EFFECTS OF ORAL ADMINISTRATION OF LEAF EXTRACT OF *UVARIA CHAMAE (MMIMI OHIA)* IN ALBINO WISTAR RATS

BY

OKAFOR EUGENIA OBIAGELI

PG/MSC/08/53505

DEPARTMENT OF MEDICAL LABORATORY SCIENCES, FACULTY OF HEALTH SCIENCES AND TECHNOLOGY, COLLEGE OF MEDICINE UNIVERSITY OF NIGERIA, ENUGU CAMPUS

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A PROJECT PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.SC) IN DEPARTMENT OF MEDICAL LABORATORY SCIENCE, FACULTY OF HEALTH SCIENCE AND TECHNOLOGY, UNIVERSITY OF NIGERIA, ENUGU CAMPUS

SUPERVISOR: DR. I. S. I. OGBU

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CERTIFICATION

DEDICATION

This research work is dedication to the Almighty God who has been my help in everything.

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I appreciate my supervisor Dr I.S.I. Ogbu for his painstaking supervision and fatherly care which help in no small measure to successful completion of this work.

My sincere thanks go to Okwuosa C., the originator of this topic and for his assistance in the animal experiment. I also acknowledge the following; Brr (Dr) Achukwu, my H.O.D, Onyeanusi J.C. for editing the work. Mrs Nubila for analyzing my data. Ijeoma, and the staff of Chemical Pathology Department, University of Nigeria teaching Hospital Ituku Ozalla,Enugu for assisting me in analyzing the samples.

I appreciate my husband Iyke, for always being there for me and Teddy who is my role model. May Almighty God reward you all adequately. Amen.

ABSTRACT

Uvaria chamae P. Beauv is known to have various medicinal and therapeutic properties. The Biochemical and toxicological effects of the leaf extract were assessed in this study using Albino wistar rats. Acute toxicity test was performed on the rats to determine the LD_{50}.Sub acute toxicity test was also carried out by oral administration of different doses of ethanolic extract of *U. chamae* leaf (EEUC) on different groups of rats for thirty (30) days. Twenty (20) male albino wistar rats were divided into four (4) groups A to D (n=5). Animals in group A served as control and received 5%tween 80. Then those in groups B, C and D received 250, 500 and 1000mg/kg of the extract. On the thirty first (31^{st}) day, 5ml of blood was collected from the animals into plain tubes for biochemical investigations. Result of acute toxicity studies showed that leaf extract of *Chamae* had no toxic effect. Administration of the extract resulted in an increases in the mean alkaline phosphatase (ALP) levels in the treated groups B (490.00 ± 38.00), C (630.00± 60.00), D (370.00±20.00) when compared with the control A (350.00±11.00).Rats in group B (250mg/kg EEUC), C (500mg/kg EEUC), and D(1000mg/kg EEUC) had mean urea values of 3.80± 0.31, 3.30±0.28 and 3.50± 0.18mg/dl respectively and these were significantly lower than the mean urea value of the control 5.00± 0.19mg/dl ($p>0.05$). However, alanine transaminase obtained for B (56.00± 1.90 iu/l) were not significantly different from 56.00± 5.20 u/l obtained for the control ($p>o.05$). Similarly, there were no significant differences in aspartate transaminase values of group B (70.00±7.30), C (64.00±3.10) and D(78.00±5.20u/l) when compared with the control of group A (76.00 ± 6.70 u/l) ($p>0.05$). Rats in the treated groups B (71.00±5.85mg/dl), C (81.00± 4.20mg/dl) and D (64.00±6.70mg/dl) had no significant increase in their creatinine levels when compared with the control (77.00±4.30mg/dl) ($p>0.05$).Sodium levels obtained were 140.00±0.68, 140.00± 0.85 and 140.00± 1.50mmol/l for groups B, C and D respectively and these were not significantly different from 140±2.00mmol/L obtained for the control ($p>0.05$). The chloride levels of groups B (110.00± 0.31); C (110.00±0.52) and D (110.00 ± 1.60mmol/l) were not significantly increased when compared with the control (110.00 ± 2.90mmol) ($P>0.05$). The mean potassium and bicarbonate levels of the extract treated groups were not significantly different compared with control group ($p>0.05$).Phytochemical analysis of the ethanolic leaf extract of *Uvaria chamae* revealed the presence of alkaloids, flavonoids, resins, proteins, reducing sugars and terpenoids. The histological profile of the liver and kidney of control animals showed normal morphological patterns of hepatocytes, renal corpusles, and tubules. In the treated groups, there was no adverse effect on kidneys. However, the liver showed evidence of mild periportal lymphocytic infiltration at concentrations of 250mg/kg, 500mg/kg and 1000mg/kg of the extract. The ethanolic leaf extract of *Uvaria Chamae* has a mild effect on the liver of albino wistar rats at low doses; hence it is safe for use in traditional medicine.

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CHAPTER ONE

INTRODUCTION

Uvaria chamae belongs to the family Annonaceae (Irvine, 1961). It is a small tree that grows to about 4.5m high. It is commonly found in the savanna and rain forest regions of Nigeria and other African countries. It is called ñM*mimi ohia*ò, ñ*Kas kaifi*ò and ñ*Akisan*ò amongst the Ibos, Hausas and Yorubas respectively (Adetunji, 1999). The fruits are yellow when ripe and have a sweet pulp which is widely eaten. The fruit carpels are in finger-like clusters. All parts of the plant are fragrant.

The root barks, stem barks and leaves have a wide spread medicinal use. In Nigeria a decoction of the stem is used for the treatment of diarrhea (Igoli *et al.*, 2005). In Ghana, severe abdominal pain is treated by a root extract with native pepper in gin. The root with Guinea grains is used in application to the fontanelle for cerebral diseases. Among the Fula people of Senegal, the root has a reputation as the ñmedicine of Richesò. It is also considered to be a womanôs medicine used to treat amenorrhea and to prevent miscarriage (Burkill, 1985), and in Togo, root decoction is given for pains of childbirth. The plant is used to make pomade in Ghana (Irvine, 1961).

U. chamae is a plant with both medicinal and nutritional values. The root bark is used for respiratory catarrh and the root extract is used in phytomedicine for the treatment of piles, menorrhagia, epistaxis, haematuria and haemolysis (Oliver 1986, Irvin 1961). The root extract is used to cure abdominal pains. The juice from the roots, stems or leaves is commonly applied to wounds and sores for quick and proper healing (Irvin 1961). Also, in Sierra Leone and Nigeria, the root is reputed for having purgative and febrifugal properties. In folklore medicine, extracts of the roots, barks and leaves are used to treat gastroenteritis, vomiting, diarrhea, dysentery, wounds, sore throats, inflamed gums and a number of other ailments (Okwu, 2007).

In spite of the various uses of *Uvaria chamae* plant *in* traditional medicine in Nigeria, there is limited information on the toxicity of the leaf extract. The present study is undertaken to investigate the effects of the plantôs leaf extract on the liver and kidney of Albino wistar rats.

1.1 AIMS AND OBJECTIVES

1. To determine the LD50 of the leaf extract albino wistar rats.
2. To determine the phytochemical constituents of the extract.
3. To assess the biochemical effects induced by the extract on the electrolytes and liver enzymes of the albino wistar rats.

4. To evaluate the effect of the administration of the extracts on the histology of the liver and kidney of the rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of herbal medicine in Nigeria

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base. Undisputedly, the history of herbology is inextricably intertwined with that of modern medicine. Many drugs listed as conventional medications were originally derived from plants. For example, Salicylic acid, a precursor of aspirin, was originally derived from white willow bark and the meadowsweet plant. (Gbile, 1985) Cinchona bark is the source of malaria-fighting quinine. Vincristine, used to treat certain types of cancer, comes from periwinkle. The opium poppy yields morphine, codeine, and

paregoric, tincture of the opium poppy, was the favored tranquilizer in Victorian times. Even today, morphine- the most important alkaloid of the opium poppy, remains the standard against which new synthetic pain relievers are measured. The use of plants as medicine is older than recorded history.

Medicinal plants in Nigeria were considered by several researchers to form an important component of the natural wealth of the country (Gbile, 1985 and Iyamabo, 1990). The tropical rainforest of Nigeria has been described by Sofowora (1986) as a reservoir of phytomedicine. Many of these plants as explained by Iwu *et al* (1993) contain substances that can be used by man. These plants have traditionally been used by Nigerians because they are natural products, environmentally friendly, easily available, cheap and more curative than many orthodox medicines imported into the country today. The health benefits of medicinal plants are attributed in part to their unique phytochemical composition (Okwu, 2004). Sometimes conventional medications have been confirmed to have toxic effects on humans (Murray, 1995).

Herbal Medicine Today

The World Health Organization (WHO, 2000) estimates that 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous

peoplesô traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine. WHO notes that of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value.

2.2 Phytochemical composition and biological activities of *uvaria chamae* parts

Phytochemical studies on the root extract of *Uvaria chamae* revealed the presence of bioactive components comprising flavonoids, alkaloids, tannins, saponins and phenols (Okwu and Iroabuchi, 2009, Olufunmilayo et al,2010).These bioactive compounds may be responsible for the medicinal properties of *U. chamae* and form the bases of its use in herbal medicine in Nigeria. The biological functions of flavonoids include protection against allergies, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors. (Okwu and Omodamiro, 2005, Farquar 1996). Flavonoids reduce the risk of estrogen-induced cancers by interfering with the enzymes that produce estrogen. However, some flavonoids behave as a powerful protective agent

against inflammatory disorders. They reduce oedma formation and inhibit the synthesis of prostaglandin E_2 and thromboxane B_2 (Okwu and Omodamiro, 2005, Samasundaram and Sadique, 1989).Okwu and Iroabuchi, (2009) discogvered that *U. chamae* root extract has a high content of flavonoids compared to alkaloids, tannins, saponins, and phenols. As a result of the availability of flavonoids in *U.chamae,* the extract prevents platelet stickiness and hence platelet aggregation. The identification of the abundant presence of saponins and tannins in the roots of *U. chamae* may be responsible for the haemostatic activity of this plant where it arrest bleeding from damaged or injured vessels by precipitating proteins to form vascular plugs. The presence of phenols in this plant indicates that it could act as anti-clotting agents, antioxidants, immune enhancer and hormone modulators. Phenols have been the subjects of extensive research as bioactive compounds used in disease prevention (Duke, 1992).Phenols have been responsible in having the ability to block specific enzymes that causes inflammatory disorders. They also modify the prostaglandin pathways, and thereby protect platelets from clumping (Duke, 1992). Okwu and Iroabuchi (2009) demonstrated that *U.chamae* root extract possesses oxytocic activities. It contracted the uterine muscle of a guinea pig at a graded dose. It has similar action to that of oxytocin though not so intense. Oxytocin is a hormone, which makes the uterus experience strong

contractions, thus producing labour (Roger, 2002). High dose of *U.chamae* root extract will prepare the uterus and ensures that fatigue disappears, producing strong, regular contraction to facilitate labour in the last month of pregnancy. Organic extract of *U. chamae* root showed strong uterine activity. *U.chamae* plant parts are used in herbal medicine to accelerate labour in south Eastern Nigeria. However, if it is used during the first month of pregnancy, they could have abortifacient properties (Okwu and Omodamiro, 2005).

