: Per cent

ACEIs: Angiotensin-converting enzymes inhibitors

AD: *Anno Domini*

AIDS: Acquired Immuno Deficiency Syndrome

ALP: Alkaline phosphatase

ALT: Alanine Aminotransferase

ANOVA: Analysis Of Variance

AP: Alkaline phosphatase

AST: Aspartate Aminotransferase

AT: Amino transferase

AV: Atrio Ventricular

B: Basophil

BBB: Blood Brain Barrier

BC: Before Christ

BNF: British National Formulary

BUN: Blood Urea Nitrogen

$Ca^{2+}$: Calcium ion

CNS: Central Nervous System

DNA: Deoxyribonucleic acid

E: Eosinophils

| | |
|---|---|
| EDTA: | Ethylene diamine tetraacetic acid |
| ESR: | Erythrocyte Sedimentation Rate |
| Fl: | Femtoliter |
| g/dl: | Gramme per deciliter |
| g/l: | gramme/litre |
| GABA: | Gamma Amino Butyric Acid |
| GFR: | Glomerular Filtration Rate |
| GGT: | Gamma Glutamyl Transpeptidase |
| H & E: | Haematoxylin and Eosin |
| $H_2SO_4$: | Tetraoxosulphate (VI) acid |
| Hb: | Haemoglobin |
| HCl: | Hydrochloric Acid |
| HDL: | High Density Lipoprotein |
| IL-18: | Interleukin-18 |
| INH: | Isoniazid |
| Kg: | Kilogramme |
| KIM-1: | Kidney Injury Molecule-1 |
| L: | Lymphocyte |
| $LD_{50}$: | Median Lethal dose |
| LDL: | Low Density Lipoprotein |
| LFTs: | Liver Function Tests |
| M: | Monocyte |
| m±SEM: | Mean Plus/minus Standard Error of Mean |

| | |
|---|---|
| MCH: | Mean Cell Haemoglobin |
| MCHC: | Mean Corpuscular Haemoglobin Concentration |
| MCV: | Mean Cell Volume |
| MDR-S: | Multi-Drug Resistant Strains |
| mg/dl: | Milligramme/deciliter |
| Mg: | Milligramme |
| Mmol/l: | Millimole/litre |
| N: | Neutrophil |
| NAG: | N-Acetyl-β-D-glucosaminidase |
| NaOH: | Sodium Hydroxide |
| NAPQI: | N- Acetyl – Para - Benzoquinone Imine |
| NGAL: | Neutrophil Gelatinase-Associated Lipocalin |
| NOAEL: | No Observable Adverse Effect Level |
| NSAIDs: | Non-Steroidal Anti-Inflammatory Drugs |
| OECD: | Organization of Economic Cooperation and Development |
| PCM: | Paracetamol |
| PCV: | Packed Cell Volume |
| Pg: | Pico liter (Number of cells per liter; $10^{12}$/L) |
| $_P$H: | Measure of Acidity or alkalinity |
| PT: | Prothrombin Time |
| PTT: | Partial Thromboplastin Time |
| RBC: | Red Blood Cell |
| REM: | Rapid Eye Movement. |

RNE: *Randia nilotica* Extract

SGOT: Serum Glutamic Oxaloacetic Transaminase

SGPT: Serum Glutamic Pyruvate Transferase

SSRIs: Selective Serotonin Re-uptake Inhibitors

TC: Total Cholesterol

TG: Triglyceride

TP: Total Protein

u/l: Unit/litre

WBC: White Blood Cell

*x g*: Gravitational force

β: Beta

μM: Micrometer

# CHAPTER ONE

## 1.0 INTRODUCTION

Toxicity studies relate the dose and duration of exposure to the biochemical, physiological, pharmacological effects that occur in an organism - animals or humans (Takayuki *et al.,* 1993). Toxicity studies are conducted to provide greater understanding of the potential intrinsic hazard of chemical substances including side effects, undesirable or adverse effects. It provides an estimate of the safety margin of agents which is often used to determine an initial safe starting dose for clinical trials and, ultimately, to achieve successful review of registration dossiers that support marketing approval and use of new medicines within the wider population (Robbinson *et al.,* 2009). Thus, historically toxicology forms the basis of drug research and therapeutics and it has continued to expand and develop in scope as both science and art (Casarette *et al.,* 1996). Toxicity evaluations which are routinely performed in the investigation of new chemical agents include the acute, sub-acute and chronic toxicity studies (Gupta *et al.,* 2012).

The use of medicinal plant extracts to cure various diseases is known since ancient times. In India, the practice of using plants for a wide variety of diseases is being carried out over ages and such medicinal plants are used to maintain and promote health, prevent and/or cure diseases (Vogel and Vogel, 1997; Siddharthan, 2007; Madhulika, 2010). Scientific research into substances used in herbal medicine practices led to the discovery of relevant plants with useful chemical constituents that can be used in treatment of various diseases (Amos *et al.,* 2001). Quite a number of medicinal plants discovered by herbal practitioners have proven to be important sources of potential therapeutic agents, e.g., quinine (antimalarial agent) from Cinchona bark; digoxin and digitoxin agents (cardiac glycosides)

from *Digitalis purpurea;* zingiberone (anti-helminthic) from *Zingiber officinalis*; vincristine and vinblastine alkaloids (anti-leukaemic agents) from *Catharantus roseus*, bourvadine (anti-cancer) from *Bourvadin ternifolia;* sinoculine (anticancer) from *Curculus tribolus* and taxol (anticancer) from *Brevifolia* (Hardman, 1991; Udupa *et al.,* 1995; Taranalli and Kuppast, 1996; Rathi *et al.,* 2004; Veerapur *et al.,* 2004; Odumegwu *et al.,* 2008; Esimone *et al.,* 2009; Patil *et al.,* 2009; Kumar *et al.,* 2012).

In Sudan, the decoction of *Randia nilotica* was reported to be used orally for mental illness, convulsion and epilepsy (Hedberg *et al.,* 1983; Chabra *et al.,* 1991) and the stem-bark was reported to be used as an anthelminthic and for jaundice and rabies (Bashir *et al.,* 1981), while in the Northern part of Nigeria, the stem-bark of the plant is claimed to be used in the management of cancer (Personal communication, 20 March, 2014), and for prophylaxis and treatment of snake bites (Ismaila and Adamu, 2012). The claimed molluscidal activity of the fruit against schistosomiasis transmitting snail *Biomphalaria glabrata* had also been authenticated as well as the CNS depressant effect of the leaves, root and stem barks of the plant (Lemmich *et al.,* 1995; Danjuma, *et al.,* 2009). However, aside the acute toxicity studies carried out for purpose of dose selections by the different researchers, no other toxicity study (sub-chronic or chronic) on the medicinal use of any part of *Randia nilotica* had been reported.

## 1.1 Statement of Research Problems

The measure of acute lethality such as $LD_{50}$ may not accurately reflect the full spectrum of toxicity or hazard associated with drugs or chemicals, thus, the sub-chronic and chronic

toxicity studies are equally important to be able to establish a wider spectrum of the toxic effects associated with the use of any drug or chemical (Casarette *et al.,* 1996).

The knowledge of the overall toxic effects of any drug often helps in the expansion of medical care whereby an identified drug can be used with the awareness of the risk/benefit effect associated with it (Timbrell, 2005).

The use of medicinal plants like *Randia nilotica* in health care without reference to long term adverse effects may constitute further health challenge; and it had been noted that reasonably large population of people in Northern Nigeria use the plant for one ailment or the other without due consideration to its toxic effect.

There is need to determine the toxic effects associated with the duration of use of herbal preparations. This has become imperative considering the widespread popularity of the use of natural products especially herbal preparations (Agrahari *et al.,* 2010).

## 1.2 Justification of the Study

Toxicity is a major pharmacological issue as it affects the overall safety of medication and so establishes safe and acceptable formulations, doses, interval and duration of therapy either in orthodox or traditional medical practice.

Vast majority of the world population depend on traditional herbal medicine for treatment of various ailments and in-fact most present day "medicines" were derived from herbal traditions (Eloff, 1998; Ezeonwumelu *et al.,* 2012).

In developed countries more recently, interest has risen in herbal medicines for healthy lifestyles Thus, pharmaceutical research interest in the value of plants as sources of new drugs is rapidly growing (Aniagu *et al.,* 2004; Chindo *et al.,* 2012).

A proper scientific evaluation of herbal medicines with emphasis on establishing their pharmacological and toxicological profiles is an imperative aspect of the efficacy and safety studies of drugs (Salawu *et al.,* 2009; Chindo *et al.,* 2012).

The need to determine the toxicity effects associated with the prolonged (sub-chronic and chronic) use of *Randia nilotica* informed this study.

## 1.3 Aim and Specific Objectives of the Study

The main aim of this study was to undertake toxicity evaluations on the ethanolic stem bark extract of *Randia nilotica* stapf in Wistar rats.

**The Specific Objectives were:**

- To evaluate Phytochemistry of the stem bark extract of the plant
- To determine acute toxicity study - median lethal dose ($LD_{50}$) determination.
- To assess the sub-chronic and chronic toxicity studies of the hydro-ethanolic stem bark extract of the plant with view to evaluate effects on:
    - Hematological parameters
    - some Renal indices
    - Some Biochemical indices

- Histopathological parameters.

## 1.4 Null Hypothesis

The ethanolic stem bark extract of *Randia nilotica* is safe for medicinal use

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Toxicity Evaluations of Chemicals

Toxicology is defined as the science of poisons and deals with the study of the action of chemicals - drugs, toxins, xenobiotics, etc. (Timbrell, 2005; Orisakwe, 2006). The toxic effects of chemicals may occur in many forms ranging from immediate death or subtle changes that take months or years to manifest. It may occur at various levels such as cells, tissues, organs or system dysfunctions (Timbrell, 2012). Progressive understanding of toxicology began way back in earliest times with the cave dwellers who recognized chemical substances from plants, animals and minerals, and used them for treatment and warfare (Timbrell, 2005). Paracelsus (1500 AD) documented that the body's response to chemical agents depends on the dose received (beneficial or harmful) in which the therapeutic doses of chemicals produce beneficial effects, while harmful doses produce toxic effects. This was inferred from the statement "All substances are poisons; the right dose differentiates a poison and a remedy". Thus, the study of the toxic effects of the harmful doses of chemical agents is referred to as toxicology (Shibamoto and Bjeldanes, 1993).

Toxicology forms the basis of drug research in evaluating the therapeutic and toxic aspects of drugs (Casarette *et al.,* 1996). It is a cascade of events starting with exposure and proceeding through absorption, distribution and metabolism and resulting in interaction with cellular macromolecules - DNA or proteins (Hodgson, 2004). Toxicity can be acute, sub-chronic or chronic, depending on the duration of exposure. Acute toxicity manifests within a relatively short period of time and could be an immediate effect or takes few days

(less than seven days) to manifest. Toxicity of a relatively prolonged effect (sub-chronic) occurs from repeated exposure that takes up to fourteen days and above, while chronic toxicity takes a very long time of up to three months and above to manifest (Casarez, 2001). Toxicity testing of new compounds or extracts is carried out in laboratory animals with a view to identify potential hazards (Rang *et al.,* 2007).

## 2.2 Spectrum of Dose-Response Relationship in Toxicology

Exposure may be via air, water, food or medication. Dose can be defined as the amount of substance taken in by an organism at specific times or intervals, while response is any change in an organism's normal state, due to the dose administered (Rang *et al.,* 2007; Alewu, 2010; Sani, 2011). The degree of response depends on the amount of toxicant at the target site, thus, toxic effects can be negligible or severe (James *et al.,* 2000). The relationship between dose and response produced follows a predictable pattern in that as the dose increases, the response also increases, until a maximum level of response is reached (Ballantyne *et al.,* 1993; Casarez, 2001).

Appropriate dose selection is essential in establishing toxicity. The tolerability of animal species to the toxic agents can be determined by obvious clinical signs as changes in behavioural patterns, reductions in body weight, decrease in food consumption and/or water intake, organ or tissue damages (histopathology), etc. Depending on the nature of the test item, other specific parameters may be used. These may include haematological, biochemical and other changes or clinical signs (Robinson *et al.,* 2009).

## 2.3. Routes of Exposure to Toxic Substances

Results obtained from toxicity studies can be different for the same dose and the level of intoxication depends on the route of exposure. Toxicants can be inhaled, ingested or absorbed through the skin (Everhard *et al.,* 1976). The route by which a substance enters the body, and the nature of the substance (polar, non polar) determines the amount, rate, and extent of absorption as well as the effects that will occur from the toxic agent (James *et al.,* 2000).

## 2.4. Physiology and Pathophysiology of toxic effects on some target organs

### 2.4.1 Liver

The liver is the largest gland in the body (Traber *et al.,* 1988) that plays important roles in detoxification, synthesis of many proteins (e.g., plasma albumin, coagulation proteins, etc.), excretion of bile etc (Hughes, 1996). The metabolic function of the liver can be affected by both diet and chemical substances. The liver's increased vulnerability to toxic attack is due to its involvement in various important functions (Mathenheimer, 1971; Berne and Levy, 1988; Cullen and Ruebner, 1991; Sharma *et al.,* 1991; Rang *et al.,* 2007).

Laboratory findings of liver injuries can be broadly divided into two patterns:

Liver Cell or Hepatocellular Injury

Obstructive bile duct or Cholestatic injury

Generally, injury (necrosis) of hepatocytes following toxicity will primarily cause an elevation of enzymes found within the hepatocytes such as the amino transferases (Giannini *et al.,* 2005). In hepatocellular diseases, the serum levels of alkaline phosphatase (AP) and Gamma glutamyl transpeptidase (GGT) do not rise in the same degree as the amino transferases (Nandi *et al.,* 1998). Cholestatsis, on the other hand, is characterized by

accumulation of compounds that cannot be excreted as a result of occlusion or obstruction of the biliary tree, and which cause increase in the serum concentration of such compounds such as bile pigments, enzymes, bile salts, etc. These substances in the serum would have otherwise been present within; or eliminated via the bile. Thus, the level of serum alkaline phosphatase (AP), Gamma glutamyl transpeptidase (GGT) and conjugated bilirubin which require a clear biliary tree for elimination will be elevated in conditions of biliary obstruction. The liver has a great functional reserve and hence abnormal laboratory values are indications of structural or activity anomalies in anatomical, biochemical or physiological components (Rang *et al.,* 2007).

*Amino Transferases (AT) level:* The level of serum alanine amino (glutamic pyruvate) transferase (ALT or SGPT) and serum Aspartate amino (glutamic Oxaloacetic transaminase) transferase (AST or SGOT) are the most reliable and sensitive biochemical tests of hepatocyte toxicity (Dobbs *et al.,* 2003). The release of high levels of these enzymes from damaged hepatocytes is indicative of hepatic injury. ALT is normally found in low concentrations in the liver and other tissue sites including cardiac and skeletal muscles, kidney, erythrocytes and pancreas, except that in liver disease, the level is abnormally high. The coenzyme for both enzymes is vitamin $B_6$ and thus, consistent abnormally low values of AST and ALT could be suggestive of underlying vitamin $B_6$ deficiency (Nandi *et al.,* 1998). The levels of AST/ALT could also be reduced below normal in certain abnormal conditions like necrosis (Nandi *et al.,* 1998).

