KAMPALA INTERNATIONAL UNIVERSITY- WESTERN CAMPUS

SCHOOL OF PHARMACY

# EVALUATION OF SUB-CHRONIC TOXICITY AND PHYTOCHEMICAL SCREENING OF AQUEOUS LEAF EXTRACT OF *OCIMUM SUAVE* [LAMIACEAE];

A RESEARCH REPORT SUBMITTED TO THE SCHOOL OF PHARMACY KAMPALA INTERNATIONAL UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF PHARMACY DEGREE.

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SEPTEMBER 2012

## DECLARATION

I ,Kioko Lucy Mutindi a fourth year student of bachelor of pharmacy hereby declare that the research proposal submitted as per University regulation is my own and has never been submitted to any institution for any academic award.

Date..... 3TH OCTOBER 2012

Signature.....

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Date..... 03/10/2012

Signature.....

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## DEDICATION

I dedicate this report to my parents ,my brother ,my sister and dean school of pharmacy for their support during my research studies.

## Acknowledgements

First great thanks are to my Lord God who has granted me this precious life and energy to be able to attain this much in my education.

Special thanks go to Mr.Yonah of the Italian laboratory-Mbarara University of Science and Technology for accepting me to conduct my study in their esteemed institution.

Sincere thanks to my supervisor Mr. Joseph Ezeonwemelu for his guidance during the proposal development and report writing; your efforts are highly appreciated. I wish to thank the entire staff of Pharmacy Department for conveying your knowledge to us and for the good guidance during our learning.

I would also like to thank my Research Assistance, Pascal our Pharmacy laboratory technician. Thank you for your tireless efforts.

Finally my heartfelt appreciations go to my dear parents for their tireless encouragement, moral and financial support during my study period.

THANK YOU ALL.

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## LIST OF ABBREVIATIONS:

ALT - Alanine aminotransferase

AST -Aspartate aminotransferase

GGT -Gamma glutamyl transpeptidase

LDH -Lactate dehydrogenase

CK -Creatinine kinase

CBC -Complete blood count

LD50 -Lethal dose that kills 50 percent of the population

NAOH -Sodium hydroxide

HCL -Hydrochloric acid

MCV -Mean cell volume

RBCS -Red blood cells

ESR -Erythrocyte sedimentation rate

Hb -Hemoglobin

MCH -Mean concentration hemoglobin

HCT -Hematocrit

TWBCS -Total white blood cells

WBC COUNT-White blood cell count

PCV -Packed cell volume

FBC -Full blood count

ALP -Alkaline phosphatase

LFTS -Liver function tests

SEM -Standard error of mean

OSE -Ocimum suave extract

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## ABSTRACT

Evaluation of sub-chronic toxicity of the aqueous leaf extract of *Ocimum suave* was administered daily for 31 days at dose levels[200, 400 and 800mg/kg] in male wistar rats .Acute toxicity of aqueous leaf extract of *Ocimum suave* was done according to Lorke,s method up to 10,000mg/kg and was found to cause no death in the two phases of the test. Thus, the LD50 of *Ocimum suave* in rats was estimated to be greater than 10,000mg/kg. This study principally aimed to assess the $LD_{50}$ of the aqueous leaf extract of *Ocimum suave*,the sub-chronic toxicity and to screen for phytochemicals present in this extract.

This study was carried out in two centers; Kampala International university-Western campus Pharmacy laboratory in Ishaka-Bushenyi Uganda and Italian laboratory in Mbarara University of Science and Technology-Mbarara Uganda.

This is an experimental study carried out between January and July 2012 at the above sites. The respondents were male wistar rats weighing 100g and above. Selection of participants was done by simple random sampling. . Effects on relative organ weights and certain hematological and plasma biochemical parameters were measured as indices of organ toxicity. The aqueous extract caused a decrease in ALT levels but AST levels were higher in the treated groups as the increment was dose dependent. The aqueous leaf extract of *Ocimum suave* affected mean cell volume ,mean cell hemoglobin and mean cell hemoglobin concentration . CK levels were increased in all OSE treated groups as compared to the control group.

The aqueous leaf extract caused mean body weight gain, but decrease in relative organ weight .The study suggests that the aqueous extract administered at normal therapeutic doses is not likely to produce severe toxic effects on some hematological and biochemical indices in rats and the organs tested[histology].