Okwu and Iroabuchi, 2008 discovered that the ethanolic extract of the roots of *U.chamae* produced a marked anti-inflammatory activity. The anti-inflammatory effects may be due to the presence of bioactive compounds such as flavonoids, saponins, and phenolic compounds.

2.3 Antibacterial properties of *uvaria chamae* parts.

The work done by Oluremi et al., 2010 on the comparative assessment of antibacterial activity of *Uvaria chamae* parts showed that the methanolic extracts of the root, stem and leaf of *Uvaria chamae* inhibited *Staphylococcus aureus, Escherichia coli*, *Klebsiella spp,* Methicillin-*resistant Staphylococcus aureus(MRSA),Protus spp,*typed strains *E.coli* ATCC 25922, *S.aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC27853 using standard agar diffusion method at 50, 100, 150, 200 and 250mg/ml. The stem bark extract

displayed highest degree of antibacterial activity, being active against all the organisms used but *Klebsiella spp* displayed lack of sensitivity at lower concentration of between 50 and 150 mg/ml. Root extract had no activity against *Klebsiella spp.* at the concentrations (50-250mg/ml) used for the study while other organisms were susceptible to the activity of the root extract. The leaf extract was active only on MRSA while the other organisms were not sensitive to the activity of the leaf.

However, the fact that all the extracts from leaf, stem bark and root showed activity against MRSA is a major breakthrough especially in the management of Methicillin-Resistant *S.aureus*, a major cause of both community-acquired and nosocomial associated infections. *Uvaria chamae* activity on MRSA has made the plant a promising source of more efficient and efficacious chemotherapeutic agent and cheaper alternative to orthodox antibiotics to which organisms frequently and continually develop resistance (Singleton, 1999).Studies on the antibacterial properties of *U. chamae* have shown that the cold, hot, and soxhlet extracts of *U. chamae* leaves inhibited the growth of *staphylococcus aureus*, *streptococcus pyogenes, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa and Vibro spp*.(Ogbulie, 2007).

The study also indicated that ethanol is a better solvent than water in the extraction of the active principles of the plant, with the soxhlet extraction method being the best.

2.4 In vitro antisickling activities of *uvaria chamae*

Uvaria chamae (Annonaceae) has been used in folklore medicine in the management of sickle-cell disease (SCD) in South-West Nigeria. Current report indicates that roots of *Uvaria chamae*(Annonaceae) and Plumbergo Zeylanica (Plumbaginaceae) are part of the constituents that are used in making traditional recipe for the management of sickle cell anaemia (Egunyomi et al.,2009). *Uvaria Chamae* root contains anthraquinones which act on the gastro-intestinal tract to increase the peristaltic action (Evans,1989.)In cases where sickle cell patients complain of constipation, the anthraquinones present in the *Uvaria Chamae* may be useful as a mild laxative.

Kenner and Yves, (1996) stated that cardiac glycosides have antispasmodic properties and act as good sedative. *Uvaria chamae* root contains cardiac glycosides which indicate that a recipe that contains *U. chamae* root may be potent to relieve symptoms of cardiac insufficiency, cough and circulatory problems (Olufunmilayo et al., 2010).

2.5 Anti-ulcer properties of *uvaria chamae* leaf

Studies on the anti-ulcer properties of ethanolic leaf extracts of *Uvaria chamae* by Chilaka et al 2010, revealed that *uvaria chamae* leaf showed a better anti-ulcer activity on albino wistar rats compared to cimetidine a standard anti-ulcer drug. The antiulcer effects of the extracts may explain the rational for the use of the plant extract in traditional medicine as a popular anti-ulcer/stomach ache.

2.6 The liver and its functions

The liver is the largest glandular organ of the body and weighs about 1.3kg.It is reddish brown in colour and is divided into four lobes of unequal sizes and shape (Crook,2006).Liver tissue is composed of thousands of lobules and each lobule is made up of hepatic cells, the basic metabolic cells of the liver(Janis,2002).

Significant liver function must be lost or impaired before some laboratory tests show abnormality. Numerous tests are used to estimate liver function. Most are not specific for a particular disease, but only reflect liver tissue damage.

2.6.1 Functions of the liver

The liver produces and excretes bile, which is a greenish liquid required for emulsifying of fats. It converts glucose to glycogen (glycogenesis) and

synthesize glucose from certain amino acid, lactate or glycerol (gluconeogenesis).Also, the liver helps in the breakdown of glycogen into glucose (glycogenolysis) and performs several roles in lipid metabolism such as cholesterol synthesis, and synthesis of lipoprotein which is made up of cholesterol, triglycerides, phospholipids and proteins(Kamath,1996).It produces coagulation factors including fibrinogen and factor ii(prothrombin),v, vii, ix, x, xii, xiii and stores fat, soluble vitamins(A,D,E and K), folate,vitaminB12 and minerals such as copper and Iron(Champee and Harvey,1994).

The liver efficiently takes up bilirubin, which is yellow pigment formed as a breakdown product of red blood cell haemoglobin and chemically modifies it to conjugated or water soluble bilirubin that is excreted into bile (Janis, 2002).It also removes harmful substances such as ammonia, toxins from the blood and breaks them down into less harmful compounds (Kamath, 1996).

2.7 Liver function tests

Liver function tests are a group of blood test used to determine if the liver is damaged or its function impaired(Perry et al,1998).Elevations of certain liver tests in relation to others aids in the determination of liver function and itôs impairment. The liver function tests include alkaline phosphatase (ALP),

Alanine and Aspartate transaminases (ALT and AST), total and direct bilirubin, Gamma-glutamyl transferase (GGT), albumin, and prothrombin time tests (Kamath, 1996).ALT and AST are notably elevated in liver damage caused by liver cell disease. However, in intrahepatic obstructive disease which may be caused by some drugs or billiarry cirrhosis, the alkaline phosphatase is most abnormal.

2.7.1 Alkaline phosphatase (ALP)

Alkaline phosphatase is an enzyme found in many tissues with the highest concentrations in the liver, biliary tract and bone. It is used to assess liver lesions that may cause biliary obstruction such as tumors or abscesses (Janis, 2002).Serum alkaline phosphatase levels may greatly increase with liver tumors and lesions, and may show a moderate increase with diseases such as hepatitis.

In the liver, the enzyme is localized to the microvilli of the bile canaliculi, and therefore it serves as a great marker of extrahepaticbiliary obstruction,such as a stone in the common bile duct, or in intrahepatic cholestasis,such as drug cholestasis or primary biliary cirrhosis.

2.7.2 Alanine aminotransferase (ALT)

Alanine aminotransferase was formerly called serum glutamic-pyruvic transaminase (GPT or SGPT).It is found in many tissues like cardiac tissue but highest concentration is in the liver. It is considered the more liver-specific enzyme of the transferases. This enzyme usually rises higher than aspartate aminotransferase in liver disease, with moderate increases (up to 10 times normal) in cirrhosis, up to 100 times normal in viral or toxic hepatitis (Barbara et al, 2000).Clinical applications of ALT assays are confined mainly to evaluation of hepatic disorders. Higher elevations are found in hepatocellular disorders than in extrahepatic or intrahepatic obstructive disorders. In acute inflammatory conditions of the liver, ALT elevations are frequently higher than those of AST and tend to remain elevated longer as a result of the longer half-life of ALT in serum(16 and 24 hours, respectively).

2.7.3 Aspartate aminotransferase (AST)

Aspartate aminotransferase was formerly called serum glutamic oxaloacetic transferase (GOT or SGOT).The highest concentrations are found in cardiac tissue, liver, and skeletal muscle, with smaller amounts found in the kidney, pancreas, and erythrocytes. It is elevated after myocardial infarction, as well as in liver disease. In liver disease, the AST increase is usually less than ALT

increase, but in liver disease caused by alcohol use, the AST increase may be two or three times greater than ALT increase(Vozarova et al, 2002)

2.8 Kidney and its functions

The kidneys form a paired organ system located in the lumber region. In an adult, each kidney is about 12cm long and weighs about 150g in men and 135g in women. Kidneys consist of a cortex and a medulla. The **nephron** is the basic unit of the kidney. It has a Malpigian corpuscle, with a vascular glomerulus within a matrix formed by mesangial cells and an epithelial Bowman's capsule. The capsule joins a series of tubules starting with the proximal tubule and followed by the loop of Henle, the distal tubule, and ending in the collecting ducts.

2.9 Functions of the kidney

The proper function of the kidney is necessary for water and electrolyte balance. The kidneys eliminate waste products, maintain water and pH balance. It also secretes hormones like rennin, erythropoietin, 1, 25-dihydroxy vitamin D3 and prostaglandins. The serum and plasma concentrations of creatinine, Blood Urea Nitrogen (BUN) and uric acid are altered in certain kidney diseases (Barbara et al, 2000).

2.9.1 Creatinine

Creatinine is a waste product of creatine phosphate, a substance stored in muscle and used for energy. Creatinine is excreted by the kidney. When renal function is impaired, blood creatinine levels rise, but more than 50% of kidney function must be lost before this happens. Creatinine levels are not affected by diet or hormone levels. Increases occur when there is impairment of urine formation or excretion, which occurs in renal disease, shock, water imbalance, or ureter blockage.

Pathophysiology

Creatinine is synthesized primarily in the liver from arginine, glycine, and methonine(Oh, 2006).It is then transported to other tissues, such as muscle, where it is converted to creatine phosphate, which serves as a high energy source. Creatine phosphate loses phosphoric acid and creatine loses water to form the cyclic compound, creatinine, which diffuses into the plasma and is excreted in the urine (Apps et al, 1992).