*Alkaline Phosphatase levels:* Elevated alkaline phosphatase (AP) in the serum usually indicates bile duct obstruction. This enzyme is however, found in other tissues including

bones. High AP values beyond the normal adult value are found in children during periods of accelerated growth such as during pubertal growth, but of which abnormally high values could be suggestive of possible bone pathology (e.g. rickets) (Nandi *et al.,* 1998).

*Hepatotoxicity*

Hepatotoxicity or liver damage from many therapeutic agents is generally manifested as hepatitis or as abnormal laboratory findings (Limdi and Hyde, 2003) (e.g. increase or decreases in plasma aspartate transaminase activity). Aside changes in the levels of the biochemical/ physiological biomarkers (ALT, AST, AP, etc) hepatotoxicity could also occur from drug-induced tissue deaths or from the unstable, highly reactive free radical intermediates that often tend to bind covalently to biomolecules including the liver to cause deleterious damages or cell dysfunctions (Alison and Samaj, 1995; Rang *et al.,* 2007) as well as immunological reaction with the antigenic components of the liver (Boobis *et al.,* 1989).

### 2.4.2 Kidney

The kidneys are bean-shaped paired organ which is located in the dorsal abdominal cavity (Rang *et al.,* 2007). The major role of the kidney is to maintain homoeostasis of the body's internal environment by regulating the volume and electrolyte contents as well as eliminating waste products of the body (Pai *et al.,* 2005; Rang *et al.,* 2007). Biotransformation also takes place in the kidney, and this makes it susceptible to toxic agents. The kidney constitutes one percent of the body's weight, and receives 20-25 percent of the blood flow. As a result, large amounts of circulating toxicants reach the kidneys quickly. The kidney filter one-third of the plasma volume that gets to it and reabsorbs 98-

99 per cent of salt and water. Changes in kidney pH may increase its processes of active secretion and/or reabsorption resulting in cellular concentrations of toxicants (Goldstein and Schnellman, 1996; Russo *et al.,* 2007). The wide involvement of the kidney in many physiological and pharmacological functions makes the kidney a sensitive body organ (Berne and Levy, 1988; Rang *et al.,* 2007).

*2.4.2.1 Nephrotoxicity*

The identification of acute kidney injury relies on tests like blood urea nitrogen, serum creatinine and electrolyte contents ($Ca^{2+}$, $Na^{+}$, $K^{+}$ etc). However, the urine may contain certain sensitive and specific biomarkers of kidney injury that are present due to either impaired tubular reabsorption or release of tubular cell proteins in response to injury. Other potential markers of kidney injury include N-acetyl-β-Dglucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1) and interleukin-18 (IL-18) (Han *et al.,* 2002; Waikar and Bonventre, 2008). Kidney injury could be secondary to altered renal haemodynamics, direct cellular damage to the tubular epithelium or tubular obstruction of urinary flow. Injuries could result in subtle changes in tubular function, including altered urine concentrating ability and/or electrolyte handling, or a significant decline in renal functional capacity (Ferguson *et al.,* 2008). Drug-induced nephrotoxicity is a common pharmacological phenomenon (Rang and Dale, 2007).

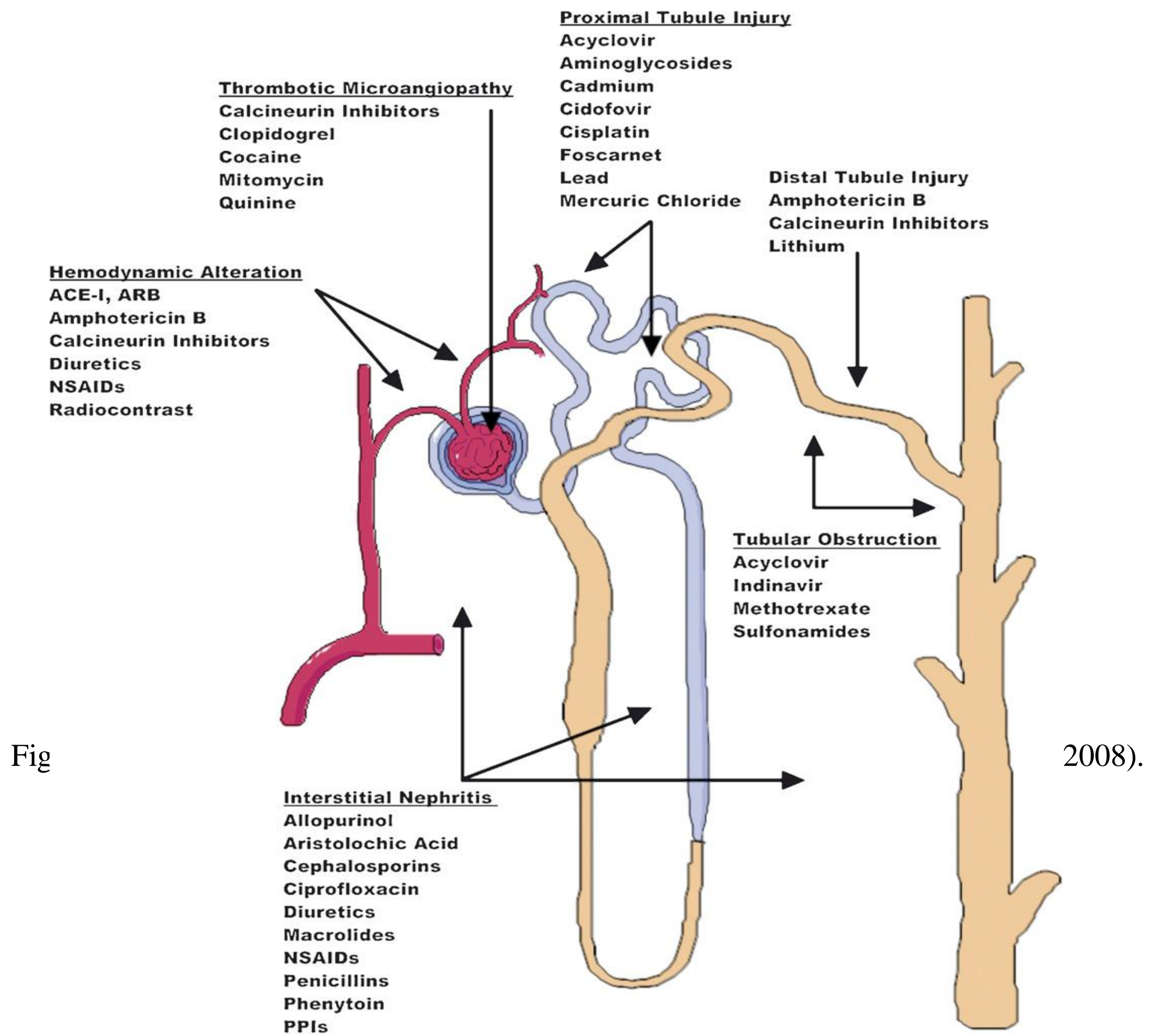



Fig ... 2008).

ACE-I: angiotensin-converting enzyme inhibitors; ARB: angiotensin II receptor blockers;
NSAID: nonsteroidal anti-inflammatory drugs

### 2.4.3 Blood

Blood is an essential tool for assessing health in humans and animals. It makes up about 6 per cent of total body weight and consists of plasma, white blood cells, red blood cells and platelets. Some cellular components and nutrients are suspended in plasma (Berne and Levy, 1988). Blood circulates through the blood vessels carrying oxygen and nutrients to the organs and removing carbon dioxide and other waste products via excretion. In addition, it is the vehicle by which hormones and other humoral transmitters reach their sites of action.

Body regions that receive a large percentage of the total cardiac output or have high blood flow/mass ratios include the liver, kidneys, heart, and brain (Hughes, 1996). The red cells, also known as erythrocytes are produced by the bone marrow in adults and have a life span of about 120 days in humans (Berne and Levy, 1988). They contain haemoglobin (Hb) – an iron-containing porphyrin compound, which takes up oxygen in the lungs and releases it to the tissues. A decrease in the circulating mass of the erythrocytes that results in hypoxia is termed anaemia, while an increase is polycythaemia. Reduction in the Hb content of erythrocytes is defined as microcytic hypochromic anemia (Hughes, 1996). A decrease may be as result of premature destruction or loss of red blood cells through haemorrhage and suppression of haematopoiesis, example, in renal failure or deficiency of iron, vitamin $B_{12}$ or folic acid which are essential for the maturation of red blood cells. Iron is needed for the formation of haem Deficiency of iron can also cause premature destruction of the red cells (Berne and Levy, 1988) resulting in hypocellularity of the marrow, direct chemical, physical or immunologic injury.

The white cells also known as the leucocytes contain 4000 – 10,000 cells per µL, of which 40 – 70% are neutrophils, 20 – 45% lymphocytes, 2 – 10% monocytes, 1 – 6% eosinophils, while less than 1% are basophils. The neutrophils, eosinophils and basophils are collectively termed granulocytes which are mostly phagocytic cells. Agents that are toxic to the bone marrow may result in reduction (leucopenia) or increase (leukocytosis) in the number of leukocytes. The qualitative disorders of white blood cells are those in which the number of white blood cells is normal, but the cells do not function normally. Neutrophils serve as major defense against infection by bacteria such as pneumococus, staphylococcus and streptococcus, which they can ingest and destroy, while increase in eosinophils (eosinophilia) may be indicative of parasitic infestations such as schistosomiasis and trichinosis (Berne and Levy, 1988). Eosinophils are also found to increase in number in cases of allergy or hypersensitivity reactions in, for example, asthma. Here, they dampen the host's immune response by limiting the antigen-induced release of mediators of inflammation. The monocytes, apart from their roles in phagocytosis, also participate in immune responses. The lymphocytes occur as B and T cells which are involved in the synthesis and secretion of specific immunoglobulin antibodies as well as in cell-mediated immune response. The platelets are the smallest cellular components and play an important role in blood clotting and it is reported that normal blood contains between 150,000 and 350,000 /µL of platelets (Berne and Levy, 1988). Reduced thrombocyte level may result in external or internal haemorrhage or loss of blood (thrombocytopenia).

Haematology and serum chemistry are important tools for assessing health in humans and animals (Boily *et al.,* 2006). Blood volume and electrolyte composition are closely regulated by complex mechanisms involving the kidneys, adrenal glands and hypothalamus

(Marcovitch, 2005). Thus, any agents that affect this regulatory system may as well affect blood cell production (Berne and Levy, 1988; Ellenhorn, 1997). Hematotoxins alter quantitative and qualitative characteristics of blood cell.

### 2.4.4 Spleen

The spleen is an important site of antibody production. It also acts as a filter, since it phagocytosize particulate matter and bacteria from blood; it probably destroys red and white cells as well as platelets at the end of their normal lifespan (Parry, 1984). The splenic pulps are the known to be the region where the red blood cells (red pulps) as well as the white blood cells (white pulps) (Weir, 1971).

In addition, the lymphoid cells are manufactured similar to those manufactured in the lymph nodes. The pulp of the spleen contains many large phagocytic reticulo-endothelial cells, and the venous sinuses are lined with similar cells. These cells function as part of a cleansing system for the blood, acting in concert with a similar system of reticulo-endothelial cells in the venous sinuses of the liver. It functions in the production of antibody and is involved in the destruction and phagocytosis of blood cells at the end of their normal lifespan. The spleen forms all types of blood cells in the embryonic stage of development while, in the adult, it produces only certain kinds of leukocytes (Parry, 1984; Zdanowicz, 2002).

The function of spleen can be affected when there is hyperplasia of the lymphoid cells or of the reticuloendothelial system (Fakunle, 1984). When the blood is invaded by infectious agents, the reticulo-endothelial cells of the spleen rapidly remove debris, bacteria, parasites,

and so forth. A decrease in white blood cell count, also termed leucopenia, results from decreased production of total white blood cells in the bone marrow or increased destruction of the cells. Leucopenia indicates bone marrow depression which occurs as a result of viral infection or toxic reactions (Goerge-Gay *et al.,* 2003).

### 2.4.5 Brain

The brain and the spinal cord make up the central nervous system. The brain is made up of the cerebral cortex which comprises of four major divisions (the occipital lobe, parietal lobe, frontal lobe and temporal lobe). The cellular components of the nervous system are glial cells and neurons. The glial cells form a mitochondrial matrix in which the neurons are embedded, insulating the neurons, and also serving some metabolic and nutritive roles (Berne and Levy, 1988; Bloom, 2006). The neurons are responsible for information processing and transfer in the nervous system. A typical neuron consists of the cell body and dendritic processes (Bloom, 2006). Blood-Brain barrier (BBB) serves as a selective permeability barrier to protect brain from injuries, but toxic substances and diseases are able to alter the permeability capacity to alter the functions or morphology of this barrier (Rang and Dale, 2007).

### 2.4.6 The heart

Although cardiac output is intermittent, continuous flow to the periphery occurs by distension of the aorta and its branches during ventricular contraction (systole) and elastic recoil of the walls of the large arteries (Berne and Levy, 1988). The heart is susceptible to anatomical or physiological pathological alterations that could be due to infections or toxic agents.

## 2.5 Acute Toxicity Studies

Acute toxicity refers to the adverse effects that occur on first exposure to a single or multiple doses of a substance within 24 hours (Rhodes *et al.,* 1993). Acute toxicity testing is the first line of toxicity assessment used in determining the toxic effect of a toxicant. Investigation of acute toxicity is the first step in the toxicological evaluation of an unknown substance where it is used to estimate the lethal dose ($LD_{50}$) and effective dose ($ED_{50}$) of drugs or xenobiotics (Turner, 1965; Kumar, 1984). $LD_{50}$ test was developed in 1927 by Trevan, J. W. in an attempt to estimate the relative poisoning potency of chemicals. $LD_{50}$ is considered the index of acute toxicity, but should not be taken as a biological constant because the result often varies due to species differences, age, sex and environment (Lorke, 1983). All chemicals of biological interest undergo acute toxicity testing to determine the order of lethality of the substance (Omaye, 2004; Lauhlou *et al.,* 2008). Thus, the objective of acute toxicity testing is to provide information for risk-benefit assessment of acute exposure (Gupta *et al.,* 2012). The more commonly used acute toxicity tests are the Lorke's method and Organization of Economic Cooperation and Development (OECD) Guidelines (420,423 and 425).