Chronic toxicity of aqueous leaf extract of *ocimum suave* should be done for histological, haematological and biochemical evaluation.

There is a need to study the effect of the extract on female wistar rats so that toxic effects can be established in both sexes.

## CHAPTER ONE

### 1.1 Background information

Pronounced m-TU-lay. In Swahili, the accent is always on the second syllable. (Mtule Basil, African Bush Basil, Perennial Bush Basil, Kilimanjaro Basil, Ocimum kilimandscharicum)

Family: Lamiaceae

- This is handsome, upright African bush basil that becomes woody with age. Within its native range, the arching, reddish seed heads are a common sight throughout the wild lands. The plant is similar to wild Vana Tulsi and is very high in Eugenol. Eugenol is oil of clove, and interestingly the local use of Mtule follows the same use that is commonly employed for oil of clove--as an antiseptic and pain reliever for dental woes. Among other uses, local people give the fresh leaves to children to allay pain of teething. Plant prefers full sun and is not picky about soil, growing well in regular garden soil, even waste places and abandoned field.

  There are several plants in sub-Saharan Africa reported to constitute effective repellent effect against arthropods of vector- borne disease. Some of these plants, for example citronella and pyrethrum, have been commercialized and are effectively used as , *Ocimum suave, Ocimum kilimandscharicum, Azadirachta indica, Eucalyptus globules and Lantana camara* plants are common and known to have provided protection against mosquitoes.osquito repellents (Berger, 1998). Several plants of this family have been proven to have insecticidal and repellent effects, used widely against blood- feeding arthropods and those feeding on crops (Curtiset *et al.*, 1998).

- cost- effective and environmentally friendly bio-products such as plant repellents can potentially be improved to supplement existing vector- control measures (Palsson *et al*,, 1999). Although there are many plant species used traditionally for protection against blood- feeding insects, there are few studies to illustrate their protective efficacy and/or contribution to disease control.

## 1.2 Problem statement

Majority of the world's population relies on plant medicine either due to unavailability of or lack of trust in orthodox medicine among the local people. This plant is particularly being used widely by the local populace without knowledge of its proper dose and toxicity. This lack of knowledge may be causing huge problems among the people without any prior note. Therefore there is need to know the toxic profile of this plant by studying the plant.

## 1.3 Objectives

### 1.3.1 Broad objective

The purpose of the study was to ascertain the acute toxicity, possible biochemical, hematological and histological effects of aqueous extract of the leaves of *Ocimum suave* and further identify the compounds responsible for the effects.

### 1.3.2 Specific Objectives of the study included;

- To ascertain whether *Ocimum suave* has acute toxic effects.
- To perform phytochemical screening to identify the constituents responsible for the biochemical, hematological and histological effects of *Ocimum suave*.
- To ascertain that *Ocimum suave* has biochemical, hematological and histological effects.

## 1.4 Hypothesis

Aqueous extract of *ocimum suave* leaves has sub-chronic toxicity on biochemical, hematological and histological studies in laboratory animals.

## 1.5 Justification of the study

Natural plant herbs have played a very important role in medicine and healthcare. Research has shown that most of the world's population still relies on traditional medicine for their healthcare needs. Unfortunately, the required information on the local species of this plant is greatly unavailable; also administration and dosage of these drugs have not been established. Therefore, in order to fill this gap in knowledge, preliminary studies have to be done to evaluate and ascertain possible risks such as undesirable effects, overdose or poisoning so that any anticipated

problems can be avoided through the use of the generated information. Traditional remedies are made up of plant extracts containing multiple chemical constituents which vary in potency. These extracts have served as valuable sources for many drug discovery programs. The dose that will be obtained from this study will help understand the desired therapeutic effects of the drug while minimizing the risk of toxic effects. It will also ensure documentation of cultural information about the drug as it is used in the study area.

# CHAPTER TWO

## 2.1 Literature review

Since ancient times, several plants and plant products have been used locally to repel or kill mosquitoes. There are several plants in sub-Saharan Africa reported to constitute effective repellent effect against arthropods of vector- borne disease. Some of these plants, for example citronella and pyrethrum, have been commercialized and are effectively used as mosquito repellents (Berger, 1998).

In Lower Moshi villages, investigations were done to establish whether whole plant and plant products derived from local areas can be used in combination with the bed nets to provide protection against malaria vectors and nuisance biting insects. Before starting such an investigation, an ethnobotanical survey was conducted to understand the common knowledge, attitude and practices, of local people, on the use of plant products for protection against mosquitoes and other biting insects (Curtiset *et al*, 1998).