Creatinine is released into the circulation at a relatively constant rate that has been shown to be proportional to an individualôs muscle mass. It is removed from the circulation by glomerular filteration and excreted in the urine (Apps et al, 1992).Small amounts of creatinine are secreted by the proximal tubule

and reabsorbed by the renal tubules (Lamb et al 2005).Daily creatinine excretion is fairly stable.

Clinical Application

Measurement of creatinine concentration is used to determine sufficiency of kidney function, severity of kidney damage, and to monitor the progression of kidney disease (Young, 2000)

2.9.2 Blood urea nitrogen (BUN)

In mammals, surplus amino acids are converted to urea and excreted by the kidneys. The surplus is measured as BUN or blood urea nitrogen. Urea synthesis occurs in the liver. Proteins are broken down to amino acids, which are then deaminated to form ammonia. Ammonia is readily converted to urea, avoiding toxicity. The kidney is the only significant route for excretion of urea.

The BUN concentration is influenced by the amount of protein degraded, diet, hormones, and kidney function. Therefore, BUN level is not as good an indicator of kidney disease as is the creatinine level.BUN levels may be low during starvation, pregnancy, and a low-protein diet. Increased BUN concentration may occur during a high-protein diet, after administration of steroids, and in kidney disease (Barbara et al 2000).

Pathophysiology

An elevated concentration of urea in the blood is called azotemia. Very high urea concentration accompanied by renal failure is called uremia, or the uremic syndrome. This condition is eventually fetal if not treated by dialysis or transplantation.

Conditions causing increased plasma urea are classified according to cause into three main categories: pre-renal, renal and post-renal (Harrisonôs online 2008). Pre-renal azotemia is caused by reduced renal blood flow. Less blood is delivered to the kidney; consequently less urea is filtered. Causative factors include congestive heart failure, shock, hemorrhage, dehydration, and other factors resulting in a significant decrease in blood volume.

Decreased renal function causes an increase in plasma urea concentration as a result of elevated urea include acute and chronic renal failure, glomerular nephritis, tubular necrosis and other intrinsic renal diseases.

Postrenal azotemia can be due to obstruction of urine flow anywhere in the urinary tract by renal calculi, tumors of the bladder or prostate, or severe infection.

2.10 ELECTROLYTES AND THEIR FUNCTIONS IN THE BODY

Ions in body fluids are called electrolytes. Major ions include the positively charged cations, sodium, potassium, calcium, and magnesium, and the negatively charged anions which include chloride, bicarbonate, and phosphate. However, a request for laboratory electrolyte measurement usually means the physician wants serum levels of four electrolytes measured. They are sodium (Na+), potassium (k+), chloride (Cl^-), and bicarbonate (HCO_3^-).

Electrolyte imbalance occurs when the serum concentration of an electrolyte is either too high or too low. This can affect all organs and the body systems.

2.10.1 Sodium (Na+)

Sodium has an important influence on osmotic concentration and determines the extracellular fluid volume. Water moves back and forth across cell membranes to maintain proper sodium balance. Rapid shifts in water volume in cells can damage or destroy cells. Sodium concentration is influenced by aldosterone levels and kidney function (Barbara et al 2000).

Hypernatremia.

Hypernatremia is defined as an increased Na+ concentration. It occurs in dehydration, hyperadrenalism (Cushingôs disease), and diabetes insipidus (a

deficiency of antidiuretic hormone). Hypernatremia is less commonly seen in hospitalized patients than hyponatremia (Kumar and Tomas 1998).

Hyponatremia

Hyponatremia is defined as decreased concentration of sodium. It occurs in severe diarrhea, acidosis of diabetes mellitus, decreased aldosterone secretion (Addisonôs disease), and renal disease in which there is poor ion exchange in the tubules. Hyponatremia is one of the most common electrolyte disorders in hospitalized and non hospitalized patients (Munger, 2007, Oh, 2007).

2.10.2 Potassium

Potassium (K^+) is the major intracellular cation in the body, with a concentration of 20times greater inside the cells than outside. Potassium is excreted by the kidney. High or low levels of potassium outside the normal range can affect muscle function, particularly in the heart. Functions of k^+ in the body include regulation of neuromuscular excitability, contraction of the heart, ICF volume, and H^+ concentration (Rose, 2001).

Hyperkalemia

Hyperkalemia is defined as increased serum potassium. It occurs when potassium leaves the cells rapidly, and may occur in anoxia and acidosis. Patients with hyperkalemia often have an underlying disorder, such as renal insufficiency, diabetes mellitus, or metabolic acidosis that contributes to hyperkalemia (Gennari, 2002).For example, during administration of KCl, a person with renal insufficiency is far more likely to develop hyperkalemia than a person with normal renal function.

The most common cause of hyperkalemia in hospitalized patients is due to therapeutic K^+ administration. This risk is greatest with intravenous k^+ replacement (Gannari, 2002).

Hypokalemia

Hypokalemia is defined as decreased serum potassium. It may be due to decreased intake of potassium, increased levels of aldosterone, or increased loss of potassium due to vomiting, diarrhea, or use of diuretics.

2.10.3 Chloride

Chloride (Cl^-) is the anion with the highest extracellular concentration. Its precise function in the body is not well understood. However, it is involved in maintaining osmolarity, blood volume, and electric neutrality. Chloride concentration varies inversely with bicarbonate (HCO_3^-).The concentration of chloride will increase in dehydration or in loss of CO_2 by hyperventilation(respiratory alkalosis). Decreased chloride concentration may occur in acidosis caused by uncontrolled diabetes, renal disease, and excessive vomiting.

2.10.4 Bicarbonate

Bicarbonate (HCO_3^-) is the second most abundant anion in ECF. Total CO_2 comprises the bicarbonate ion (HCO_3^-), carbonic acid (H_2CO_3), and dissolved CO_2,with HCO_3^- accounting for more than 90% of the total CO_2 at physiologic pH. Because HCO_3^- composes the largest fraction of total CO_2 measurement is indicative of HCO_3^- measurement. Acid-base imbalances cause changes in HCO_3^- and CO_2 levels. Plasma HCO_3^- levels are increased in metabolic alkalosis due to severe vomiting in pyloric stenosis, hypokalemia states or excessive intake of alkali and in compensated respiratory acidosis. The values are decreased in metabolic acidosis of renal origin, diabetic

acidosis, diarrhea/intestinal fistula, hypertension, dehydration, compensated respiratory alkalosis.

2.11 REFERENCE RANGES

Reference ranges vary from laboratory to laboratory, and also it depends on the method used. Reference ranges for the methods in this work are as follows:

Alkaline phosphatase (ALP): 25- 92u/l (Kind and King method, 1954)

Alanine transaminase (ALT): 3-15 u/l (Reitman and Frankel method, S1957)

Aspartate transaminase (AST): Serum up to 12u/l (Randox kit)

Creatinine:0.6-1.1mg/dl for male

0.5-0.9mg/dl for female (Randox kit)

Urea: 10-55mg/dl (Randox kit)

Sodium (Na^+): 135-145mmol/L (Ion Selective Electrode Analyzer method)

Potassium (K^+): 3.5-5.0mmol/L (Ion Selective Electrode Analyzer method)

Chloride (Cl^-): 97-108mmol/L (titrametric method)

Bicarbonate (HCO_3^-): 24-28mmol/L (titrametric method)

CHAPTER THREE

MATERIALS AND METHOD

3.1 SAMPLE COLLECTION

3.1.1 Animals

Thirty two male albino wistar rats were procured from Anatomy Department and housed in the animal House of college of medicine, University of Nigeria Enugu Campus. They were fed with guinea feed and water *ad libitum*. These animals were acclimatized for two weeks. All the rats used for this study were handled according to institutional and international guidelines for experiments involving the use of vertebrates.

3.1.2 PLANT COLLECTION

Uvaria chamae leaves were collected from a farmland near Senior Staff quarters of University of Nigeria, Enugu Campus. They were authenticated by a Botanist at University of Nigeria, Nsukka and a voucher specimen was kept in their Herbarium for future reference. Voucher number 95 was given to it.

3.2 PLANT EXTRACTION

Five hundred grams (500g) of dried leaves of *U. chamae* was grounded into fine powder and kept in a leak-proof container. Five (5) litres of absolute

ethanol were used to extract the active ingredients in the fine powder by macerating the powder in this solvent for 48 hours with intermittent agitation. After 48hours, the extract was sieved with a muslin cloth and filtered using a whatman No.1 filter paper and filtrate evaporated to dryness. The ethanol extract had a yield of 12.6 %(w/w). From this stock, fresh solution of the extract dissolved in 5% aqueous suspension of tween 80 was made to give a final concentration of 100mg/ml.

3.3 ACUTE TOXICITY TEST (LD50 DETERMINATION)

The Lorke (1983) procedure of LD_{50} determination was used. This method of acute toxicity determination makes the following assumptions:

1. Substances more toxic than 1mg/kg are so highly toxic that it is not important to calculate the $LD_{50.}$
2. LD_{50} values greater than 5,000mg/kg are of no practical interest.
3. An approximate figure for the LD_{50} is usually adequate to estimate the risk of acute intoxication.

The experiment involved a preliminary trial with three different doses of the plant extract. The animals (rats) were placed in three groups of three rats per group. The first group (A), received 1000mg/kg of body weight of the ethanolic leaf extract. Group B received 2000mg/kg of body weight. Group C

received 2500mg/kg of body weight. The rats were constantly observed for the first 2 hours, intermittently for the next 4 hours, and then overnight. The number of dead rats per group was recorded at the end of 24 hours. Then the LD_{50} was calculated using the formula;

$$LD_{50} = \sqrt{a \times b}$$

Where, a= minimum dose that caused 100 %(LD_{100}) death.

b=maximum dose that did not cause any death.

3.4 EXPERIMENTAL DESIGN

Twenty male Albino wistar rats were used for the experiment. They were divided into four groups of five rats each. The first group (Group A) served as the control and received 5mg/kg of tween80 (vehicle).Groups B, C and D received the extract at doses of 250, 500 and 1,000 mg/kg respectively. Administration was done orally and lasted for a period of thirty (30) days during which the animals were given water and standard pellets (Guinea-feed^{R}) *ad libitum.* They were observed daily for physical and behavioral signs of toxicity.

On the 31th day, 5ml of blood was collected from each animal through the retro orbital vein into plain tubes and their sera separated for biochemical analysis.

The animals were euthanized and their liver and kidney excised and preserved in 10% formol saline for histological assessment.