## 2.6 Sub - chronic and Chronic Toxicity Testing

Sub-chronic testing is usually a study ranging from several days to weeks. According to the British Home Office (2004), sub chronic toxicity studies are carried out with a view to identify toxicity that develops only after weeks of continuous exposure to a chemical. Test animals are monitored for changes in physical appearance, behavior, body weight, and food intake. Blood and excreta (urine, faeces) are also routinely obtained and tested for

hematological, urinary, biochemical and histopathological changes. Chronic toxicity testing refers to long-term studies over a period of three to several months or years (Gupta *et al.,* 2012; Monosson, 2013). The objective of such testing is because some adverse effects take longer time to manifest such as carcinogenesis. Extra testing beyond chronic toxicity testing could be carried out for clinical trials. For chronic toxicity evaluations, larger proportions of test objects are often used (Omaye, 2004).

## 2.7 Medicinal plants

Traditional and folklore medicines play an important role in health services around the globe. Plants, as illustrated throughout the history of civilization have served as the major source of medication for the treatment of human ailments. Until recently medicinal plants have been known to have greater appeal mostly among the rural poor, who may not be able to afford orthodox medicines. Herbal medicines are being accepted and used increasingly worldwide not only as medicines, but also as dietary supplements, along with modern chemotherapeutic agents (Agrahari *et al.,* 2010).

The use of medicinal plant extracts to cure various diseases is known since ancient times. In India, the practice of using plants for a wide variety of diseases is being carried out over ages; and the use of such medicinal plants to maintain and promote health, prevent and or cure diseases are well documented in Ayurveda (Siddharthan, 2007; Madhulika, 2010).

## 2.8 The plant, *Randia nilotica*

Plate I: The leaves and stem of *Randia nilotica*

### 2.8.1 Taxonomy of *Randia nilotica*

| | |
|---|---|
| Family: | *Rubiaceae* |
| Genus: | *Catunaregam* |
| Specific epithet: | *nilotica* - (Stapf) |
| Botanical name: | *Catunaregam nilotica* (Stapf) |
| Common names: | *Lachnosiphonium niloticum* (Stapf) Dandy, *Randia nilotica* Stapf, *Xeromphis nilotica* (Stapf) Kea, *Catunaregam nilotica* |
| Local names: | Barbaji, tsibra or kwanarya (Hausa); Gial goti (Fulani) (Dalziel, 1937; www.zipcodezoo.com., 2014). |

### 2.8.2 Medicinal uses of *Randia nilotica*

The use of medicinal plant extracts to cure various diseases is known since ancient times (Morton, 1987; Anonymous, 1992; Dewan *et al.,* 2000; Sangraula *et al.,* 2002; Akinyemi *et al.,* 2005; Dahiru and John-Africa 2006; Fegert *et al.,* 2006; Goyal *et al.,* 2006; Adejumobi *et al.,* 2008; Ya'u *et al.,* 2013).

In the Northern part of Nigeria, oral communication claim has it that the stem bark is used in the management of cancers, as well as in the prophylaxis and treatment of snake bites in humans and livestock (Ismaila and Adamu, 2012). In Sudan, the fruits show a very strong activity against the schistosomiasis transmitting snail *Biomphalaria glabrata* (Lemmich *et al.,* 1995), also well known for their antispasmodic, anti-infertility, and antidysenteric properties (Varshney *et al.,* 1978). The bark is also used as a fish poison and in the treatment of jaundice and rabies (Bashir *et al.,* 1981). Danjuma *et al.,* (2009), demonstrated the central nervous system depressant effect of the as claimed in folklore. a decoction is

used orally for mental breakdown and for convulsions (Chabra *et al.,* 1991). Dried root in Tanzania is used against epilepsy and for madness (Hedborg *et al.,* 1983).

### 2.8.3 Geographical Distribution and Description of *Randia nilotica*

*Randia nilotica* is a low land shrub or tree widespread in the Sudan and central east and West Africa (Lemmich *et al.,* 1995). Branches of the plant are whitish and have sharp spines 1-3 cm long. Its leaves and flowers are borne in pairs below the spines. Flowers are paired or solitary and terminate at the lateral branchlets. Fruits are 1.2 – 2 cm long, 1 – 1.5 cm broad, ellipsoid, glabrous and two-celled (Hutchinson *et al.,* 1963).

# CHAPTER THREE

# 3.0 MATERIALS AND METHODS

## 3.1. Collection and Identification of the Plant Material

The stem bark of the plant *Randia nilotica* was collected at Galadimawa village in Giwa Local Government of Kaduna State, Nigeria in the month of April, 2013. It was identified and authenticated by Mall. Umar Shehu Galla, a taxonomist with the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna, which was compared with a voucher specimen number of 2867 and deposited in the herbarium section of the same Department.

## 3.2. Experimental Animals

Wistar rats of both sexes weighing between 180 – 270 g obtained from the Animal House facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used. The animals were maintained on standard rodents' feed and water *ad libitum*. The female nulliparous rat groups were kept in separate cages to avoid pregnancy.

## 3.3 Equipment

The following equipment were used:

- Mettler scale analytical weighing balance (P162, Gallenkamp)
- Weighing scale (W.T. Avery, U.K.)
- Microscope (Fisher Science Education, China)
- Biochemical Analyser (Selectra XL; Vitascientifics, Netherlands)
- Hetteich Universal Centrifuge (Universal 32, Austria)
- Evaporating dish with water bath (Biotech. Laboratories, U.K.)

Others were: cotton wool, separating funnel, conical flask, measuring cylinder, hand gloves, cages, plain and Ethylene diamine tetra acetic Acid (EDTA)-buffered sample bottles, syringes and Needles, stop watch, mortar and pestle etc.

### 3.4 Chemicals and Reagents

The following chemicals, which were of standard grades, were used:

- Tetraoxosulphate (VI) acid ($H_2SO_4$), acetic anhydrite, Magnesium, NaOH, Potassium Bismuth Iodide, Potassium Mercuric Iodide, ferric chloride, lead sub-acetate, bromine water, Distilled water, α-naphthol (BDH Chemicals limited, England)
- 10 % buffered formalin (900 ml distilled water + 100 ml 40% formalin) (BDH Chemicals limited, England)
- Bouin's fluid (250 ml 40 % formalin + 750 ml saturated solution of picric acid + 50 ml acetic acid, for brain preservation) (BDH Chemicals limited, England)
- Ethyl Alcohol (M&B, UK)

## 3.5.0 Methods

### 3.5.1 Extraction of the Plant Material

The stem bark of *Randia nilotica* was cleaned and air-dried under shade. It was then pounded into coarse powder using pestle and mortar and 1000 g of the obtained powder was cold macerated in 2.5 litres of 70 % ethanol for about 72 hours. The resulting mixture was filtered, and the filtrate evaporated to dryness at 40 $^oC$ over an evaporating dish.

$$\text{Percent (\%) yield} = \frac{\text{Weight of Concentrate}}{\text{Weight of powdered sample}} \times 100$$

### 3.5.2 Preliminary Phytochemical Screening of Stem Bark Extract of *Randia nilotica*

The extract was screened for the presence of saponins, carbohydrates, cardiac glycosides, triterpenes, flavonoids, tannins, alkaloids, steroids, cardenolides and anthraquinones, according to Trease and Evans (1989).

#### 3.5.2.1 Test for Steroid and Triterpenes (Liebermann- Burchard's Test)

About 0.5 g of the extract is dissolved in 2 ml of acetic anhydrite and cooled in the ice then concentrated Tetra-oxo-sulphate (VI) acid ($H_2SO_4$) carefully added. Development of colour from violet to bluish-green will indicate the presence of a steroidal ring of the glycosides, while a colour change from pink to violet will be an indication of the presence of triterpenes.

#### 3.5.2.2 Tests for Flavonoids

**Shinoda's Reduction Test**: About 0.5 g of each extract is dissolved in 1-2 ml of ethanol, warmed and then filtered. Three pieces of magnesium chips is be added to the filtrate followed by few drops of concentrated HCl. A pink orange or red to purple colouration will indicate presence of flavonoids.

**Sodium hydroxide test:** Few drops of aqueous NaOH are added to 5 ml of extract. A yellow colouration will indicate the presence of flavonoids.

#### 3.5.2.3 Test for Saponins (Frothing test)

To 1 g of powdered bark in a test tube, 10 ml of distilled water was added and the tube shaken vigorously for 30 seconds. Observance of honeycomb froth that persists for 10-15 minutes in the drug sample will indicate the presence of saponins.

#### 3.5.2.4 Test for carbohydrates

***Fehling's test:*** 0.1 g of the crude extract will be dissolved in 5 ml of water. Equal mixture (2.5 ml each) of Fehling's solution A and B will be added and boiled for few minutes. A brick red precipitate will indicate the presence of free reducing sugar.

***Molisch's test:*** 0.5 g of the crude extract was dissolved in 3 ml of water, with slight heating. To this will be added few drops of Molisch's reagent, and carefully added small amount of concentrated tetra-oxo-sulphate VI ($H_2SO_4$) acid from the side of the test tube to form a lower layer. With a soluble carbohydrate, this will appear as a purple ring when the sulphuric acid is gently poured in to form a layer below the aqueous solution.

#### 3.5.2.5 Test for Alkaloids

0.5 g of the crude extract was stirred with 5 ml of 1 % aqueous Hydrochloric acid on water bath and filtered. 3 ml of the filtrate will be taken and divided equally into 3 portions in test tubes. To the first test tube, Dragenddorff's reagent (solution of Potassium Bismuth Iodide) was added and no occurrence of orange-red precipitate was seen. To the second test tube, 1 ml of Mayer's reagent (Potassium Mercuric Iodide) was added and appearance of buff

colour or cream precipitate was not seen. To the last test tube, few drops of Wagner's reagent (Iodine in Potassium Iodide) was added. There was absence of a dark- brown precipitate.

#### 3.5.2.6 Test for Tannins

**Ferric chloride test:** addition of 2-3 g of the crude extract in a test tube with 10-15 ml of 50 % alcohol then filtered and the filtrate divided into four portions. To a portion of the extract, 3-5 drops of ferric chloride solution is added. A green or greenish-black precipitate was observed. For hydrolysable tannins, a blue or blue-black precipitate was observed.

**Bromine water test:** Three drops of bromine water will be added to a solution of the extract in a fumed cupboard. A buff-coloured precipitate will indicate the presence of condensed tannins only.

**Lead sub-acetate test:** Three drops of lead sub-acetate were added to a solution of the extract. A colour precipitate observed will indicate the presence of tannins.

#### 3.5.2.7 Test for anthraquinones derivatives (modified Borntrager's Test)

1 g of powdered crude powder of the stem bark of *Randia nilotica* is boiled with 5 ml of 10 % hydrochloric acid for 2-3 minutes. This will hydrolyze the glycosides to yield aglycones, which are soluble in hot water only. The hot solution was filtered into a test tube, the filtrate was cooled and extracted with 5ml of benzene. The upper (benzene) layer was pippetted off and shaken gently in a test tube with half of its volume in 10 % ammonium

hydroxide. The non-formation of rose-pink or cherry red colouration meant that the extract contains no anthraquinone derivatives (free or in combined state).

#### 3.5.2.8 Test for cardiac glycoside (Kella-Killiani Test)

1 g of extract was dissolved in glacial acetic acid containing traces of ferric chloride. The test tube was held at an angle of 45°. 1 ml of concentrated tetraoxosulphate VI acid was added down the side. Formation of purple ring colour at the interface was observed

#### **3.5.2.9** Test for cardenolides (Kedde's Test)

1 ml of 2 % 3, 5- dinitrobenzoic acid in 95 % alcohol was added to the extract. This was further made alkaline by the addition of 5 % sodium hydroxide. The absence of purple blue colour was observed

### 3.5.3. Acute Toxicity Study: Median Lethal Dose ($LD_{50}$) determination

The median lethal dose ($LD_{50}$) of the extract was determined using Lorke's method (1983). The study was carried out in two phases. In phase 1, three groups of three animals each were used. The extract was administered orally in widely differing doses (10 mg/kg, 100 mg/kg and 1000 mg/kg). The treated animals were observed frequently for up to twenty four hours post administration for signs of toxicity. The second phase of the experiment was based on the outcome of the first phase (presence or absence of death). After 24 hours, in the absence mortality, phase 2 was carried out. In phase 2, four groups of one animal each were orally treated with extract in widely increased doses (1200 mg/kg, 1600 mg/kg, 2900 mg/kg and 5000 mg/kg). The animals were then observed for signs of toxicity for the first 4 hours and mortality for 24 hours. The square root of the smallest dose that caused

mortality and the highest dose that did not was taken as the media lethal dose ($LD_{50}$) of the extract.

### 3.5.4 Sub-chronic and Chronic Toxicity Studies

The repeated dose (28 and 90 days) oral toxicity studies of the organization of Economic Cooperation and Development (OECD) Guidelines number 407 and 408 of 1995 and 1998 respectively, in Wistar rats, were used for sub-chronic and chronic effects respectively. Four groups of 10 and 20 Wistar rats of both sexes, matched for weight and sex per group were orally treated daily for 28 and 90 days respectively with distilled water (group 1) and *Randia nilotica* extract doses of 250, 500 and 1000 mg/kg for groups 2-4. Blood samples were collected by cardiac puncture at the end of treatment periods of 28 days for sub-chronic and 90 days for chronic toxicity tests. The blood from each group was divided between two sample bottles of plain for biochemical analysis and EDTA for haematological analysis.

The brain, heart, kidney, liver and spleen of all the rats in each group were removed after draining the blood. The excised organs were weighed prior to fixing in 10% buffered formalin and Bouin's fluid (for brain only) for histopathological examinations. The biochemical parameters evaluated for toxic effects of *Randia nilotica* extract include aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels, blood urea nitrogen (BUN), creatinine, calcium, total protein (TP), albumin, total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL). Haematological parameters evaluated were: packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) count, mean corpuscular haemoglobin concentration (MCHC), mean

cell haemoglobin (MCH), mean cell volume (MCV), white blood cell count (WBC) and differentials – neutrophils, lymphocytes, monocytes, basophils, eosinophils; erythrocytes sedimentation rate (ESR). The anatomical histopathological analysis of the brain, heart, kidney, liver and spleen were undertaken. Also, organ-weight variation of the excised organs was evaluated

### 3.5.5. Statistical Analysis

Data were expressed as mean ± standard error of mean (M±SEM) and results presented as tables. Data were analyzed using one way Analysis of variance (ANOVA) followed by Dunnett's Post-Hoc test for multiple comparisons. Level of significance was taken at $p \leq 0.05$.

# CHAPTER FOUR

# 4.0 RESULTS

## 4.1. Percentage Yield of the Extraction of the Stem bark of *Randia nilotica*

The extraction process yielded a brownish sticky solid residue of constant weight 56.28 g (5.628 %).

## 4.2 Phytochemical Screening of the Stem Bark Extract of *Randia nilotica*

**Test for Steroids and Triterpenes:** Change from violet to bluish green colouration indicated the presence of steroids, while colour change from pink to violet indicated presence of triterpenes.