At Lower Moshi, *Ocimum suave, Ocimum kilimandscharicum, Azadirachta indica, Eucalyptus globules and Lantana camara* plants are common and known to have provided protection against mosquitoes. These aromatic plants, *Ocimum suave* (OS) *and Ocimum kilimandscharicum* (OK) locally known as a broom "Ufagio" in the Kiswahili language, belong to the family Lamiaceae and are the focus of this study. Several plants of this family have been proven to have insecticidal and repellent effects, used widely against blood- feeding arthropods and those feeding on crops (Curtiset *et al*, 1998).

Although, treated mosquito nets have been proved to be effective in reducing child morbidity and mortality, there are still operational problems slowing down the scaling up of Insecticides Treated bed Nets (ITN) usage such as seasonal variation of ITN use in the community, equity and access constraints, low rates of net re-treatment with insecticides and reports of insecticide resistance in malaria mosquitoes. With such problems facing the existing control measures against vector- borne diseases, there is a need to look for alternative and supplementary means to support existing control measures. Alternative, cost- effective and environmentally friendly bio-products such as plant repellents can potentially be improved to supplement existing vector-control measures (Palsson *et al*, 1999).

Although there are many plant species used traditionally for protection against blood- feeding insects, there are few studies to illustrate their protective efficacy and/or contribution to disease

control. Following a survey conducted, OS and OK were the most common plants used as insects repellent by local communities at Lower Moshi, north-eastern Tanzania. This study evaluates deterrence, and feeding inhibition effects of *Ocimum suave* (OS), *Ocimum kilimandscharicum* (OK), *Azadirachta indica* (AI) *Eucalyptus globules* (EG) and *Lantana camara* (LC) on three mosquito species, *Anopheles arabiensis* (Patton), *Anopheles . gambiae* (Giles) and *Culex quinquefasciatus* (Say) in the field and experimental huts (Palsson,1999).

The use of repellent materials from plants against nuisance insects is common with great potential to complement existing malaria control programmes and this requires evaluation in the field. Ocimum plant species, *Ocimum suave* (Willd) and *O. kilimandscharicum* (Guerke) materials and their essential oils extracted by steam distillation were evaluated in the field and experimental huts for repellence, feeding inhibition effects against three mosquito species, *Anopheles arabiensis* (Patton), *Anopheles. gambiae* (Giles) and *Culex quinquefasciatus* (Say). The protective effect of essential oils from Ocimum plants were compared with N, N-diethly-3- methylbenzamide (DEET), a standard synthetic repellent. Also, the protective effect of fumigation by burning of repellent plants; *Ocimum suave*, *Ocimum kilimandscharicum*, *Azadirachta indica*, *Eucalyptus globules* and *Lantana camara* were tested in experimental huts and selected local houses (Palsson, 1999).

In the field, protection by Ocimum plants from mosquito bites was high and there was small variation among different mosquito species. Protection efficiency was 93.4%, 91.98% and 89.75% for *An. arabiensis* while for *Cx. quinquefaciatus* it was 91.30%, 88.65% and 90.50% for DEET, *Ocimum suave* and *O. kilimandscharicum* respectively. In the experimental hut, deterrence induced by burning of Ocimum and other plants ranged from 73.1.0% to 81.9% for *An. arabiensis* and 56.5% to 67.8% for *Cx. quinquefaciatus*, while feeding inhibition was 61.1% to 100% for *An. arabiensis* and 50% to 100% for *Cx. quinquefaciatus*. Evaluations under field conditions confirmed high protective efficacy, enhanced feeding inhibition and house entry inhibition (Palsson, 1999).

A study conducted by [Kweka *et al*,2008) shows that the potential of *Ocimum suave and Ocimum kilimandscharicum* crude extracts and whole plants of *Ocimum suave, Ocimum*

*kilimandscharicum*, *Azadirachta indica*, *Eucalyptus globules and Lantana camara* for use in protecting against human biting while the burning of plants reduces significantly the indoor resting mosquitoes (Chogo *et al*,1991).

The plant materials of OS, OK, AI, EG and LC were the first five common plants used as repellents mentioned by the community members in previous study in this area. The method used for insect protection is mainly burning of dry plant material. In this test, one kilogram of each plant material was burnt between 7 pm and 10 pm in selected houses in the community and experimental huts as commonly used in the community (Chogo *et al*, 1991).