3.5 PHYTOCHEMICAL ANALYSIS OF THE ETHANOLIC LEAF EXTRACT.

These were carried out according to the methods described by Trease and Evans (1989) for determination of tannins, saponins, flavonoids, and anthraquinone. Test for alkaloids was performed using Wagnerôs and Dragenendoffôs reagents (Soforowa, 1986).Also, reducing sugars, Steroids, Terpenoids, Carbohydrate, resins, and Proteins were tested.

METHOD

100mg of the ethanolic extract was reconstituted with 40ml of ethanol. It was filtered and 30ml of the filterate was dispensed 3ml each into ten test tubes labeled 1 to 10.The filtrates in the test tubes 1 to 7 were evaporated to dryness using water bath at 75^{o}C.

3.5.1. Test for Reducing Sugars

To the residue in test tube 1, 3ml of water was added; 3ml of Fehlingôs solution was also added into it. The content was then mixed and incubated for 2 minutes.

Observation: Green suspension and red precipitate.

Result: + + +

3.5.2 Test for Flavonoids

To the residue in test tube 2, 3ml of ethylacetate was added; 3ml of 10% dilute ammonia was also added. Presence of green colour at the lower segment indicates the presence of flavonoids.

Observation: Green colour at the lower segment.

Result: + +

3.5.3. Test for Terpenoids

To the residue in test tube 3, 2mls of acetic anhydride and 2mls of conc. sulphuric acid were added.

Observation: Interphase colour: redish

Supernatant: Light green.

Result: +

3.5.4 Test for Alkaloids

1ml of 10% dilute sulphuric acid was added to the residue in test tube 4. It was mixed and filtered. To 1ml of the filterate, 2 drops of Dragendorff was added and mixed. It was allowed to stand for sometimes.

Observation: Brick red precipitate.

Result: + +

3.5.5 Test for Anthraquinones

To the residue 5, 4ml of diethyl ether was added. Then 4ml of dilute ammonia was also added.

Observation: Absence of wine red at the lower layer.

Result: Negative (-)

3.5.6 Test for Resins

To the residue in test tube 6, 10ml of ethanol was added. It was shaken and filtered through water moistened filter paper.

Observation: Presence of white cloudy precipitate.

Result: + + +

3.5.7 Test for Saponins (Fronting Test)

Preparation of aqueous filtrate.

15ml of water was added to the residue in test tube 7.It was shaken well and filtered.

4ml of water was added to 4ml of the aqueous filtrate. It was shaken very well and observed for foamy appearance.

Observation: Clear solution shown.

Result: Negative (-)

3.5.8 Test for tannins

2 drops of ferric chloride was added to 1ml of aqueous filtrate in test tube 8 .It was mixed and allowed to stand for some minutes. Greenish-black or bluish-black indicates the presence of tannins.

Observation: No colour.

Result: Negative (-)

3.5.9 Test for Protein

The filtrate in test tube 9 was reduced to 1ml and a few drops of millionôs reagent were added. It was mixed and allowed to stand for some minutes. White precipitate indicates the presence of protein.

Observation: White precipitate present.

Result: + +

3.5.10 Test for Carbohydrate

To 2ml of aqueous filtrate, few drops of Molisch reagent was added, 1ml of conc. Sulphuric acid was also added. It was mixed. Purple mauve interphase indicates the presence of carbohydrate.

Observation: No colour

Result: Negative (-).

3.6 ESTIMATION OF ALKALINE PHOSPHATASE

Alkaline phosphatase was estimated using Kind and King method (1954).

PRINCIPLE.

Alkaline phosphatase is a group of phosphomonoester enzymes, which have optimal activity when the pH is in the neighbourhood of 9.8. They act on a large variety of physiological and non-physiological substrates. In this method, disodium phenyl phosphate is used as the substrate. This is hydrolyzed by the enzyme liberating phenol and phosphoric acid. The phenol is estimated by its reaction with 4-aminoantipyrene to produce a colour in the presence of alkaline oxidizing agent (Alkaline ferricyanide).

PROCEDURE

Four tubes were set up for test, blank, standard and standard blank.2mls of buffered substrate were added to the test and blank tubes and warmed for 5 minutes in a 37^{o}C water bath.0.1ml of serum was added to the ñtestò tubes and both tubes incubated at 37^{o}C for exactly 15 minutes.1.1ml buffer was added to the standard tube followed by 1ml of working phenol standard, while 1.1ml of buffer and 1ml of water was added to standard blank tube.

0.8ml of 0.5N NaOH was added to all the tubes followed by 1.2ml of 0.5N $NaHCO_3$, 0.1ml of serum was then added to the test and blank tubes.1ml of 4-aminoantipyrene was added to all the tubes followed by 1.0ml of potassium ferricyanide. The tubes were mixed thoroughly after each addition. The absorbance of the tubes was determined calorimetrically at 520nm using water to zero the colorimeter.

CALCULATION

$$\frac{T-B}{S-B} \times 0.01 \times \frac{100}{0.1} \times 7.1 = \frac{T-B}{S-B} \times 71$$

Where,
T=Test
B=Test blank
S=Standard
B=Standard blank

3.7 ESTIMATION OF TRANSAMINASES (ALANINE AND ASPARTATE)

These were done by end point colorimetric method of Reitman and Frankel (1954). The procedure outlined in Randox Kit was used.

PRINCIPLE

The transaminases are metabolic enzyme, which catalyse transamination reactions in the body.

Alanine + Oxoglutarate $\xrightarrow{ALT}$ pyruvate + glutamate

Aspartate + Oxoglutarate $\xrightarrow{AST}$ oxaloacetate + glutamate

The Keto-acids produced, pyruvate and oxaloacetate are estimated by complying with 2.4 dinitrophenylhydrazine to form hydrazones. In alkaline medium, the colour of the hydrazones is reddish brown and its intensity is measured at 540nm.The colour intensity is directly proportional to the amount of keto acids formed, which in turn depends on the enzyme activity in the sample. The oxoglutarate is responsible for the blank reading since it also forms hydrazone with 2,4 dinitrophenylhydrazine(2,4 DNPH).

PROCEDURE

0.5ml of ALT and AST reagent1(buffer) were added to two test tubes each labeled ótestô andô blankô.0.1ml of sample was added to the tube labeled átestô. These were mixed and then incubated at 37^{o}C for 10 minutes (to allow the substrate to attain a constant physiological temperature).After which 0.5ml of reagent2 (2,4-dinitrophenylhydrazide) was added to both tubes (Test and Blank).0.1ml of the sample is added to the blank tube. The tubes were then mixed and incubated at room temperature for 20minutes. 5.0ml of sodium

hydroxide (NaOH) solution was added, the contents were mixed by inversion and the absorbance of the tubes determined calorimetrically at 540nm wavelength using distilled water to zero the instrument.

The absorbance of the blank was then subtracted from that of the test and the result read off from the AST activity table and ALT activity.

3.8 ESTIMATION OF SERUM UREA

Diacetylmonoxime method for Serum urea (Rosenthel, 1980) as described in Randox kit.

PRINCIPLE

In hot acid medium, diacetylmonexime is hydrolysed to diacetyl and monoxime. Diacetyl condenses with urea to produce a pink product. The intensity of the colour is proportional to the concentration of urea.

PROCEDURE

Three test tubes (Test, Standard and Blank) were set in a rack.100µl of reagent1 was added into the tubes and 10µl of each of sample, standard, and water were added into the tubes respectively. The content of the test tubes were mixed and incubated at 37^0c for 10 minutes. Then 2.5mls of reagent2 was added and 2.5mls of reagent3 was also added. They were mixed immediately

and incubated at 37^0c for 15minutes.Absorbance of the sample and standard were read against blank at 550nm wavelength.

Calculation

$$\frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard (97mg/dl)}}{1}$$

3.9 ESTIMATION OF SERUM CREATININE.

Serum creatinine was estimated using Jaffe reaction method, (1945) as described in Randox kit.

PRINCIPLE

Creatinine reacts with picric acid in alkaline medium to form a red complex of creatinine picrate. The colour intensity is due to creatinine and other chromogens. The creatinine colour can be eliminated by the addition of acetic acid and the interference estimated.

PROCEDURE

Three test tubes (Test, Standard and Blank) were set in a rack.1000µl of working reagent (equal volumes of picric acid and sodium hydroxide) were added into the test tubes.100µl each of sample, standard and distilled water were added into the tubes respectively. The content was mixed and absorbance

(A_1 of test and standard) was read after 30 seconds at 550nm wavelength. The absorbance (A_2 of test and standard) was read again after 2minutes incubation at room temperature.

CALCULATION

A_2 ï A_1 = △A (Change in Absorbance of test and change in absorbance of standard)

$$\text{Concentration of creatinine} = \frac{\triangle \text{A Test}}{\triangle \text{ A Std}} \times \frac{\text{Conc. of Std (1.97mg/dl)}}{1}$$

3.10 ESTIMATION OF SODIUM AND POTASSUM

Sodium and Potassium were estimated using Ion Selective Analyzer (ISE Analyzer)

PRINCIPLE: The instrument measures the electrode potential and the data is processed by the microprocessor to obtain the concentration of a given ion (Na+ and K+) .The method is called ñstandard comparisonò. It uses two kind standard and solutions. One for calibration of the base point, and other for the calibration of the slope. The result is obtained from the potentials of the sample and two standard solutions.

The equations are as follows:

$$C_x = C_A \times E \times P \left(\frac{E_x - E_A}{S} \right)$$

$$S = \frac{E_B - E_A}{\text{Log} \left(\frac{C_B}{C_A} \right)}$$

Note: C_x, E_x : the concentration and potential of the Sample.
C_A, E_A: the concentration and potential of standard A.

C_B, E_B : the concentration and potential of standard B.
S: the slope of electrode.

METHOD

The machine was pressed on standby. It was cleaned (deproteinized) and options like test, control, and work list were displayed. Test was pressed on displaying numbers (1-10) for the patientôs laboratory number. The sample was presented through the aspirator and run was pressed on. Test in progress was displayed and result was printed out.

3.11 ESTIMATION OF CHLORIDE

Chloride was estimated using Schales and Schales method.

PRINCIPLE

Chloride ions (Cl^-) are estimated by titration with Hg $(NO_3)_2$using diphenyl carbazone as an indicator. Cl^- in the serum combines with Hg^{2+} from $Hg(NO_3)_2$ to form $Hgcl_2$. The excess Hg^{2+} reacts with diphenyl carbazone complex forming blue violet colour.