**Tests for steroidal alkaloids:** absence of orange-red precipitate with Dragenddorf's reagent. Also, absence of a cream precipitate with Mayer's reagent as well as absence of brown precipitate with Wagner's reagent indicated absence of alkaloids

**Test for flavonoids:** Formation of a pink orange colouration in the Shinoda's test indicated the presence of flavonoids

**Test for Saponins:** Formation of honeycomb froth which persisted for about fifteen minutes following vigorous shaking indicated the presence of saponins.

**Tests for Carbohydrates:** formation of a brick red precipitate in Fehling's solution A and B indicated the presence of free reducing sugars. Also, formation of a layer below the acquous solution using Molish's reagent indicated the presence of soluble carbohydrate

**Tests for Tannins:** Formation of blue-black precipitate with the Ferric chloride test and a brown coloured precipitate in the Lead sub-acetate test as well as buff-coloured precipitate in the Bromine water test indicated the presence of Tannins

**Test for anthraquinones:** The absence of rose-pink or cherry red colour in the modified Bontrager's test indicated absence of anthraquinone derivatives

**Test for cardiac glycoside (Kella-Killiani Test):** Formation of purple ring colour at the interface was indicated the presence of cardiac glycosides.

**Test for cardenolides (Kedde's Test):** The absence of purple blue colour indicated the absence of cardenolides

A summary of the result obtained from the preliminary Phytochemical screening of the stem bark extract of *Randia nilotica* is presented in Table 4.1

**Table 4.1: Preliminary Phytochemical Screening of Ethanol Stem Bark Extract of *Randia nilotica***

| Constituents | Test | Result |
|---|---|---|
| Saponins | Frothing | + |
| Carbohydrates | Molisch'reagent | + |
| Cardiac glycoside | Kella-killiani | + |
| Triterpenes | Liebermann Burchard's | + |
| Flavonoids | Shinoda, NaOH | + |
| Tannins | $FeCl_3$, bromine water, Lead subacetate tests | + |
| Alkaloids | Mayer,s reagent, Dragendorff, Wagner | _ |
| Steroids | Liebermann Burchard's | _ |
| Cardenolides | Kedde's test | _ |
| Anthraquinone | Borntrager | _ |

**Key:** + = Present; - = Absent

## 4.3 Acute Toxicity Study for Oral Median Lethal Dose ($LD_{50}$)

There were no observable signs of toxicity following post treatment observation in both the first and second phase experiments of Lorke's toxicity test. The oral median lethal dose ($LD_{50}$) for the extract was found to be greater than 5000 mg/kg body weight.

## 4.4. Sub-chronic and Chronic Toxicity Studies

### 4.4.1 Effect of 28 and 90 days daily Oral Administration of Ethanol Extract of *Randia nilotica* on Haematological Parameters in Rats

The result of the haematological analysis as shown in table 4.2 (sub-chronic) and 4.3 (chronic) below showed a significant ($P \leq 0.05$) decrease in packed cell volume (PCV) at 1000 mg/kg following 28 day oral administration.

For the 90 day oral administration, both the 500 and 1000 mg/kg doses caused significant ($P \leq 0.05$) decrease in PCV, Hb and RBC counts. The mean cell volume (MCV) was also significantly ($P \leq 0.05$) increased, but only at 1000 mg/kg

**Table 4.2: Effect of Ethanolic Stem Bark Extract of *Randia Nilotica* on Haematological Indices in Rats Following 28 Days Daily Oral Administration**

Data are presented as Mean±SEM; *= $p \leq 0.05$ compared with distilled water. One-way

| Haematological Indices | Treatments(mg/kg) | | | |
|---|---|---|---|---|
| | Distilled Water (1ml/kg) | RNE 250 | RNE 500 | RNE 1000 |
| PCV (%) | 44.70±0.99 | 45.00±0.85 | 45.50±0.64 | 40.88±1.09* |
| Hb (g/dl) | 14.54±0.43 | 14.98±0.33 | 15.18±0.36 | 13.59±0.50 |
| RBC( x $10^{12}$/L) | 4.72±0.20 | 4.88±0.26 | 5.17±0.28 | 3.96±0.16 |
| MCHC(g/dl) | 35.20±1.60 | 33.56±0.24 | 34.00±0.37 | 33.25±0.56 |
| MCH(pg) | 29.50±1.46 | 28.78±0.36 | 28.00±0.33 | 29.25±0.75 |
| MCV(Fl) | 87.80±0.98 | 86.44±0.67 | 84.10±0.97 | 85.00±1.02 |
| Neutrophil (%) | 20.30±2.47 | 22.33±2.01 | 23.80±2.15 | 16.00±4.36 |
| Lymphocytes (%) | 79.40±2.44 | 77.33±2.04 | 75.30±2.15 | 84.38±3.46 |
| Monocytes (%) | 0.00±0.00 | 1.00±0.00 | 4.00±0.00 | 0.00±0.00 |
| Eosinophils (%) | 2.00±0.00 | 2.00±1.00 | 1.67±0.33 | 2.00±0.00 |
| WBC ( $10^9$/L) | 8.44±0.38 | 8.76±0.43 | 9.37±0.29 | 8.70±0.53 |
| ESR (mm/Hr) | 2.30±0.26 | 3.22±0.40 | 2.20±0.33 | 2.63±0.46 |

ANOVA, Dunnett's post hoc test; n= 10; RNE= *Randia nilotica* extract

**Table 4.3: Effect of Ethanolic Stem Bark Extract of *Randia Nilotica* on Haematological Indices in Rats Following 90 Days Daily Oral Administration**

| Haematological indices | Treatments (mg/kg) | | | |
|---|---|---|---|---|
| | Distilled Water (1ml/kg) | RNE 250 | RNE 500 | RNE 1000 |
| PCV (%) | 40.75±1.23 | 41.39±1.85 | 26.20±3.60* | 26.50±3.50* |
| Haemoglobin(g/dl) | 13.32±0.39 | 13.61±0.60 | 8.50±1.20* | 8.65±1.35* |
| RBC($10^{12}$/l) | 4.77±0.17 | 4.97±0.23 | 3.08±0.51* | 2.90±0.40* |
| MCHC(g/dl) | 32.40±0.29 | 40.69±5.27 | 32.20±0.37 | 32.50±0.50 |
| MCH(pg) | 28.15±0.23 | 27.62±0.21 | 28.60±0.75 | 30.00±1.00 |
| MCV (fl) | 85.35±0.55 | 83.08±0.54 | 86.20±2.94 | 91.50±0.50* |
| Neutrophil (%) | 25.65±1.25 | 28.46±0.91 | 27.40±1.72 | 26.50±3.50 |
| Lymphocytes (%) | 72.20±1.26 | 68.62±1.15 | 72.00±1.64 | 73.00±3.00 |
| Monocytes (%) | 2.00±0.27 | 2.33±0.33 | 1.00±0.00 | 0.00±0.00 |
| Eosinophils (%) | 3.00±0.47 | 3.44±0.29 | 2.00±0.00 | 1.00±0.00 |
| WBC ($10^9$/l) | 14.53±0.87 | 14.94±1.20 | 11.02±2.14 | 8.95±0.35 |
| PLATELETS($10^9$/l) | 229.37±8.94 | 256.31±19.57 | 175.20±10.37 | 170.50±2.50 |

Data are presented as Mean±SEM; * $p \leq 0.05$ compared with distilled water. One-way ANOVA, Dunnett's post hoc test; n=20; RNE= *Randia nilotica* extract

### 4.4.2 Effect of 28 and 90 Days Daily Oral Administration of Ethanolic Stem Bark Extract of *Randia Nilotica* on Biochemical Parameters in Rats

The 28 days oral daily administration caused no alteration in blood urea-nitrogen, creatinine and calcium levels at all doses tested compared with distilled water– 250, 500 and 1000 mg/kg (Table 4.4). Daily oral administration for 90 days caused significant ($P \leq 0.05$) increase in blood urea nitrogen at all doses tested (Table 4.5). No significant ($P \leq 0.05$) variations were observed in the other biochemical indices including the liver enzymes, total protein, albumin, total cholesterol, triglycerides and high density lipoprotein for the 28 days treatment compared with distilled water (Table 4.6), but the 90$^{th}$ day treatment showed significant ($P \leq 0.05$) increase in the high density lipoprotein (HDL) at the 250 mg/kg of extract compared with distilled water (Table 4.7).

**Table 4.4: Effect of Ethanolic Stem Bark Extract of *Randia Nilotica* on Some Renal Indices Following 28 Days Daily Oral Administration in Rats**

| Renal indices (mmol/L) | Treatment (mg/kg) | | | |
|---|---|---|---|---|
| | Distilled Water (1ml/kg) | RNE 250 | RNE 500 | RNE 1000 |
| BLOOD UREA NITROGEN | 4.39±0.31 | 4.38±0.35 | 4.30±0.34 | 4.58±0.36 |
| CREATININE | 55.40±3.23 | 55.60±3.92 | 58.00±3.30 | 64.22±4.12 |
| CALCIUM | 2.31±0.04 | 2.32±0.06 | 2.42±0.06 | 2.43±0.04 |

Data are presented as mean±SEM; One-way ANOVA, Dunnette's post hoc test. No statistical significance observed, compared with distilled water; n=10, RNE= *Randia nilotica* extract

**Table 4.5: Effect of Ethanolic Stem Bark Extract of *Randia Nilotica* on Some Renal Indices Following 90 Days Daily Oral Administration**

| Renal Indices (mmol/L) | Treatments (mg/kg) | | | |
|---|---|---|---|---|
| | De-ionized water (1 ml/kg) | RNE 250 | RNE 500 | RNE 1000 |
| BLOOD UREA NITROGEN | 3.32±0.13 | 4.12±0.22* | 4.42±0.41* | 4.75±1.05* |
| CREATININE | 49.33±1.78 | 50.75±4.04 | 49.50±4.66 | 57.50±0.50 |
| CALCIUM | 2.32±0.03 | 2.38±0.05 | 2.32±0.03 | 2.46±0.00 |

Data are presented as mean±SEM; * $P \leq 0.05$ compared with distilled water. One-way ANOVA, Dunnett's post hoc test; n=20; RNE= *Randia nilotica* extract

**Table 4.6: Effect of Ethanolic Stem Bark Extract of *Randia Nilotica* on Some Liver Indices following 28 Days Daily Oral Administration**

| | Treatments (mg/kg) | | | |
|---|---|---|---|---|
| Liver indices | Distilled Water (1ml/kg) | RNE 250 | RNE 500 | RNE 1000 |
| AST(u/l) | 68.70±7.80 | 69.80±5.95 | 81.44±8.19 | 81.00±7.38 |
| ALT(u/l) | 81.40±5.54 | 75.90±6.33 | 84.63±7.49 | 85.44±7.46 |
| ALP(u/l) | 87.70±6.95 | 73.80±9.78 | 97.00±7.05 | 102.00±8.24 |
| ALBUMIN(g/l) | 38.50±3.25 | 41.50±4.06 | 34.11±1.01 | 38.11±1.35 |
| TP(g/l) | 64.50±0.97 | 64.50±0.81 | 62.89±0.96 | 66.33±0.75 |
| TRIGRD(mg/dl) | 0.98±0.83 | 0.95±0.10 | 0.92±0.07 | 0.83±0.07 |
| H D L(mg/dl) | 0.91±0.09 | 0.75±0.08 | 0.84±0.07 | 0.84±0.07 |
| TC(mg/dl) | 2.34±0.09 | 2.42±0.06 | 2.39±0.07 | 2.42±0.09 |
| LDL(mg/dl) | 1.23±0.08 | 1.48±0.10 | 1.36±0.04 | 1.41±0.08 |

Data are presented as mean±SEM; One-way ANOVA, Dunnett's post hoc test. No statistical significance compared with distilled water; n=10; RNE = *Randia nilotica* extract

**Table 4.7: Effect of Ethanolic Stem Bark Extract of *Randia Nilotica* on Some Liver Indices following 90 Days Daily Oral Administration**

| Liver Indices | Treatments(mg/kg) | | | |
|---|---|---|---|---|
| | Distilled Water (1ml/kg) | RNE 250 | RNE 500 | RNE 1000 |
| AST (u/l) | 64.16±3.63 | 63.31±4.55 | 73.60±4.89 | 66.00±20.00 |
| ALT (u/l) | 70.32±3.84 | 70.85±5.21 | 87.00±5.65 | 72.50±21.50 |
| ALP (u/l) | 85.00±4.38 | 103.46±8.77 | 113.00±11.81 | 90.50±31.50 |
| ALBUMIN (g/l) | 37.00±0.64 | 37.33±1.18 | 39.80±0.66 | 42.00±0.00 |
| TP (g/l) | 64.94±0.87 | 66.67±1.28 | 65.50±1.85 | 67.00±0.00 |
| TRIGLRD (mg/dl) | 0.85±0.05 | 0.91±0.06 | 0.88±0.08 | 1.15±0.15 |
| HDL (mg/dl) | 0.78±0.04 | 0.99±0.05* | 0.76±0.07 | 0.85±0.05 |
| TC (mg/dl) | 2.38±0.05 | 2.39±0.07 | 2.60±0.12 | 2.75±0.55 |
| LDL (mg/dl) | 1.42±0.06 | 1.22±0.10 | 1.66±0.08 | 1.67±0.57 |

Data are presented as Mean±SEM; * $P \leq 0.05$ compared with distilled water. One-way ANOVA, Dunnett's post hoc test; n=20; RNE = *Randia nilotica* extract

### 4.4.3 Effect of Ethanolic Stem Bark Extract of *Randia Nilotica* on Organ Weights

No significant ($p \leq 0.05$) increase or decrease in weight was observed for all the organs following 28 days daily oral administration (Table 4.8). Significant ($p \leq 0.05$) decrease in brain weight was observed for the 90 days daily oral administration (Table 4.9).

**Table 4.8: Effect of Stem Bark Extract *Randia Nilotica* on Organ Weights Following 28 Days Daily Oral Administration in Rats**

| Organs (g) | Treatments (mg/kg) | | | |
|---|---|---|---|---|
| | Distilled water (1ml/kg) | RNE 250 | RNE 500 | RNE 1000 |
| HEART | 0.57±0.04 | 0.56±0.04 | 0.51±0.07 | 0.56±0.04 |
| SPLEEN | 0.69±0.05 | 0.62±0.04 | 0.83±0.09 | 0.66±0.03 |
| LIVER | 4.86±0.29 | 4.46±0.32 | 5.02±0.41 | 4.26±0.16 |
| KIDNEY | 0.90±0.05 | 0.87±0.04 | 1.06±0.08 | 0.92±0.04 |
| BRAIN | 1.49±0.04 | 1.45±0.07 | 1.52±0.07 | 1.38±0.04 |

Data are presented as Mean±SEM; $P \leq 0.05$, One-way ANOVA, Dunnett's post hoc test. No statistical significance compared with distilled water; n=10; RNE = *Randia nilotica* extract

**Table 4.9: Effect of Stem Bark Extract *Randia Nilotica* on Organ Weights Following 90 Days Daily Oral Administration in Rats**

| Organs (g) | Treatments (mg/kg) | | | |
|---|---|---|---|---|
| | Distilled Water (1ml/kg) | RNE 250 | RNE 500 | RNE 1000 |
| HEART | 0.54±0.54 | 0.54±0.03 | 0.55±0.07 | 0.58±0.03 |
| SPLEEN | 0.57±0.04 | 0.61±0.06 | 0.77±0.06 | 0.60±0.00 |
| LIVER | 4.17±0.11 | 4.37±0.24 | 4.72±0.71 | 4.70±0.20 |
| KIDNEY | 0.81±0.03 | 0.81±0.06 | 0.98±0.11 | 0.90±0.01 |
| BRAIN | 1.72±0.04 | 1.50±0.07* | 1.42±0.11* | 1.30±0.10* |

Data are presented as Mean±SEM; * $P \leq 0.05$ compared with distilled water. One-way ANOVA, Dunnett's post hoc test; n=20; RNE = *Randia nilotica* extract

### 4.4.4: Histopathology of Some Isolated Organs Following 28 And 90 Days Daily Oral Administration in Rats

The histopathology of the organs showed both dose and duration dependent alterations.