In the experimental hut trials, two huts were selected, the test hut and control hut. The experiment had a binary setting. One hundred (100) female mosquitoes of 3 to 6 days old of the same species were released in each hut with a person sleeping under an untreated bed net. The plant materials, particularly the leaves were picked up within the community areas then dried in the sun for a day before use in this experiment (Chogo *et al*, 1991).

Plant materials were burnt only in the test hut. The next day mosquitoes were collected in window traps and verandahs. Physiological conditions (unfed, blood fed and gravid) of mosquitoes collected were observed, then provided with 10% sugar solution for 24 hrs to score mortality (Chogo *et al*, 1991).

In the village trial, eight houses were selected and grouped into four pairs each with two houses, i.e. the control and experimental houses. Volunteers slept under an untreated net in each of the trial houses, to protect them from being exposed to wild mosquitoes that might be infected. Effects of plant repellents on mosquitoes were observed for four consecutive days (Chogo *et al*, 1991).

**2.2 Field trial of extracts of Ocimum plants (community study)**

Four houses and four pairs of volunteers performing man-landing catch (MLC) at each house were involved to evaluate the protective effect of essential oil extracts from OS and OK. The volunteers were provided with anti-malarial prophylaxis during the study period. The first group was treated with OK (a solution with 20% OK essential oil); second group treated with DEET at similar concentration (20%), third group with OS (a solution with 20% OS essential oil) and the fourth, a control group treated with a mixture of glycerine and acetone. The 20% solution of OK,

DEET and OS were prepared by dissolving crude essential oil into glycerine and acetone to a final concentration of 20%. The proportion of major active ingredients in that sample (OK, OS and DEET) was used to derive the concentration of 20% which was used in this evaluation. The amount of oil used by volunteer was determined by measuring the weight of oil with bottle before and after application on feet. The repellent and control were applied on feet below the knee. Volunteers seating on chair 5 meters apart outside the house collected mosquitoes landing on their lower legs and on their feet using an aspirator. Collected mosquitoes were grouped in hourly intervals and identified using a morphological key. Experiments were 4 by 4 Latin squ[illegible] arrangement, for four days per week for sixty-four weeks. The exercises started at 18:00 h and ended at 22:00 h. Both insect collectors and treatments were interchanged to prevent bias. Experiments were done for 4 days in a week and each treatment was rotated with same pair of volunteers. Treatments were interchanged between the groups alternatively in every week of trial. DEET, a known standard repellent was used for comparison in this evaluation (Jembere *et al*, 1995).

**2.3 The efficacy of Ocimum plant extracts in the community field study**

In the field study, 1708 *Anopheles gambiae* s. and 1093 *Culex quinquefasciatus* were collected in 64 weeks of Man Landing Catch. *Anopheles arabiensis* is the commonest Anopheline species accounting for 61.2% of all collected mosquitoes. All *An. gambiae* s.l. were presumed to be *An. arabiensis* following previous identification records in the area (Omolo *et al*, 2004).

All tested compounds showed significant protection efficiency (PE) to human volunteers against all mosquito species. The PE for *An. arabiensis* was 93.44% for DEET, 91.98% for OS and 89.75% for OK (Table 1). The mean number of *An. arabiensis* caught per night for each treatment and control are shown in Table 1. The lowest and highest numbers of mosquitoes landing per night by type of treatment are indicated as 95% CI.

Protective efficiency of standard repellent DEET, extracts of *Ocimum suave* and *Ocimum kilimandscharicum* to *Anopheles arabiensis* in the field evaluation estimated by human landing catch conducted for a total of 64 weeks (Omolo *et al*, 2004).

In *Cx. quinquefasciatus*, the PE was 91.30%, 88.65% and 90.50% for DEET, OS and OK respectively. Although the PE of all products was more than 88%, the magnitude of protection

by OK and DEET against *Cx quinquefasciatus* was comparable. The lowest and highest numbers of mosquitoes landing per night by type of treatment are indicated as 95% CI.

Protective efficiency of standard repellent DEET, extracts of *Ocimum suave* and*Ocimum kilimandscharicum* to *Culex quinquefasciatus* in the field evaluation estimated by human landing catch conducted for a total of 64 weeks (Omolo *et al*, 2004).