$2Nacl + Hg(NO_3)_2 \longrightarrow Hgcl_2 + 2\ NaNO_3$

PROCEDURE

Two test tubes were set up in a rack for test and standard. 0.2ml of buffer ($Hgcl_2$) was added to the tubes.0.2ml of serum was also added to the ótestô test tube and 0.2ml of standard to the óstandardô test tube. Four drops of indicator (diphenyl carbazone) were added and titration was done using mecuric nitrate and reading was taken at the violet end point.

CALCULATION

$$\frac{\text{Titre of test}}{\text{Titre of Standard}} \times \frac{\text{Concentration of Standard (100mmol/l)}}{1}$$

3.12 ESTIMATION OF BICARBONATE

Bicarbonate was estimated using titrametric method by Van Slyke (1917).

PRINCIPLE:

Carbon dioxide is released from plasma bicarbonate with dilute hydrochloric acid. Excess hydrochloric acid is then titrated against Sodium hydroxide using phenol red as indicator.

METHOD

A universal container was set in a rack.0.2ml of non-haemolysed serum was added in the universal container.2.0ml of CO_2 free deionized water was added. 1.0ml of 0.01N HCL was also added. Then 2 drops of indicator was added. The container was whirled round properly to release the CO_2. The solution was titrated against the 0.01N NaOH to a faint pink colour.

CALCULATION:

$$HCO_3 = \frac{(1\text{-titre}) \times 10}{0.2} = \frac{(1\text{-titre}) \times 100}{2} (\text{mEq/l})$$

3.13 HISTOPATHOLOGICAL EXAMINATION

The method described by Krause (2001) was used for histopathological examination. Liver and kidney were fixed for 15 minutes; they were washed with normal saline to remove fixatives. They were then dehydrated through ascending grades of ethanol to absolute alcohol (70%, 90%, 95% and absolute).The liver and kidney were cleared in xylene to remove the alcohol thereby imparting to them a degree of transparency. They were then transferred from clearing agent to a bath of paraffin wax for impregnation and embedded

with paraffin wax (melting point, 56^{o}C),The embedded tissue were trimmed and mounted on a wooden block, tissue sections were cut at 5µm on a rotator microtone. These sections were floated out on clean microscope slides which had previously been lightly albumerized (to avoid detachment from slides during staining procedure) after which they were dried for 2 hours at 37^{o}C (Drury and Wallington,1973).The slides were observed using the Leitz, DIALUX research microscope and photomicrographs produced in bright field at a magnification of x 400.

CHAPTER FOUR

RESULTS

The result of acute toxicity studies showed that the leaf extract of *Uvaria chamae* had an LD_{50} greater than 2500mg/kg in rats. Phytochemical analysis of the leaf of *uvaria chamae* revealed the presence of flavonoids, alkaloids, resins, proteins, reducing sugars and terpenoids (Table 4.1).

Alkaline phosphatase (ALP) showed a statistically significant increase in groups B ($P<0.01$) and C ($P<0.001$) when compared with the control (A) (Table4.1). Alanine aminotransaminase (ALT), Aspartateamino transaminase (AST) and creatinine mean values showed no statistically significant different in all the test groups when compared with the control($P>0.05$)Table 4.1.

Groups B, C, and D had significantly lower urea levels when compared to the control ($P<0.05$) Table4.1.The mean values of Sodium, Potassium, and Chloride were not significantly different when compared to those of the control ($P>0.05$) Table4.2.There was a significant increase in the mean values of bicarbonate of the treated groups when compared with the control ($P<0.05$) Table 4.2.

The histological profile of the liver and kidneys of control rats sowed normal morphological patterns of hepatocytes, renal corpuscles and tubules (Fig1 and Fig5).In the treated groups, there were no adverse effects on kidneys (Fig6, Fig7, and Fig8).

The liver showed evidence of mild periportal lymphatic infiltration at concentrations 250mg/kg, 500mg/kg and 1000m/kg of the extract (Fig2, Fig3 and fig4).

Table 4.1: The effect of the ethanol extract of *Uvaria chamae* leaf on some biochemical indices of hepatic and renal injury in treated rats

PARAMETER A	GROUP control	GROUP B 250 mg/kg EEUC	GROUP C 500 mg/kg EEUC	GROUP D 1000 mg/kg EEUC
ALP(i u/l)	350.00±11.00	490.00±38.00**	630.00±60.00***	370.00±20.00^{ββ}
ALT (u/l)	56.00±5.20	56.00±3.30	56.00±4.10	56.00±1.90
AST (u/l)	76.00±6.70	70.00±7.30	64.00±3.10	78.00±5.20
UREA (mg/dl)	5.00±0.19	3.80±0.31**	3.30±0.28***	3.50±0.18*** ^{β}
CREATININE (mg/dl)	77.00±4.30	71.00±5.80	81.00±4.20	64.00±6.70

Results expressed as Mean ± SEM; n = 5

* Compared with the control group. *P<0.05, **P<0.01 and ***P<0.001.

^{β} One ï Way Analysis of variance ^{β}P<0.05, ^{ββ}P<0.01

Table 4.2: The effect of the ethanol extract of *Uvaria chamae* leaf on serum electrolytes in treated rats

PARAMETER	GROUP A Control	GROUP B 250 mg/kg EEUC	GROUP C 500 mg/kg EEUC	GROUP D 1000 mg/kg EEUC
Sodium (mmol/l)	140.00±2.00	140.00±0.68	140.00±0.85	140.00±1.50
Potassium(mmol/l)	6.10±0.48	5.80±0.14	6.40±0.09	6.10±0.20
Chloride (mmol/l)	110.00±2.90	110.00±0.31	110.00±0.52	110.00±1.60
Bicarbonate(mmol/l)	26.00±0.53	28.00±0.60*	27.00±1.10	29.00±1.10*

Results expressed as Mean ± SEM; n = 5

* Compared with the control group. *P<0.05, **P<0.01 and ***P<0.001.

$^{\beta}$ One ï Way Analysis of variance $^{\beta}$P<0.05, $^{\beta\beta}$P<0.01

Table 4.3.PHYTOCHEMICAL ANALYSIS RESULT

CONSTITUENTS	INFERENCE
FLAVONOIDS	++
ANTRAQUINONE GLYCOSIDES	_
ANTRACENE GLYCOSIDES	_
ALKALOIDES	++
SAPONINES	_
TANNINS	_
RESINS	+++
PROTEINS	++
CARBOHYDRATES	_
REDUCING SUGARS	++
STEROIDS	_
TERPENOIDS	+

KEY

- Absent
+ Present
++ Moderately present
+++ Abundantely present

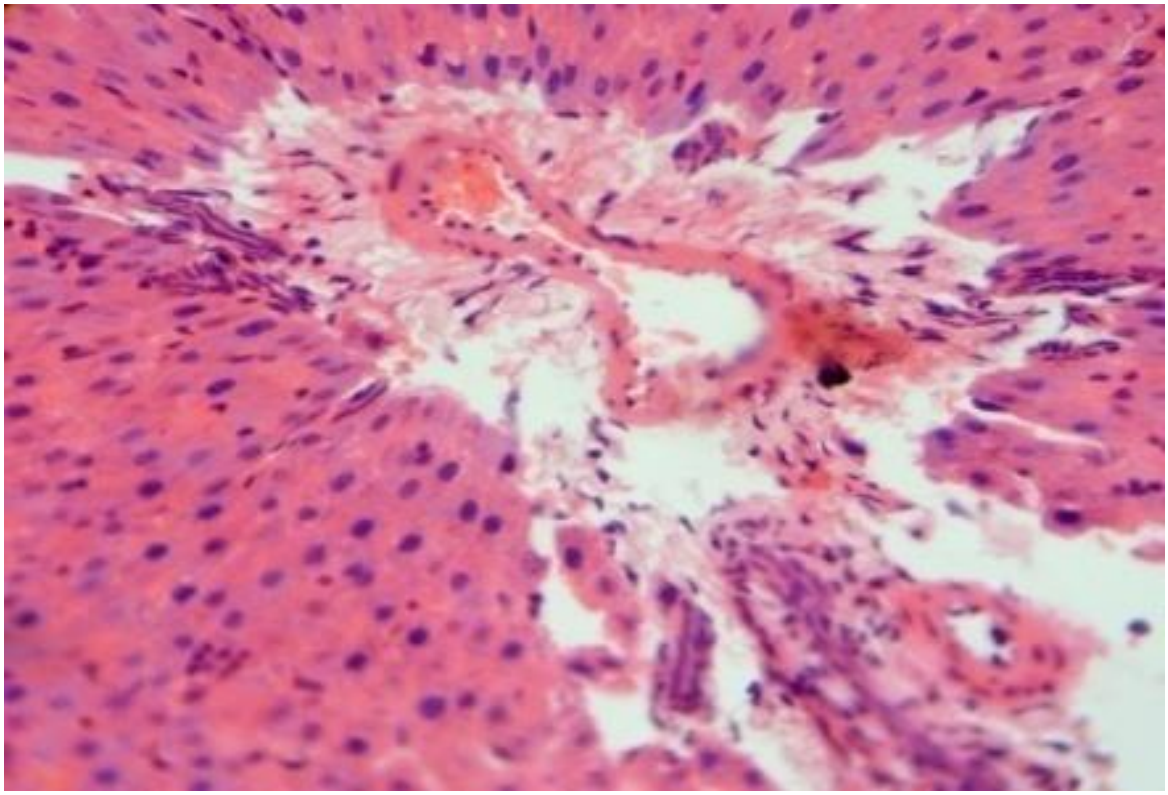

Fig1. Photomicrograph of control rat liver showing a portal tract with no lymphocytic infiltration (H & E stain x 400).