The 28 day oral administration with the stem bark extract of *Randia nilotica* at all doses – 250 mg, 500 mg and 1000 mg showed neuronal degeneration and congested blood vessels of the brain, while for the 90 day daily oral administration, cerebral congestion with oedema occurred, when compared with the control group (Plates II and III).

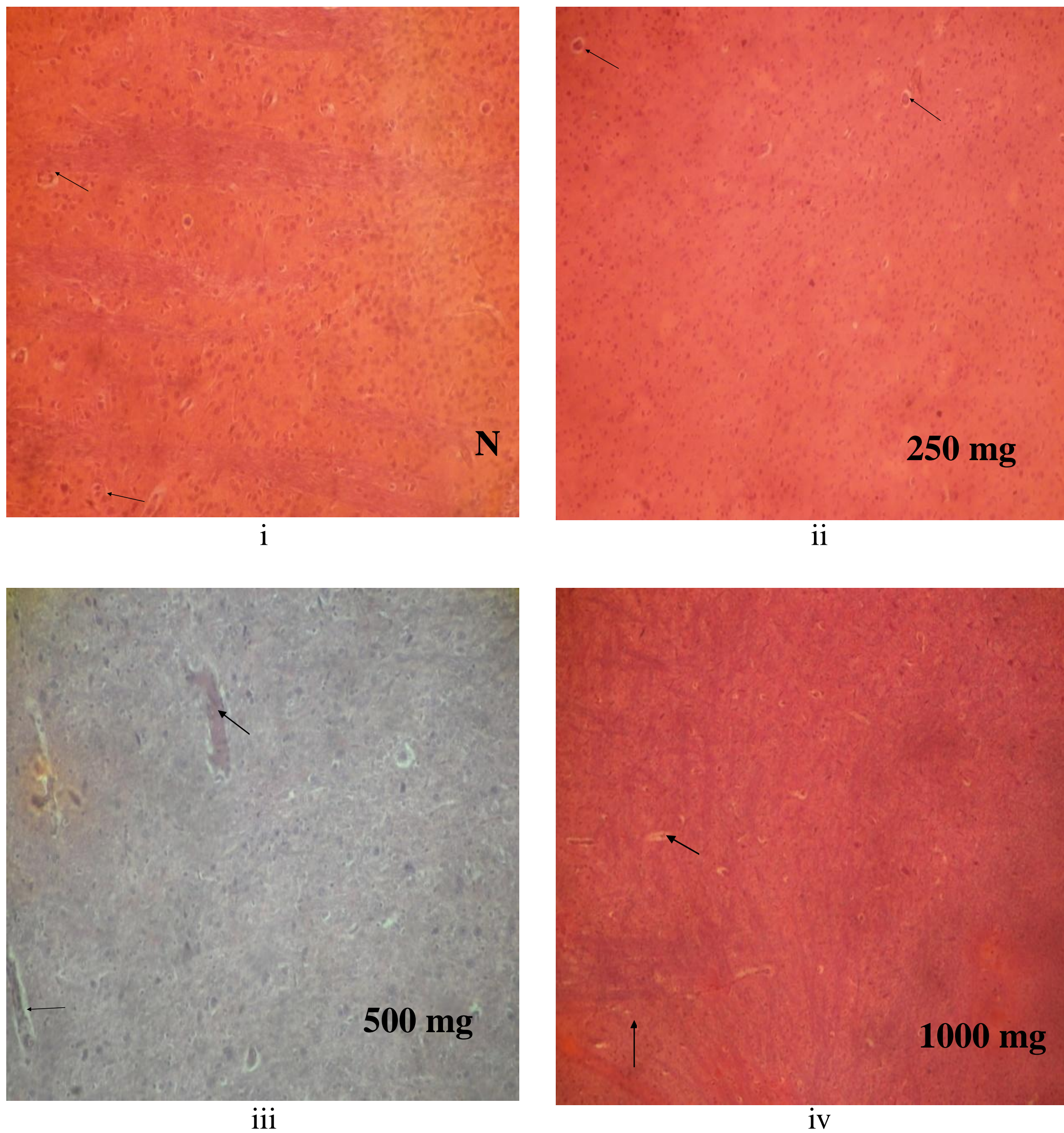
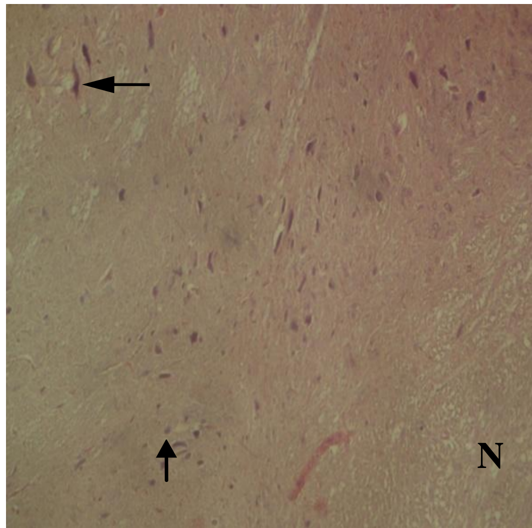



**Plate II**: Photomicrograph of section of the brain of Wistar Rat showing intact neurons (i) (H & E x 400); slight neuronal degeneration (ii) (H & E x 200); congestion of blood vessels (iii) (H & E x 200) and neuronal degeneration (iv) (H & E x 150), after 28 days daily oral administration.

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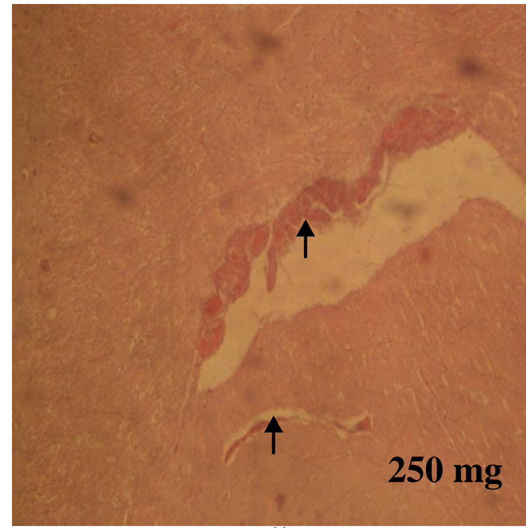


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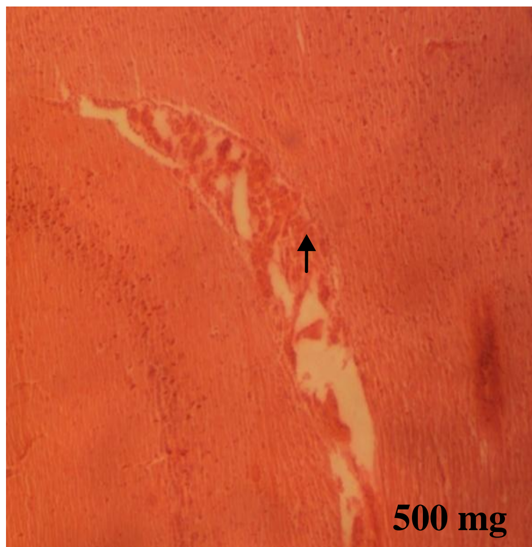


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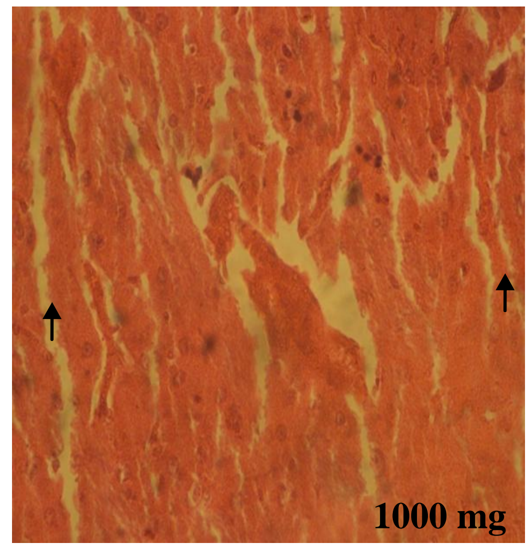
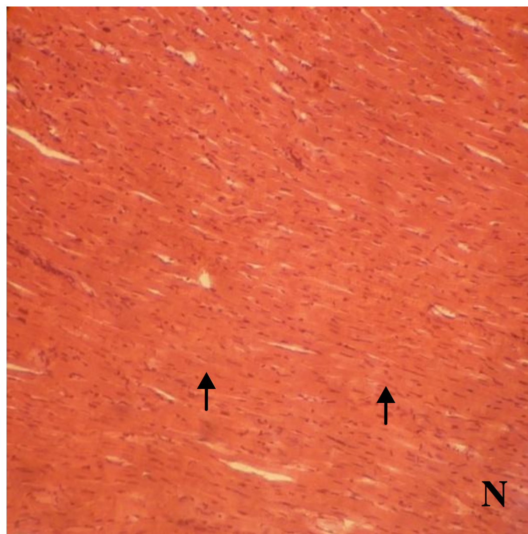



iv

**Plate III**: Photomicrograph of section of the brain of Wistar Rat showing intact neurons (←) and dendrites (↑) (i) (H & E x 230); cerebral congestion (ii) (H & E x 200); marked cerebral congestion (iii) (H & E x 200); cerebral congestion (iv) (H & E x 720), after 90 days daily oral administration.

The heart had myocardial congestion, infiltration of inflammatory cells and myocardial haemorrhage for sub chronic effect and in addition, the 90 day treatment caused fragmentation of muscle fibres and oedema when compared to control group in each case (Plates IV and V).



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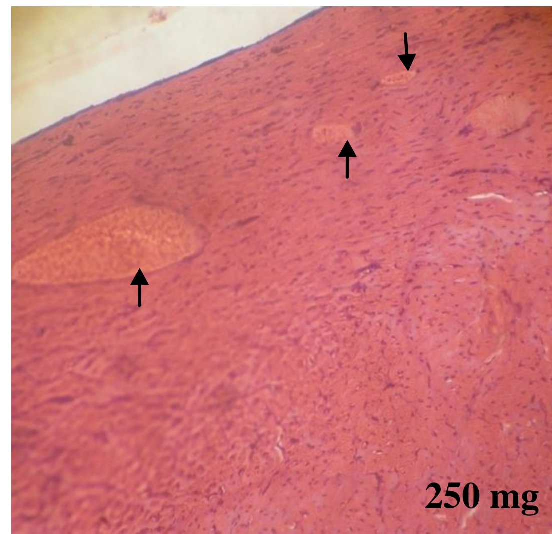

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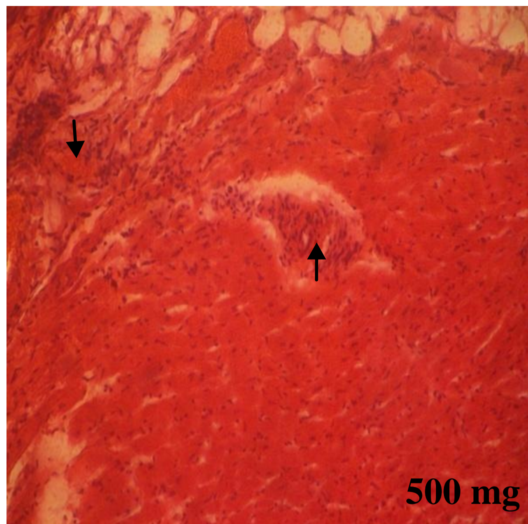

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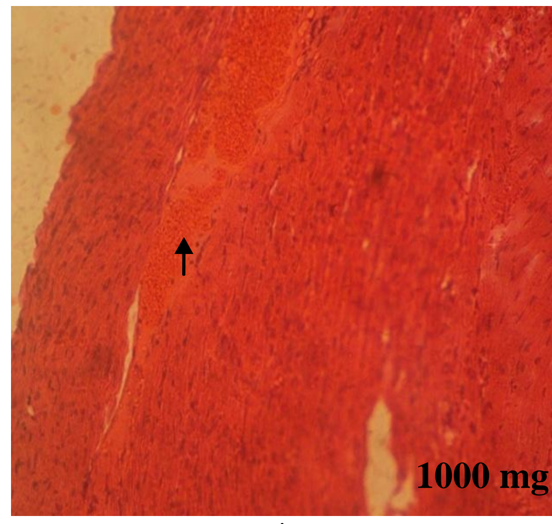
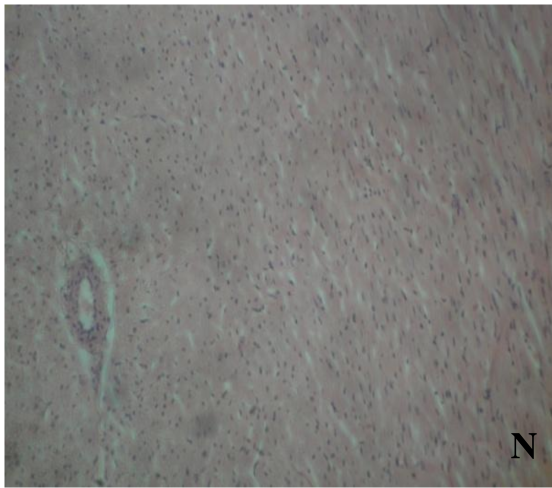


iv

**Plate IV:** Photomicrograph of section of the heart of Wistar Rat showing no observable lesions (intact nuclei) (i) (H & E x 200); congestion of the myocardium (ii) (H & E x 400); severe congestion ( ↑ ) and infiltration of inflammatory cells ( ↓ ) (myocarditis) (iii) (H& E x 360) and myocardial haemorrhages (iv) (H & E 360), after 28 days daily oral administration.