**2.4 The efficacy of Ocimum and other plants in experimental huts**

The impact of smoke from burned repellent plant materials in experimental huts was observed in 24 hours after burning. An increase in exophily behaviour (i.e. reduced indoor resting mosquitoes) and blood- feeding inhibition was observed. In experimental huts, high deterrence and feeding inhibition rates of *O. suave*, *O. kilimandscharicum*, *Azadirachta indica*, *Eucalyptus globules* and*Lantana camara* on *An. arabiensis* and *Cx. quinquefaciatus* were observed by collection of large numbers of these mosquitoes in window and verandah traps. Deterrence ranged from 79.4% to 88.9% and 71.2% to 86.9% while feeding inhibition ranged from 60% to 98.4% and 18.5% to 85.4% in *An. arabiensis* and *Cx.quinquefaciatus* respectively. The OK induce high deterrence in *An. arabiensis* while AI in *Cx. quinquefasciatus* and feeding inhibition (> 90%) in both species of mosquito tested. Performance of OS in terms of deterrence and feeding inhibition of *An. arabiensis* and *Cx. quinquefaciatus* (range from 80% to 98%) was much higher than those of EG and LC (range from 18.5% to 88.1%), in particular LC induced the lowest effect in reducing feeding in Culex . In general, the protective effects of the four plant repellents were much higher on *An. arabiensis* than *Cx. quinquefasciatus* mosquitoes.

Deterrence and feeding inhibition rates of *Ocimum suave*, *Ocimum kilimandscharicum*, *Azadirachta* indica, *Eucalyptus globules* and *Lantana camara* to *An. arabiensis* and *Cx. quinquefaciatus* in experimental huts (Alonso *et al*, 2000).

**2.5 The efficacy of Ocimum and other plants in selected community houses**

In the selected community houses, feeding inhibition ranged from 61% to 100% for *An. arabiensis*and from 50% to 100% for *Cx. quinquefasciatus* . Likewise, deterrence ranged from 73.1% to 81.9% for *An. arabiensis* and from 56.5% to 67.8% for *Culex*. In particular, the EG induce high deterrence (89.1%) while OS and OK have shown higher feeding inhibition (100%) in both species of mosquito tested. Although the performance of other plants used in terms of (deterrence and) feeding inhibition of *An. arabiensis* and *Cx. quinquefasciatus* (range

from 50% to 100%) was higher on treated houses, the difference was comparable to the effect recorded from control houses (range from 55% to 86%). In general, the protective effect of plant repellents was much higher on *Anopheles* than *Culex* mosquitoes (Alonso *et al*, 2000).

Deterrence and feeding inhibition rates of *Ocimum suave*, *Azadirachta indica*, *Eucalyptus globules* and *Lantana camara* to *An. arabiensis* and *Cx. quinquefaciatus* in village houses. However, the deterrence effect of burning repellents in village houses have shown variations between treatment day and days before next treatment in reducing indoor resting mosquito. In general, day 0 of treatment recorded significantly low numbers of mosquitoes resting indoors than subsequent days of observation after treatment. The number of mosquitoes caught increased gradually on subsequent days after treatment, suggesting a lack of residual effect of smoke from repellent plant materials (Alonso *et al*, 2000).

**2.6 TRADITIONAL USES**

*Ocimum suave is used as a traditional medicine for the treatment of stomachache, cough and* influenza. It is also used as a perfume, an insect repellent (particularly against mosquitoes) and a grain protectant.

Another species *Ocimum kilimandscharicum* Baker ex Gürke Traditonally is used for the treatment of serious colds and coughs, abdominal pains, measles and mild diarrhoea in children. It is also used as a grain protectant in East Africa.

A species *Ocimum canum* Sims, Uses: The leaves are used as a traditional medicine in West Africa for the treatment of fevers, dysentery and to relieve toothache. Leaves used as flavouring and as an insect repellent. It is also used in Rwanda to protect against post-harvest insect damage (Omolo *et al*, 2004).

## 2.7 BIOCHEMICAL STUDIES

These include the studies of enzymes and other biological factors such as urea, bilirubin, creatinine and potassium plasma levels that are physiologically important for normal functioning of the body systems. A large number of enzymes are synthesized in the cell and are continuously released in circulation in small amounts as a result of the normal wear and tear of cells. They are removed from circulation by degradation or excretion. They are present in circulation in very minute amount. The liver function tests (LFTs) give information about the activity or concentration of enzymes and compounds in serum rather than quantifying specific hepatic functions, and they are divided into two:

Functional plasma enzymes or plasma specific enzymes, these enzymes are purposely secreted into circulation to perform specific catalytic functions, and includes lipoprotein, lipase, blood coagulation factors, complement proteins and others.