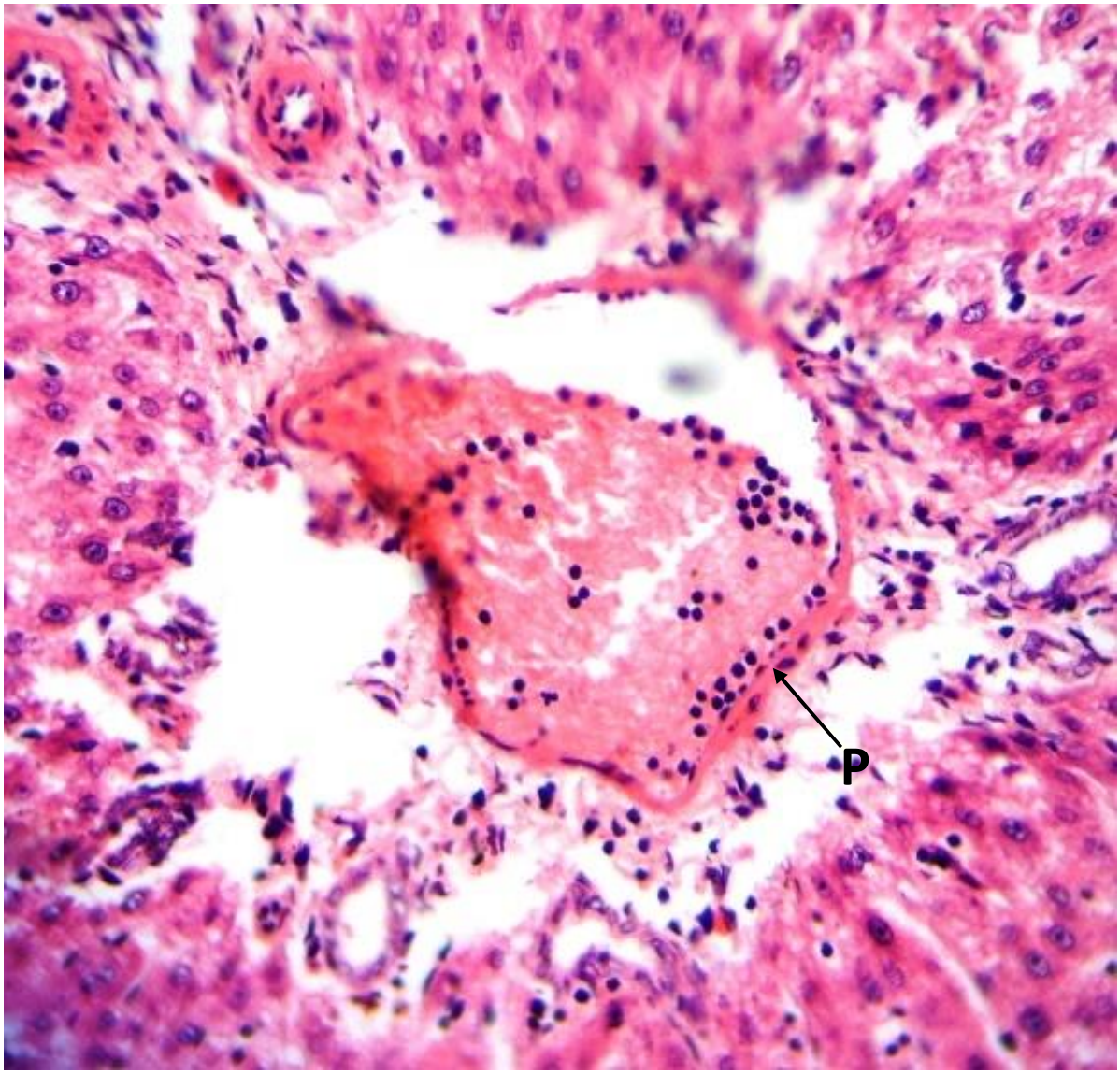

Fig 2. Photomicrograph of rat liver treated with 250mg/kg of EEUC showing mild lymphocytic cell infiltration of portal tract. (H &E stain X400)

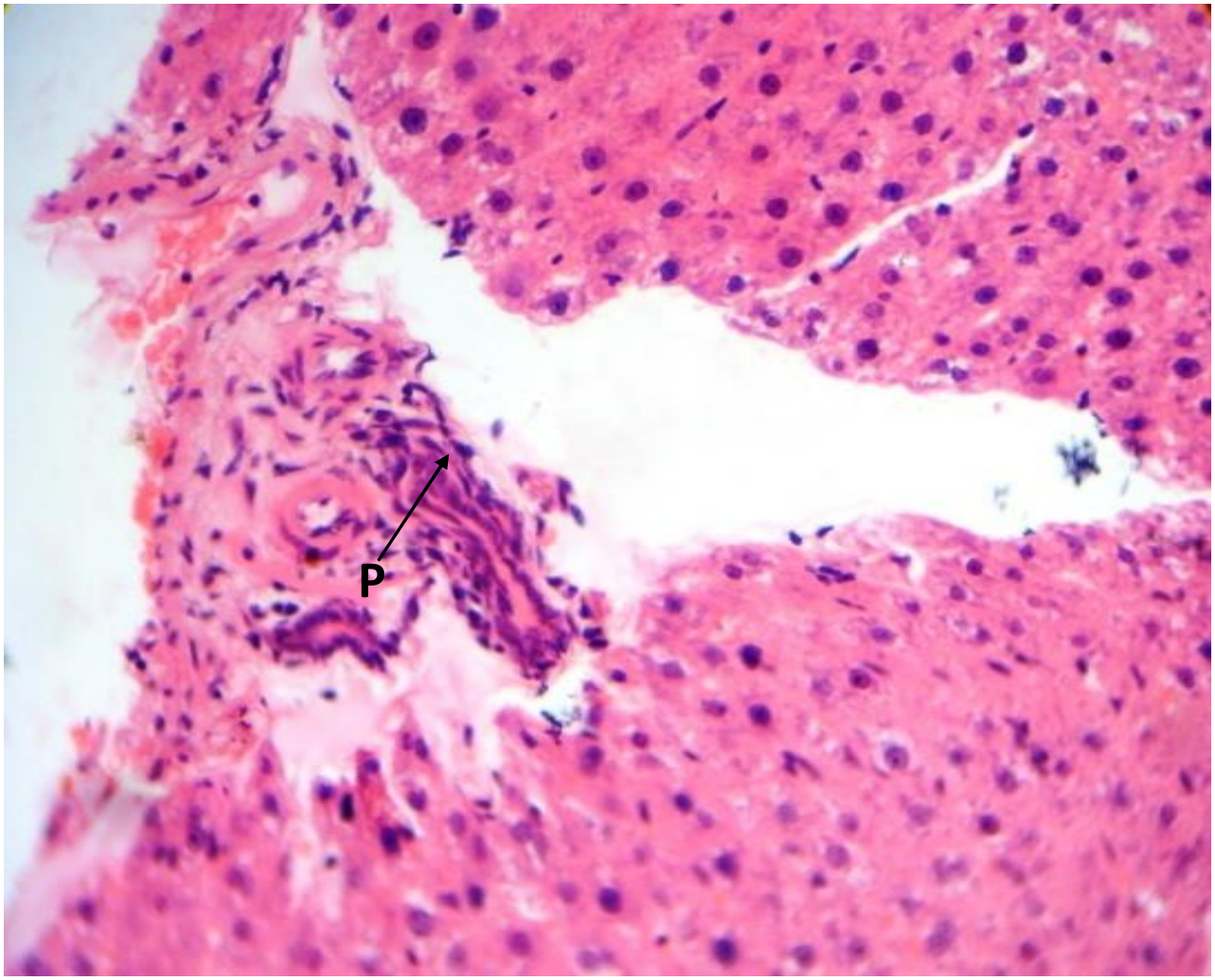

Fig 3 Photomicrograph of the liver of the rat treated with 500mg/kg of EEUC showing mild lymphocytic cell infiltration of portal tract.(H & E stain X400)

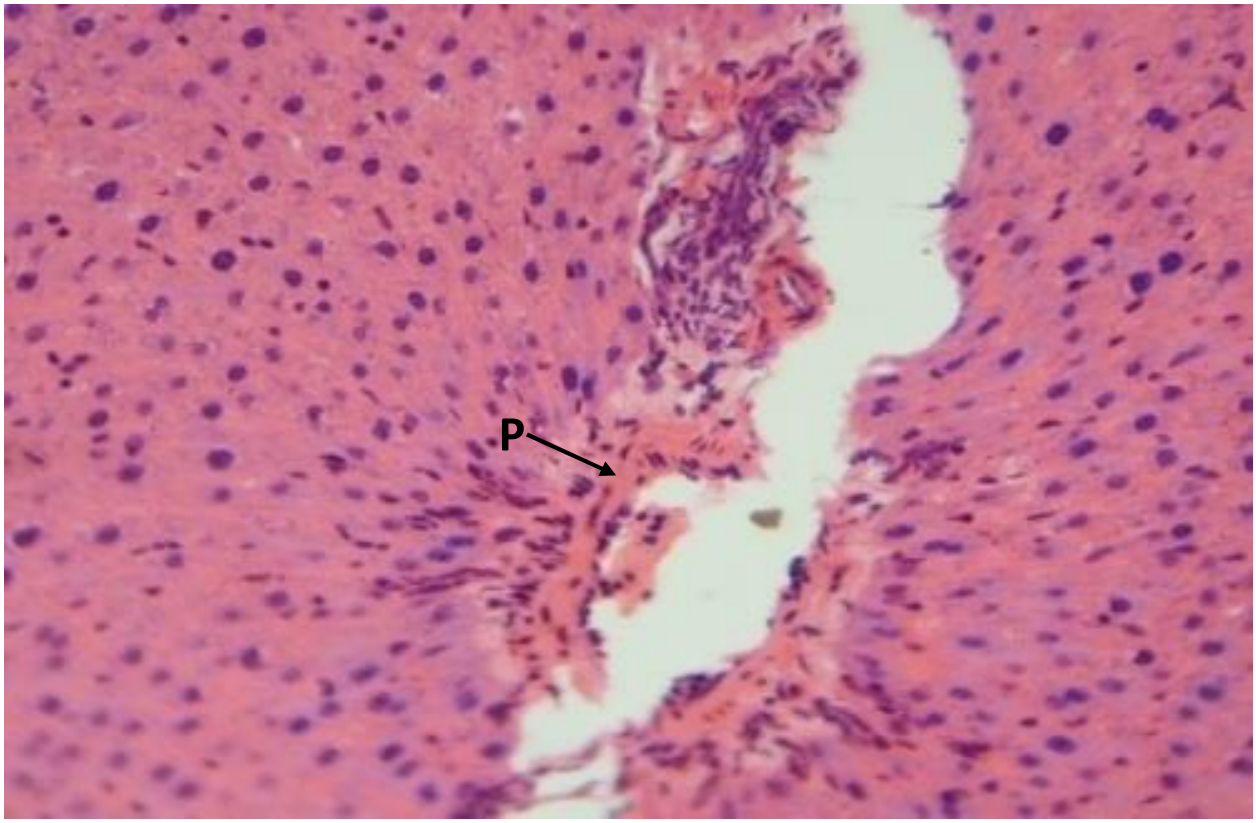

Fig 4 Photomicrograph of the liver of rat treated with 1000mg/kg of EEUC showing mild lymphocvtic cell infiltration of portal tract (H & E stain X400)