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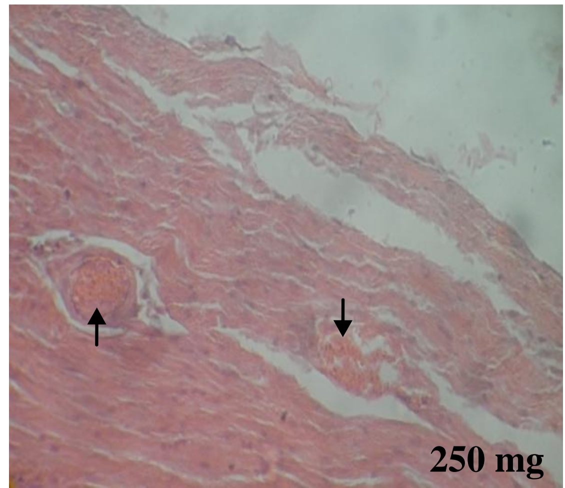


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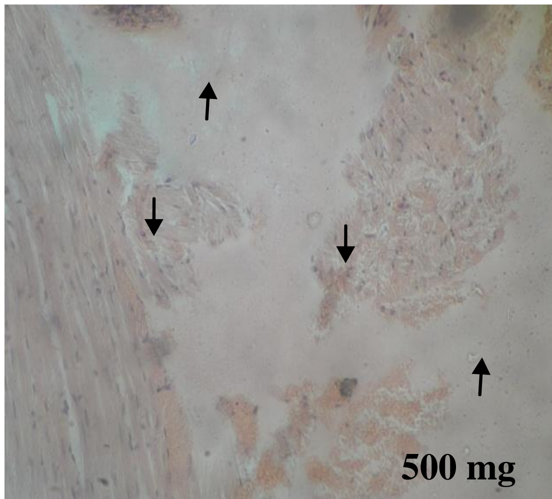


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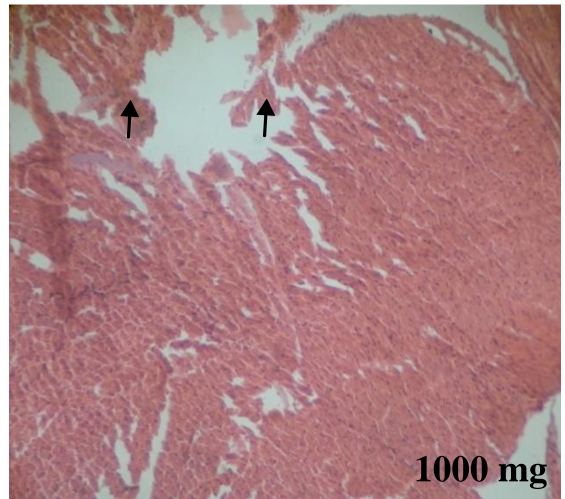


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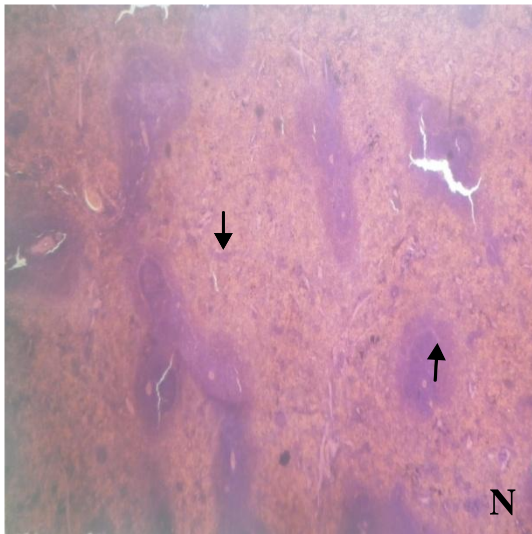
**Plate V:** Photomicrograph of section of the heart of Wistar Rat showing no observable lesions (intact nuclei)(i) (H & E x 400); congestion of blood vessels ( ↑ ) and haemorrhages ( ↓ ) (ii) (H & E x 400); oedema ( ↑ ) and fragmentation of muscle fibres ( ↓ ) (iii) (H & E x 400) and fragmentation of muscle fibres (iv) (H & E x400), after 90 days daily oral administration.

The alterations on the spleen were congestion of blood vessels and depletion of lymphocytes, while the liver had congestion of blood vessels, perivascular infiltration of inflammatory cells and necrosis of hepatocytes (Plates VI and VII).

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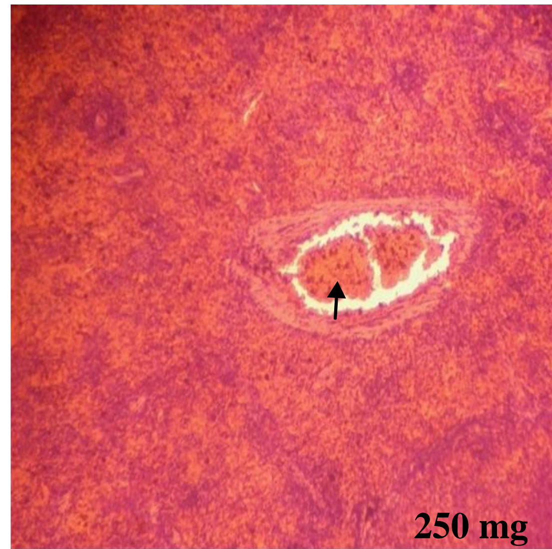


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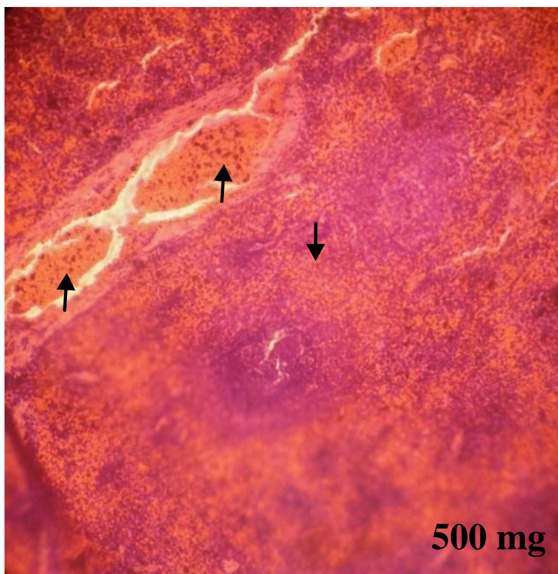


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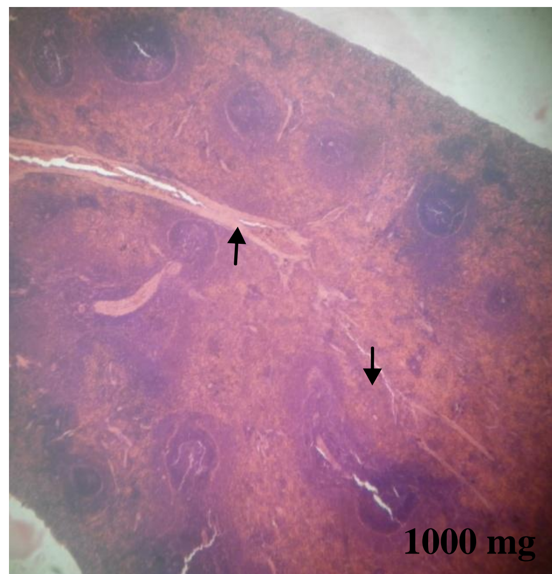


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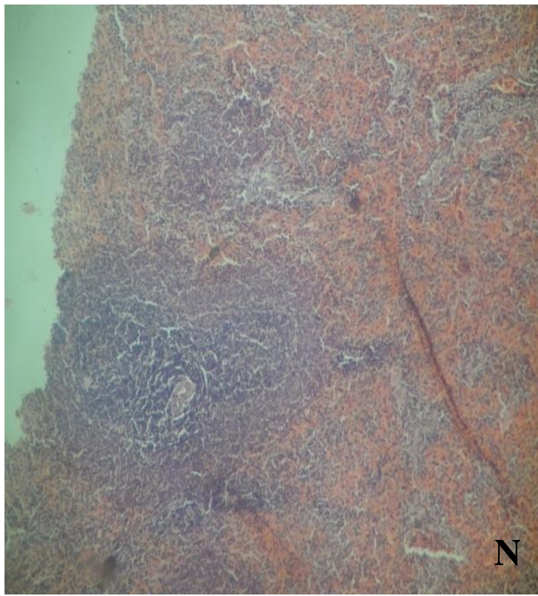
**Plates VI:** Photomicrograph of section of the spleen of Wistar Rat showing white pulp ( ↑ ) and red pulp ( ↓ ) (i)(H & E x 40)**;** congestion of blood vessels (ii) (H & E x 80); severe congestion ( ↑ ) and slight depletion of lymphocytes ( ↓ ) (H & E 200); congestion ( ↑ ) and slight depletion of lymphocytes ( ↓ ) (H & E x 240), after 28 days daily oral administration.



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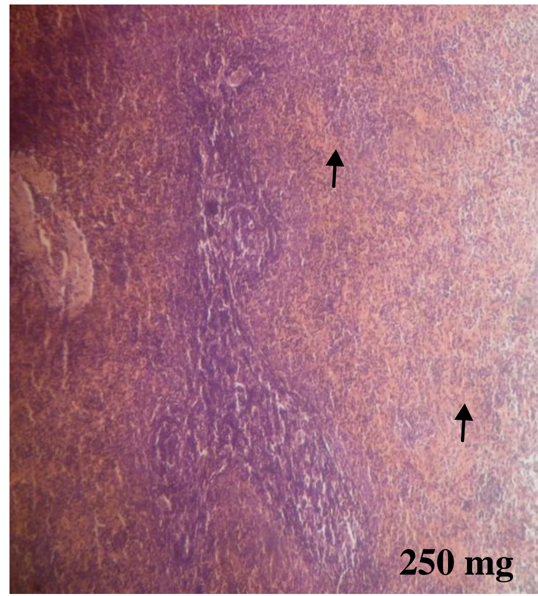

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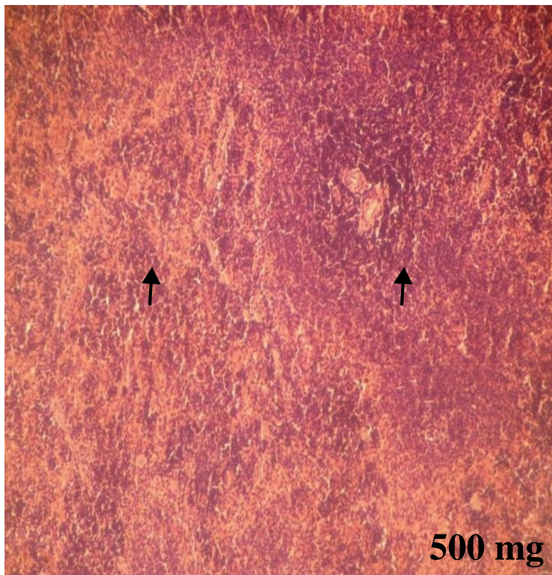

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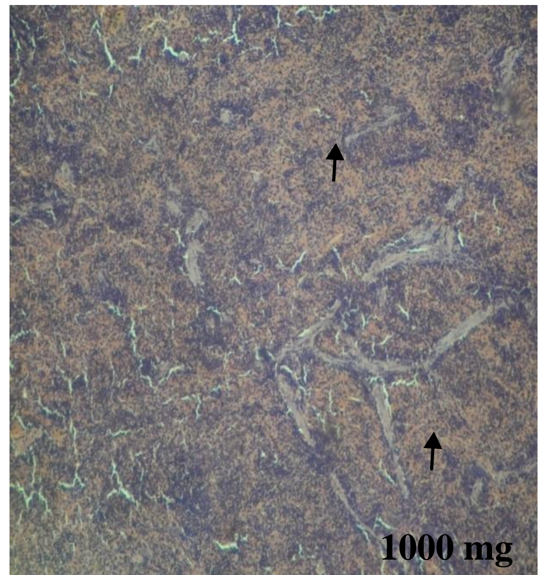

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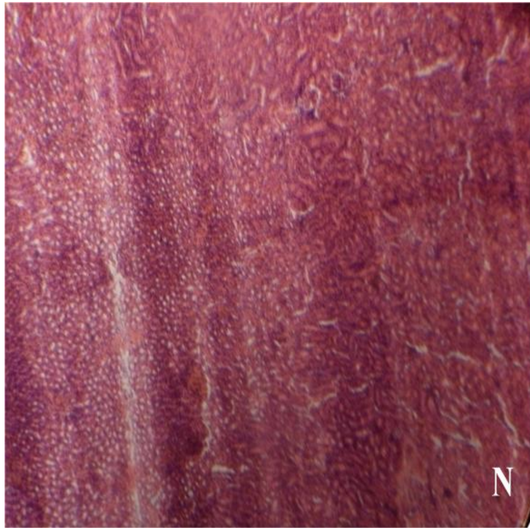
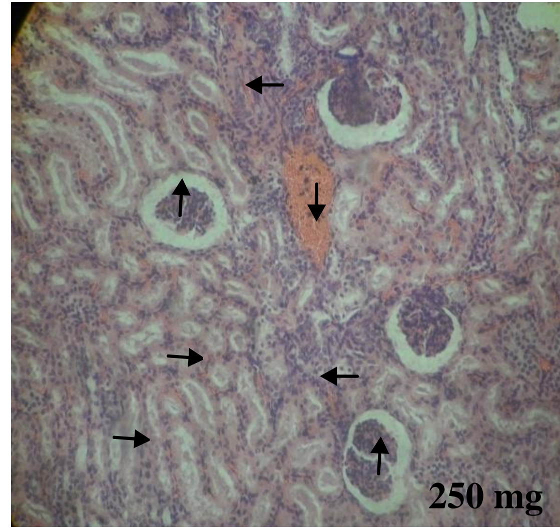
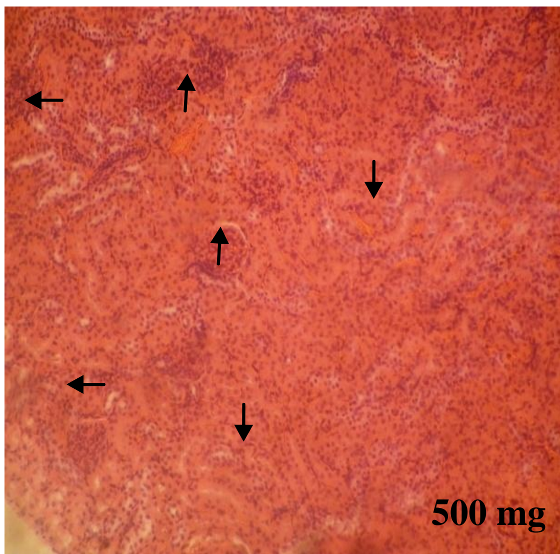
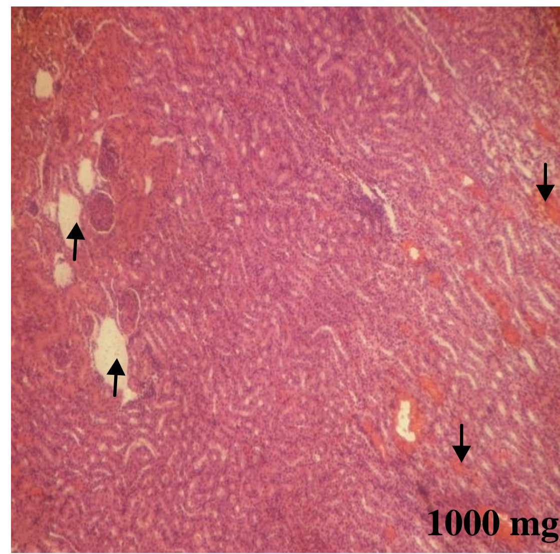
**Plates VII:** Photomicrograph of section of the spleen of Wistar Rat showing no observable lesions (i) (H & E x 200); depletion of lymphocytes (ii) (H & E x 200); depletion of lymphocytes (iii) (H & E x 200); depletion of lymphocytes (iv) (H & E x 200), after 90 days daily oral administration.