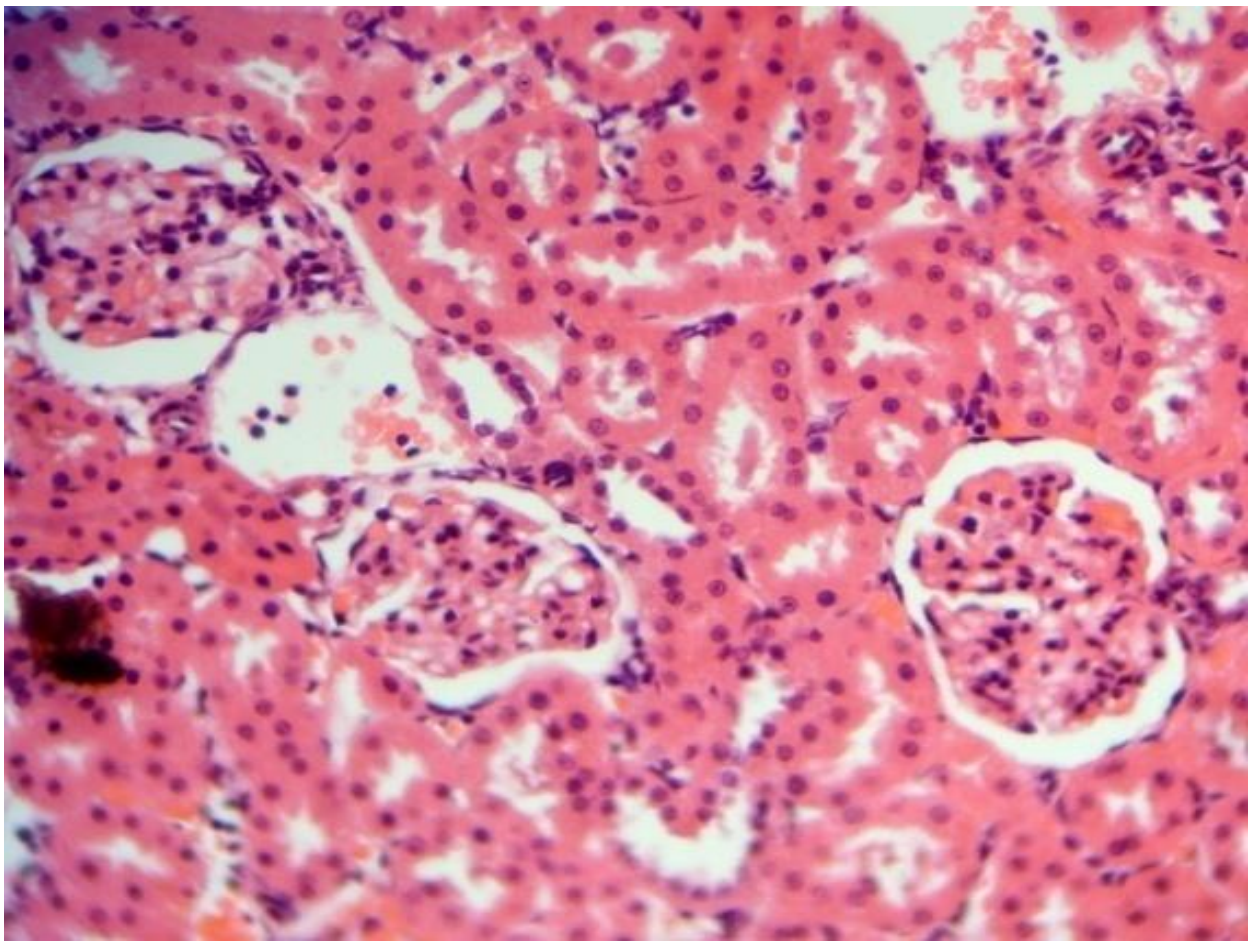

Fig 5 Kidney micrograph of control rat showing unremarkable glomeruli and renal tubules (H & E stain x 400).

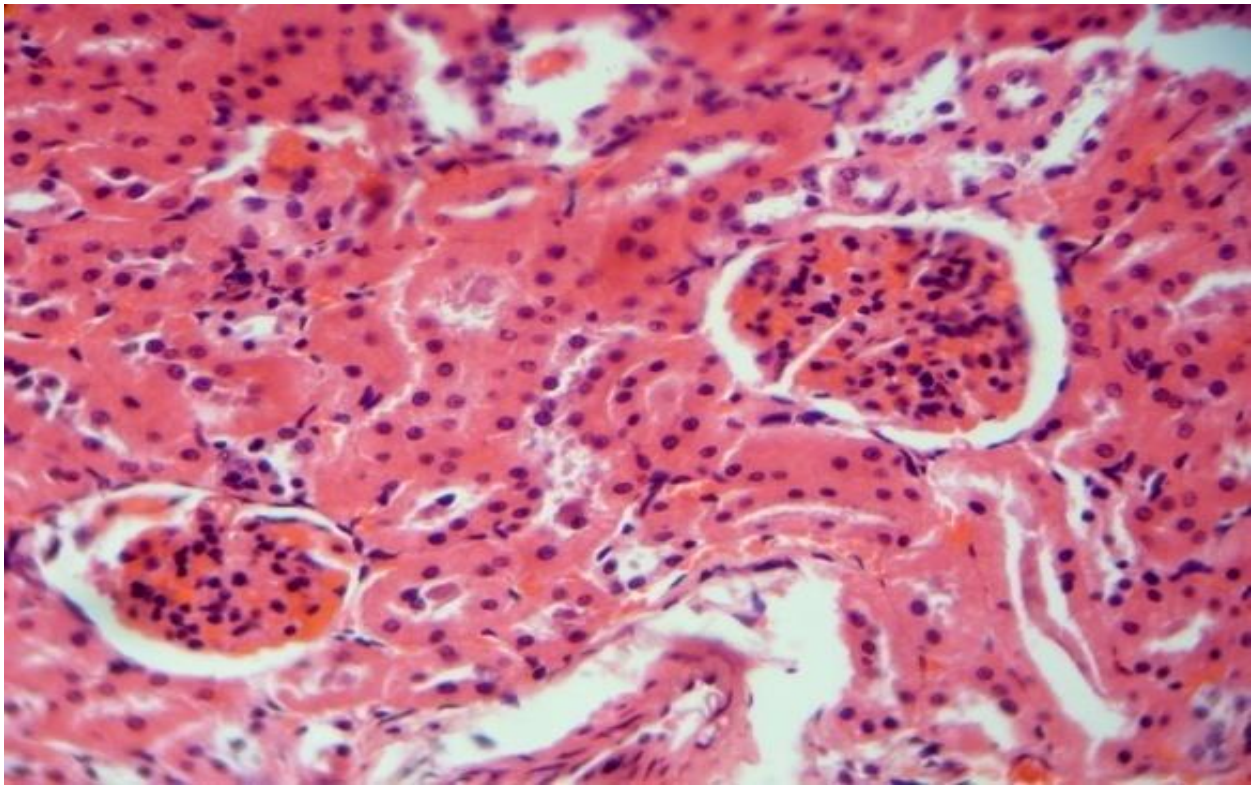

Fig 6 Kidney micrograph of rat treated with 250mg/kg of EEUC (H & E stain x 400). No abnormality detected.

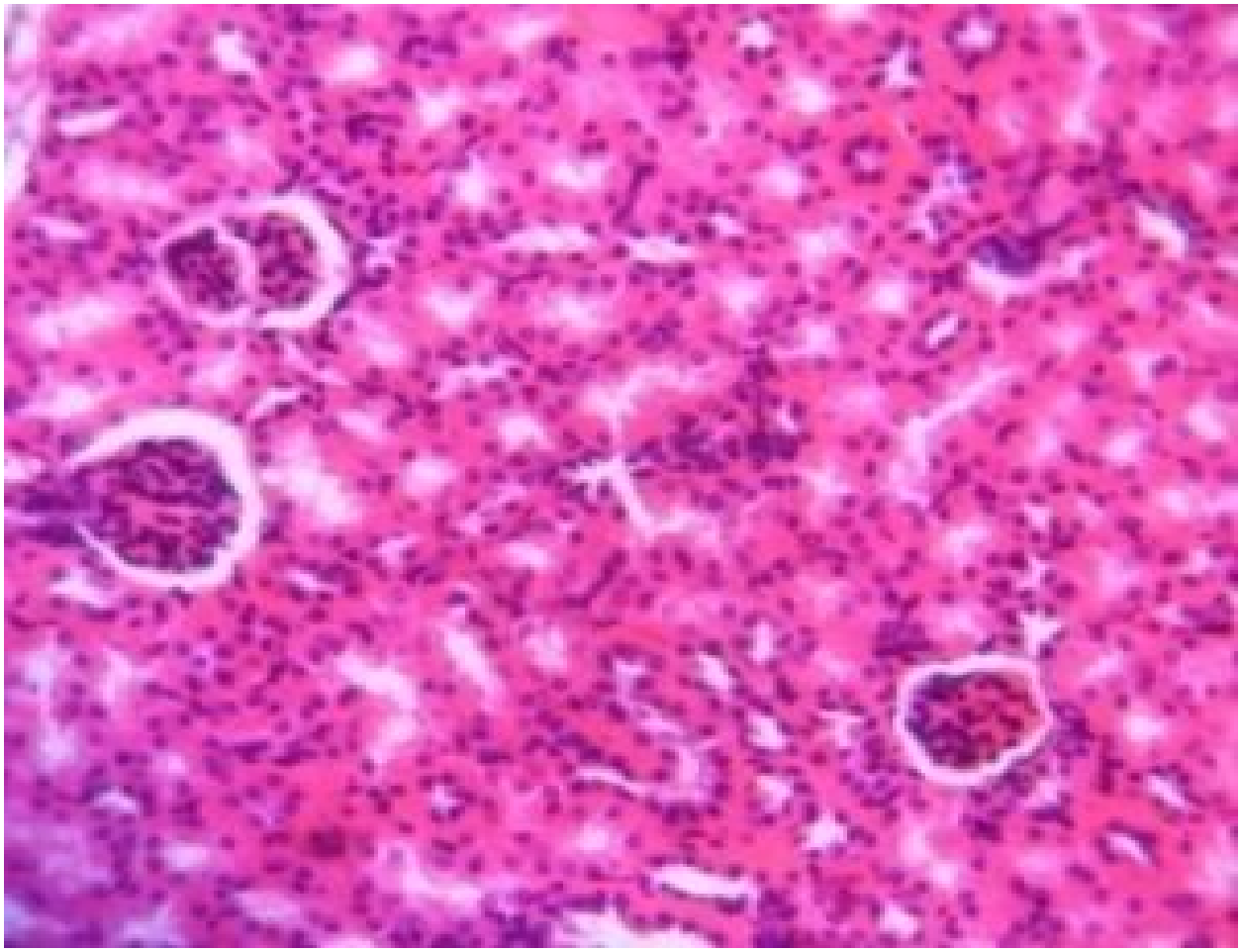

Fig 7 Kidney micrograph of rat treated with 500mg/kg of EEUC (H&E stain X 400) No abnormality detected.

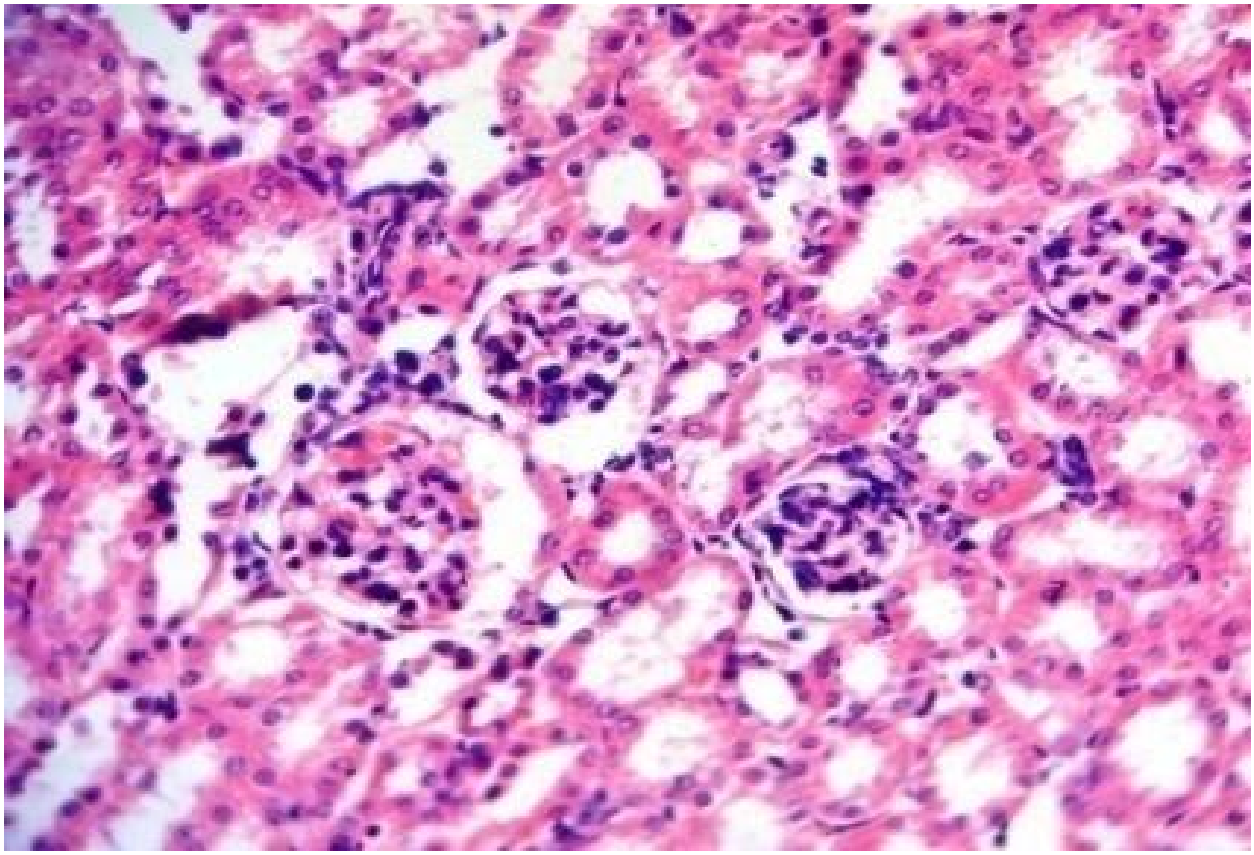

Fig 8 Kidney micrograph of rat treated with 1000mg/kg of EEUC (H&E stain X 400) No abnormality detected

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Herbal medicine is gaining popularity in developing countries as it has been estimated that 80% of the world population still depend mainly on traditional medicine and traditional treatment involving the use of plant extract (WHO, 2000). Therefore, there is need to provide information on the safety or adverse effect of these medicinal plants. Few scientific studies have been undertaken to ascertain the safety of *Uvaria chamae* plant in Nigeria.

Result of acute toxicity studies showed that the leaf extract of *Uvaria chamae* had an oral LD_{50} > 2500mg/kg in rats. No death was observered during the experiment. Phytochemical analysis of the leaf of *Uvaria chamae* revealed the presence of flavonoids, alkaloids, resins, proteins, reducing sugars and terpenoids (Table 4.3). These results are in agreement with the report of Egunyonmi et al, (2009,), with an exception of antraquinone and anthracene glycosides which are not present in the leaf extract. Alkaloides are nerve stimulants, convulsants and muscle relaxant (Kenner and Yves, 1996). The biological function of flavonoids include protection against allergies, platelet aggregation ,microbes, ulcers, hepatotoxins, viruses and tumours (Okwu and

Omodamiro 2005, Farquar,1996).However, some flavonoids behave as powerful protective agents against inflammatory disorders. They reduce oedema formation, and inhibit synthesis of prostaglandinsE_2, Prostagland F_2 and thromboxane B_2 (Okwu and Omodamiro2005, Delrio et al 1986).The biochemical indices monitored in the liver and kidney of albino wistar rats in this study are useful markers for accessing the functional capacities of these organs. Biochemical indices of organ if altered will impair the normal function of the organ (Afolayan and Yakubu, 2009). ALT levels obtained for groups B, C and D were not significantly different from that obtained for the control. Similarly, the AST for groups B, C and D were not significantly difference when compared to the control mean value.

Aminotransferases, ALT and AST are useful markers of liver cytolysis (Yakubu et al., 2005).The normal values of liver ALT and AST observed in the treated rats can be adduced to the normal functional activity of the organ. Serum alkaline phosphatase (ALP) level may greatly increase with liver tumors and lesion, and may show a moderate increase with diseases such as hepatitis (Barbara et al 2005).There was an increase in ALP values with increase in concentration of the extract in the treated rats. Rats in groups B (250mg/kg), C(500mg/kg) and D(1000mg/kg) had mean ALP values which were significantly higher than that of the control. The increase in ALP values is an indication of liver lesion (Kaneko, 1980, Tietz, 1994).

However, 250mg/kg (B), 500mg/kg (C) and 1000mg/kg (D) treated groups had significantly lower urea levels when compared to the control.

Low urea levels are not common and are not usually a cause for concern. They can be seen in severe liver disease or malnutrition but are not used to diagnose or monitor these conditions. The creatinine levels of the treated groups (B, C, and D) were not significantly different from that of the control. Measurement of creatinine concentration is used to determine sufficiency of kidney function and severity of kidney damage and to monitor the progression of kidney disease (Young, 2000).The normal values of creatinine obtained in this study indicates normal kidney function. Also mean values of sodium in B, C and D were not significantly different when compared to the control. Potassium mean values in B, C and D, and chloride mean values in B, C, and D were not significantly different when compared to the controlôs mean values respectively.

These showed that the rats have normal renal functions after taking the extract.

There was a significant increase in the mean values of bicarbonate of the treated groups B and D when compared with the control A. Electrolyte imbalance (too high or too low) can affect all organs of the body.

The histological profile of the liver and kidney of control animals showed normal morphological patterns of hepatocytes, renal corpuscles and tubules (Fig.1 and Fig. 5). In the treated groups, there were no adverse effects on the kidneys (Fig.6, 7, and 8) suggesting a normal functional capacity of the kidneys.

However, the liver showed evidence of mild periportal lymphocytic infiltration at concentrations above. These were not dose dependent. The mild periportal lymphatic infiltration observed may have occurred due to metabolism of toxic material contained in the extract in the liver which is the primary organ of biotransformation. This study shows that *Uvaria chamae* leaf extract has no toxic effect at low doses. Therefore; it is safe for consumption at the doses investigated.

5.2 Conclusion

In conclusion, this study showed that acute administration of *U.Chamae* leaf extract is safe. However, further studies should be directed towards extending the duration of the experiment to check if there will be an adverse effect on the liver.

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APPENDIX I

REAGENT COMPOSITION

Urea

Contents	Concentrations of solution.
Urea standard	79.27mg/dl
R1a (Picric acid)	35mmol/l
R1b (Sodium Hydroxide)	0.32mmol/l

Creatinine

Creatinine Standard	1.97mg/dl
R1a (Picric acid)	35mmol/l
R1b (Sodium Hydroxide)	0.32mmol/l

Working reagent

Mix equal volumes of R1a and R1b.Stable for 3days at 15 to 20^{o}c

APPENDIX II

MATERIALS AND EQUIPMENT

1. 5ml sterile Syringe for oral intubation of the extract
2 Plain bottles
3 Capillary tubes
4 Cotton wool
5 Weighing balance
6 Refrigerator
7 *Uvaria Chamae* leaves
9. Cuvette
10. Colorimeter-Photoelectric colorimeter APEL 101.
11. Measuring cylinder
12 Micro pipette
13 Measuring cylinder
14 Test tubes
15 Racks

APPENDIX 111

STATISTICAL FORMULAE

Data obtained were analyzed with statistical package for social sciences (SPSS) computer software.

The statistical methods used are**:**

1 Mean $(\bar{x}) = \frac{\Sigma \overline{x}}{n}$

Where $\Sigma \overline{x}$ = sum of all the values obtained

n = number of values obtained.

1. Standard deviation (SD) = $\sqrt{\frac{(x-\bar{x})^2}{n-1}}$

Where x = Values obtained

$\bar{x}$ = Mean of values obtained

n = Number of values obtained

3. SE (x) = $S/\sqrt{n}$

Where, SE = standard Error

S = Sample standard deviation

X = Sample mean

$\sqrt{n}$ = Square root of sample size

(4) Studentôs t ï test analysis

$$Tcal = \frac{X_1 - X_2}{\sqrt{\frac{SD_1^2}{n_1} + \frac{sd_2^2}{n_2}}}$$

Where n_1 = Number of sample study for control values.

n_2 = Number of sample study for test

x_1 = Mean of control values

x_2 = Mean of test values

SD_1 = Standard deviation of control values

SD2 = Standard deviation of test values