The kidney showed atrophy of glomeruli, haemorrhage, tubular necrosis, cellular infiltration, hyper cellular glomeruli and adhesion of the parietal surface with Bowman's capsule following 28 days daily oral administration of the stem bark extract of *Randia nilotica*, and the further effect on the 90 day included vacuolation of glomeruli, infiltration of inflammatory cells and congestion of glomeruli (Plates VIII and IX).

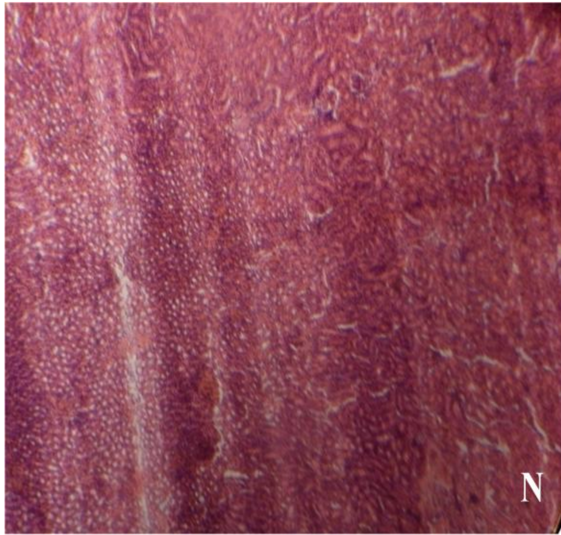









**Plates VIII:** Photomicrograph of section of the kidney of Wistar Rat showing no observable lesions (i) (H & E 400); atrophy of glomeruli ( ↑ ), haemorrhages ( ↓ ), renal tubular necrosis (←) and cellular infiltration (→) (ii)(H & E x 400); hypercellular glomeruli in which there was adhesion of the parietal surface with the Bowman's capsule ( ↑ ), renal tubular necrosis ( ↓ ) and cellular infiltration (←) (iii) (H & E x 400); glomerular vacoulation ( ↑ ) and haemorrhages ( ↓ ) (iv) (H & E x 200), after 28 days daily oral administration.

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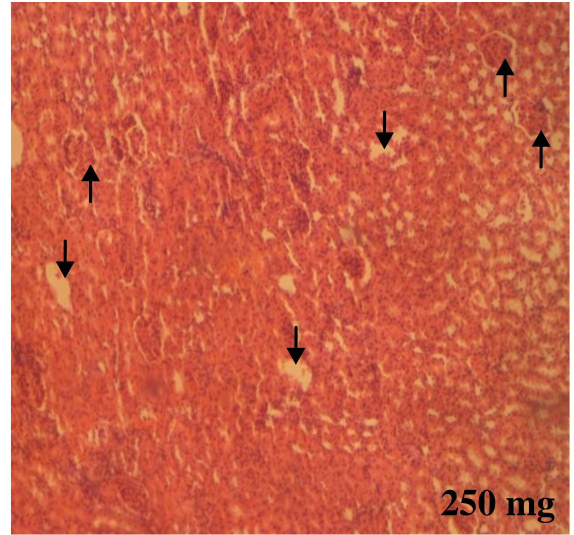
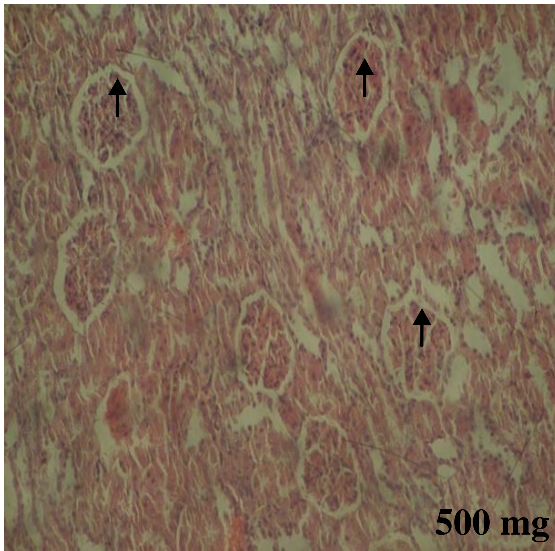
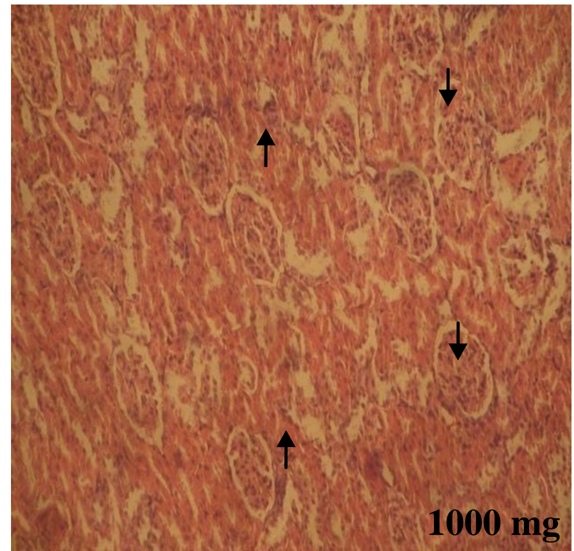



ii




iii




iv

**Plates IX:** Photomicrograph of section of the kidney of Wistar Rat showing no observable lesions (i) (H & E 400); hypertrophy ( ↑ ) and vacuolation ( ↓ ) of the glomeruli (ii) (H & E 360); hypertrophy of glomeruli (iii) (H & B x 360); infiltration of glomeruli and parenchyma with inflammatory cells ( ↑ ) and hypertrophy of glomeruli ( ↓ ) (iv) (H & E x 400), after 90 days daily oral administration.

Histopathology of the liver at 250 mg/kg, 500 mg/kg of the stem bark extract of *Randia nilotica* showed congestion of blood vessels, perivascular infiltration of inflammatory cell and necrosis of the hepatocytes. The 1000 mg/kg showed severe hepatic necrosis, cellular infiltration and haemorrhages following 28 and 90 days daily oral administration of extract of the stem bark of Randia nilotica (Plates X and XI).

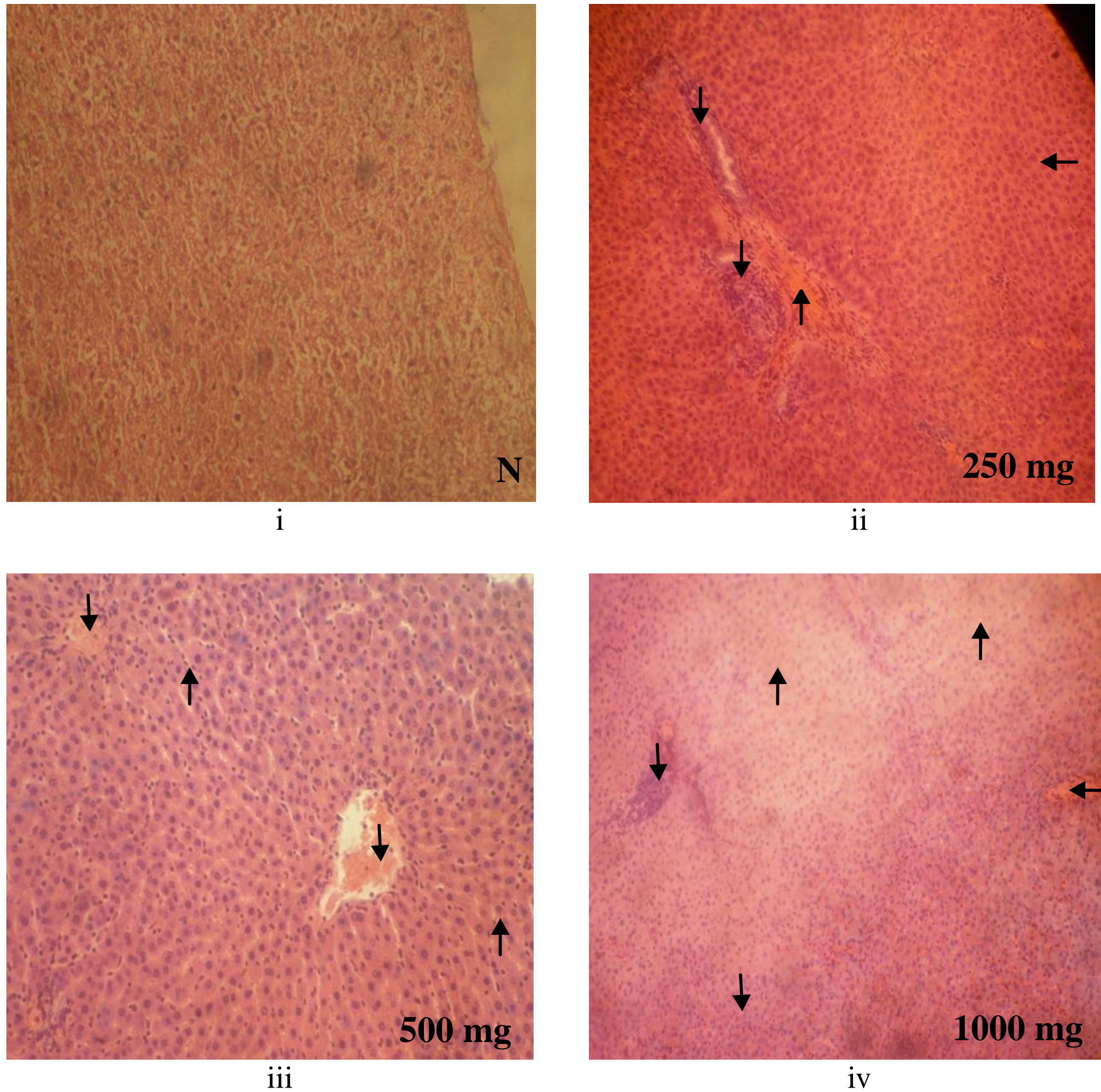
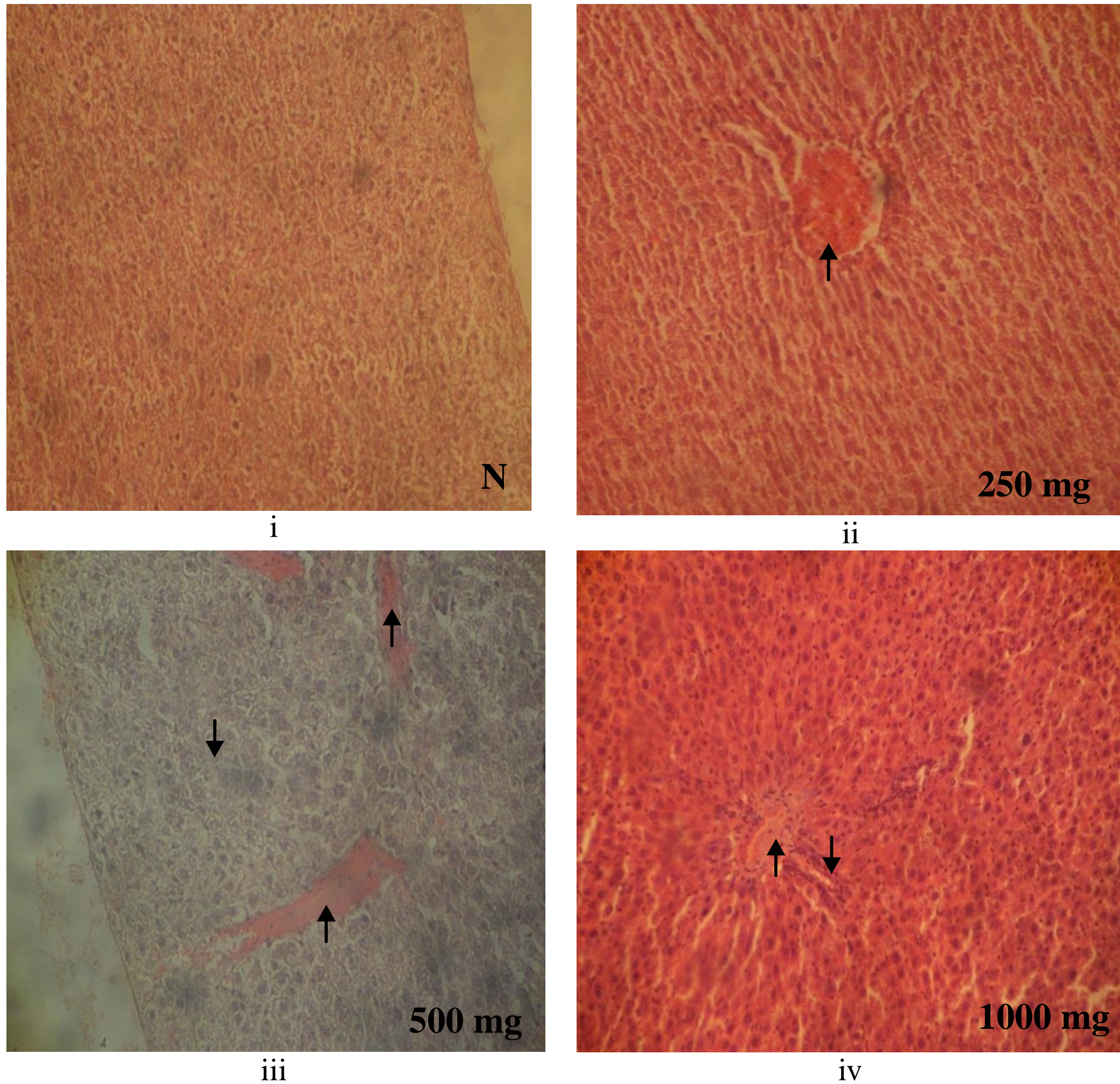



**Plate X:** Photomicrograph of section of the liver of Wistar Rat showing no observable lesions (i) (H & E 200); congestion of blood vessels ( ↑ ), perivascular infiltration of inflammatory cells ( ↓ ) and necrosis of hepatocytes (←) (ii) (H & E x 240); necrosis of hepatocytes ( ↑ ) and congestion of blood vessels ( ↓ ) (iii) (H & E x 140); severe hepatic necrosis ( ↑ ), cellular infiltration ( ↓ ) and haemorrhages (←) (iv) (H & E x 140), after 28 days daily oral administration.




**Plates XI:** Photomicrograph of section of the liver of Wistar Rat showing no observable lesions (i) (H & E 200); congestion of blood vessels (ii) (H & E x 400); congestion (↑) and necrosis of hepatocytes (↓) (iii) (H & E x 400); congestion (↑) and perivascular infiltration with inflammatory cells (↓) (iv) (H & E x 400), after 90 days daily oral administration.

# CHAPTER FIVE

## 5.0 DISCUSSION

The phytochemical screening of the stem bark extract of *Randia nilotica* in this study revealed the presence of saponins, carbohydrates, cardiac glycosides, triterpenes, flavonoids and tannins, while anthraquinones, steroids and cardenolides were absent. Previous report by El-Kheir and Salih (1980) and Danjuma *et al.,* (2009) on the constituents of the hydroethanolic stembark extract of this plant showed similar findings.

Saponins had been reported to possess CNS effects (Wagner *et al.,* 1983; Dubois *et al.,* 1986; Stables and Kupferberg, 1997; Taesotikul *et al.,* 1998; Guyton and Hall, 2000; Monti and Monti, 2000; Curtis and Jermaine, 2002; Monaca, 2003; Nicoll, 2004). Danjuma *et al.,* (2009) also reported sedative and antidepressant activities of the saponin fraction of *Randia nilotica* stem bark extract. Saponins had also been reported to have antimalarial, diuretic, expectorant, haemolytic and anti-inflammatory effects (Evans, 1989). Other secondary metabolites with pharmacological activities include tannins, which is locally used in the management of varicose vein, heavy menstrual flow as well as inflammatory conditions of the gastrointestinal tract (Evans, 1989). Similarly, cardiac glycosides have been shown to increase the force of myocardial contraction and to reduce conductivity of the atrio-ventricular (AV) node. The glycosides had also been employed in the management of supraventricular tachycardias and heart failures (Rang *et al.,* 2012). The afore-mentioned claims strongly support the varied and heavy ethnomedicinal use of the plant in the folkloric management of many conditions – although without adequate or documented consideration for toxicity

The observed oral $LD_{50}$ result of >5000 mg/kg obtained in this study was considered as no practical risk (Lorke, 1983). $LD_{50}$ value is used for assessing the safety margin of substances and it often gives an idea of the toxic level of a chemical substance or compound. It provides the prospect for discovering and maximizing the clinical benefits of test compounds (Van Boxtel and Buitenhuis, 2001). Any compound with an oral $LD_{50}$ between 500 – 2000 mg/kg body weight had been reported to be practically non toxic (Hodge and Steiner, 1943; Zbinden and Flury-Roversi, 1981; Hayes, 1989; Grossel and Crowl, 1994). Based on this classification therefore, the oral $LD_{50}$ in this study indicated relative oral safety of the stem bark extract of *Randia nilotica*.

The significant variations observed in the haematological parameters are suggestive of anaemic condition of some sort (Adewuyi, 2007; Katzung *et al.,* 2009). Blood volume and electrolyte composition are closely regulated by complex mechanisms involving the kidneys, hypothalamus and other organs (Marcovitch, 2005). Thus, any agents that affect these organs as is the case with this extract may as well affect blood cell production. This is further seen in the histopathological effects on the spleen, brain and kidney.

Haemorrhage and severe circulatory congestion of blood vessels was noted in the histopathological examinations of the various organs in this study and this may have resulted in such haematological alterations, congestions of blood vessels, haemorrhages and depletion of lymphocytes. Blood vessels branch extensively to form circuits that supply blood to many organs and tissues of the body and any resistance to such flow of blood will invariably increase the total vascular resistance and could result in oedema and fragmentation of muscle fibers as observed especially for the heart in this study (Guyton

and Hall, 2006). In the histopathological study, the heart also had myocardial congestion, infiltration of inflammatory cells and myocardial haemorrhage. Myocarditis has been noted to decrease the heart's ability to pump blood and causing reduced efficiency that could lead to reduction in blood parameters (Parry, 1984; Dixon, 1987; Guyton and Hall, 2006).

The 28 days daily oral administration caused no alteration in blood urea-nitrogen, creatinine and calcium levels at all dose levels, but oral administration for 90 days caused significant increase in blood urea nitrogen. Usually, kidney damage is indicated by the level of excreted substances like creatinine, urea, albumin etc. Consistent elevation of blood urea nitrogen (BUN) level occurs only when the renal function, specifically the glomerular filtration rate (GFR) is reduced by 40 to 60 percent and which is indicative of renal impairment (Treseler, 1995; Chen *et al.,* 2008). This implies a likelihood of reduction in glomerular filtration rate from prolonged administration of the stem bark extract of *Randia nilotica* in rats. The histopathological finding for the kidney following 90 days daily oral administration showed infiltration of the glumeruli and parenchyma with inflammatory cells, and this could also explain the reason for such increase in blood urea nitrogen. Urea is the totality of the protein metabolism waste product of the kidney in proportion to the concentrating power of the kidney (Ferguson *et al.,* 2008). Low urea concentration could be an indication of low protein diets or kidney malfunction. In dogs, cats, rabbits and other mammalian species creatinine clearance can be used to determine glomerular filtration rate (GFR) as an index of renal function, but it is often not used for humans due to intermediate urinary creatinine filtrate reabsorption and plasma creatinine secretion. The nonspecificity of these standard diagnostic metrics suggests that more specific test be carried out to

confirm preliminary results of creatinine and the blood urea nitrogen assessment of kidney injury (Ferguson *et al.,* 2008).

The 90 days daily oral administration of the stem bark extract of *Randia nilotica* showed significant ($p \leq 0.05$) increase of high density lipoprotein (HDL) level only at the 250 mg/kg dose. The 28 days daily oral administration did not show any significant ($p \leq 0.05$) increase or decrease in the liver enzymes, total protein, albumin, total cholesterol, triglycerides and high density lipoprotein for the 28 day treatment was significant. There was actually slight increase in the transaminase (ALT, AST) levels, although this effect was not significant. Generally, necrositic injuries of the hepatocytes from toxic agents primarily often cause an elevation of enzymes found within the hepatocytes such as the amino transferases (Nyblom *et al.,* 2004). Such injuries were noted from the histopathological studies of the liver in this study, and might probably be responsible for the slight increases observed in the transaminases. However the observed significant ($p \leq 0.05$) increase in high density lipoprotein (HDL) level as well not significant ($p \leq 0.05$) reduction in low density lipoprotein (LDL) that occurred over a long time use at its lower dose level (250 mg/kg) seemed to be a boosting effect on the liver. Flavonoid, which is one of the constituents of this extract, had been reported to possess hepatoprotective activity and thus, may be responsible for this observed effect (Chaterrjee, 2000). The HDLs are secreted by the liver and small intestines and they acquire cholesterol from peripheral tissues, thus protecting the cholesterol homeostasis of cells (Katzung *et al.,* 2009). High density lipoprotein (HDL) decreases the clotting potentials of blood, participate in the retrieval of bad cholesterol from the arterial wall and also help to inhibit the oxidation of atherogenic lipoproteins (Akubue *et al.,* 2006; Katzung *et al.,* 2009). HDL - Cholesterol acts as an anti-inflammatory agent

as well as an antioxidant for LDL – cholesterol as to reduce its harmful effects (Akubue, 2009). Thus, its reduced level (hypoalphalipoproteinaemia) is a risk factor for atherosclerotic disease.

While significant ($p \leq 0.05$) decrease in brain weight was observed for the 90 days daily oral administration of the stem bark extract of *Randia nilotica*, the 28 days daily oral administration did not reveal any significant ($p \leq 0.05$) increase or decrease.

The histopathological examination showed both dose and duration-dependent alterations in morphology of all the organs examined – brain, heart, kidney, spleen and liver – clearly revealing injury at cellular level.

The 28 days daily oral administration of stem bark extract of *Randia nilotica* showed neuronal degeneration and congested blood vessels of the brain, while the 90 days daily oral administration, cerebral congestion with oedema occurred. Injuries or inflammations of any sort from toxic compounds could cause disturbances in the homeostatic mechanism of the body system. Injury to neuronal cells is known to cause necrosis which manifests as cell swelling, vacuolization and lysis, because it is often associated with $Ca^{2+}$ overload and membrane death of the involving cells. Necrotic cells spill their content into the surrounding tissue, thereby evoking inflammatory response (Rang *et al.,* 2007).

The heart had myocardial congestion, infiltration of inflammatory cells and myocardial haemorrhage for sub chronic effect and in addition, the 90 days daily oral administration of stem bark extract of *Randia nilotica* caused fragmentation of muscle fibres and oedema.

Injuries on the heart could hinder the circulatory activities of the heart and restriction of blood flow. Haemorrhage results in anaemia and in addition to being important as a cause of severe circulatory congestion of blood vessels, anaemia may aggravate existing heart disease or may unmask clinically inapparent disease. Oedema forms in patients with cardiac failure if the hydrostatic pressure in the veins exceeds the plasma oncotic pressure and tissue pressure. It tends to form in areas with a high hydrostatic pressure due to gravity and low tissue pressure, and it is also favoured by a low plasma protein concentration (Parry, 1984; Dixon, 1986). Myocarditis has been proven to decrease the heart's ability to pump blood thereby causing hypoeffectivity thus, portending the risk for shock. Blood vessels branch extensively to form circuits that supply blood to many organs and tissues of the body. Increasing the resistance of these vessels invariably increases the total vascular resistance (Guyton *et al.,* 2006). This could possibly account for the oedema observed as well as fragmentation of the muscle fibres.

Histopathology of the kidney following 28 days daily oral administration of the stem bark extract of *Randia nilotica* revealed atrophy of glomeruli, haemorrhages, tubular necrosis, cellular infiltration, hyper cellular glomeruli and adhesion of the parietal surface with Bowman's capsule. The further effect following the 90 days daily oral administration include vacuolation of glomeruli, infiltration of inflammatory cells and congestion of glomeruli. There is indication of reduced – if any – or lack of antioxidant properties of the extract (Duarte *et al.,* 2001), thereby resulting in tubular necrosis and desquamation of the cells, forming casts (Dixon, 1986). The wide involvement of the kidney in many physiological and pharmacological functions makes the kidney a sensitive body organ (Berne and Levy, 1988; Rang *et al.,* 2007).

The alterations on the spleen were congestion of blood vessels and depletion of lymphocytes following both 28 and 90 days daily oral administration of the extract. The function of spleen can be affected when there is hyperplasia of the lymphoid cells or of the reticuloendothelial system (Parry, 1984). This study suggests that there may be damage to the spleen over prolonged exposure to the stem bark extract of *Randia nilotica*.

The liver had congestion of blood vessels, perivascular infiltration of inflammatory cells and necrosis of hepatocytes. The liver's increased vulnerability to toxic attack is due to its involvement in various important functions including direct involvement in the metabolism of most toxic agents.

These findings on the long term daily oral administration of the stem bark extract of *Randia nilotica* which resulted in morphological, physiological and pathological alterations on vital organs calls for caution in the use of the stem bark extract of the plant.

# CHAPTER SIX

## 6.0 SUMMARY, CONCLUSSION AND RECOMMENDATIONS

### 6.1 Summary and Conclusion

*Randia nilotica* is used traditionally for various diseases, but information on its toxicity profile is not known. This study has shown that:

- oral acute administration of the extract up to 5000 mg/kg did not cause mortality and other observable toxic effects in rats
- the hydroethanolic stem bark extract of *Randia nilotica* has bioactive constituents such as saponins, carbohydrates, cardiac glycosides, triterpenes, flavonoids and tannins.
- at low oral dose of 250 mg/kg the extract increased the level of high density lipoprotein cholesterol following prolonged use, probably due to its flavonoids content
- the extract significantly decreased ($p \leq 0.05$) packed cell volume (PCV) level (44.70 – 40.88) following 28 day oral administration at its 1000 mg/kg dose
- a significant decrease ($p \leq 0.05$) in packed cell volume (PCV), Haemoglobin (Hb) count and red blood cell (RBC) count occurred following 90 day oral administration with both the 500 and 1000 mg/kg doses, as well as significant ($p \leq 0.05$) increase (85.35 – 91.50) in mean cell volume (MCV) at 1000 mg/kg, following 90 days daily oral administration.
- statistically significant increase ($p \leq 0.05$) in the level of blood urea nitrogen (BUN), occurred across all dosages, following daily oral administration for 90 days

- significant reduction ($p \leq 0.05$) in the weight of the brain occurred in a progressive manner, following oral administration for 90 days
- dose and duration-dependent toxic effects occurred on vital body organs in both short and long term use of the extract, consisting in significant alteration in morphology and histology of the brain, kidney, heart, liver and spleen.

The major inference that could be drawn from the above work is in the fact that the stem bark extract of *Randia nilotica* is toxic to the organs at the doses tested over prolonged period of time. It is therefore conclude that, while it has pharmacological utility, *Randia nilotica* merits further attention with respect to toxicity as it is seen to be toxic.

## 6.2 Recommendation for further study

- There is the need to isolate and characterize the bioactive constituents in the stem bark of *Randia nilotica* with a view to ascertaining the metabolites of its toxic principle for structural modification that may still permit its beneficial use.

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# APPENDICES

I. Conversion of 'rpm' to 'g'

G-force= $1.118 \times R \times (rpm/1000)^2$ or G – force = $0.0000118 \times R \times RPM^2$

Where R = radius of rotation in millimeters

rpm = revolutions per minute

G = Gravitational force "g"

II. Extractive yield from stem bark of *Randia nilotica*

Weight of container = 135.42g

Crude extract + container = 191.70

Weight of extract = 56.28

$$\%\text{age yield} = \frac{\text{Weight of extract (g)}}{\text{Powdered plant (g)}} \times 100$$

Where: weight of powdered plant = 1000g

Weight of extract = 56.28

Thus, $\frac{56.28g}{1000g} \times 100$

= 5.63%

III. Low density lipoprotein values were calculated using Friedworld Formula (in mg/dl) (Randox Laboratories, 2014):

$$\text{LDL Cholesterol (mg/dl)} = \text{Total Cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL Cholesterol}$$

IV. Calculation of $LD_{50}$

$LD_{50}$ √ Minimum toxic dose X maximum tolerated dose

Or

$LD_{50}$√Maximum dose for all survival X minimum dose for all death

V. Calculation of organ weight ratio

The heart, spleen, liver, brain and kidney of the rats were removed and weighed using a sensitive weighing balance, and the relative organ body weight ratio (ROW) was determined using the formula:

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day (g)}}$$