Non-functional plasma enzymes or non-plasma specific enzymes. These enzymes do not perform their catalytic function in plasma. They are intracellular enzymes that enter circulation when cells in which they are synthesized disintegrate. When the cell breakdown is at normal rate, the enzymes are found to be very minute in the circulation, and if the cell destruction increases due to pathological conditions, these enzymes will be released into circulation in large amounts and their concentration in plasma will rise many times above normal. However, if the enzyme has a selective tissue distribution or if it is present in far higher concentration in some tissues than elsewhere in the body, it can pinpoint the site of the disease, and this makes these non functional plasma enzymes having a selective tissue distribution to be of diagnostic importance. The following enzymes have become of diagnostic importance:

Lactate dehydrogenase (LDH): This enzyme catalyses the interconversion of pyruvate and lactate. Its tissue distribution is very wide; however, its concentration is much higher in myocardium, muscle and liver than in any other tissues. Therefore, LDH plasma levels rises in myocardial infarction, viral hepatitis and muscles injuries. There are five isoenzymes, $LDH_1$ and $LDH_2$ appear primarily in the heart, $LDH_3$ appear primarily in the lungs, $LDH_4$ and $LDH_5$ are also primarily in the liver and skeletal muscles.

Transaminase: The serum transaminases are increased if cells are damaged and enzymes released into the circulation. The two most common transaminases are aspartate amino transferase (ASP) and alanine amino transferase (ALT), and they are present in high concentrations in myocardium, liver and muscles. ASP is more concentrated in the myocardium than ALT, and ALT is more concentrated in the liver than ASP.

Creatine kinase (CK): is present in myocardium, muscles, and brain. It catalyses the reaction, creatine + ATP creatine P + ADP. Plasma CK rises in myocardial injuries, it is an early indicator in myocardial infarction.

Alkaline phosphatase (ALP): this is a group of enzymes that hydrolyze organic phosphate esters at an alkaline $_{p}H$. It is released into circulation mainly from bones and liver. Its level indicates or estimates the amount of bile flow impedance.

Amylase: this is a digestive enzyme, synthesized in the pancreas and the parotid gland. In acute pancreatitis there is a sharp rise of plasma amylase.

Gamma glutamyl transpeptidase (GGT): Is found in hepatobiliary, pancreatic, and kidney cells. This enzyme catalyses the transfer of the gamma-glutamyl residue of glutathione to other substrates. Its plasma concentration rises in most of the hepatocellular and hepatobiliary disease, although elevations correlate with obstructive disease than with pure hepatocellular damage. An elevated GGT level is often one of the early indicators of alcoholic liver disease.

Lipases: this lipolytic enzyme is release into circulation from the pancreas. Plasma lipase rises in acute pancreatitis.

## 2.8 HEMATOLOGICAL STUDIES

Hematological studies deals with the scientific studies of blood and its diseases.

Blood is a connective tissue in a fluid form. It is made up of three kinds of cells which are:

Red blood cells (ERYTHROCYTES)

White blood cells(LEUKOCYTES) and

Platelets (THROMBOCYTES)

A complete blood count (CBC) or full blood count (FBC) comprises;

Hemoglobin (Hb), the Hb test measures the grammes of Hb contained in 100ml (1dl) or 1L of whole blood and provides an estimation of the oxygen-carrying capacity of the red blood cells. The Hb values depend on the number of red blood cells and the amount of Hb in each red blood cell. The normal values are 14-18g/dl for men and 12-16g/dl for women. Low values indicate anaemia.

Hematocrit (Hct) or packed cell volume (PCV): this measures the percentage by volume of packed red blood cells in a whole blood sample after centrifugation. Its value is three times the Hb value, and it's given in percentage or fraction.

Total white blood cells (TWBCs) report the number of white blood cells in a cubic millimeter of whole blood. The normal value range from 4000WBC/$mm^3$ to 11,000WBC/$mm^3$, an increase in WBC count (LEUKOCYTOSIS) usually indicates infection, and may also result from leukaemia or from tissue necrosis, most found in bacterial infection and a decreased WBC count (LEUKOPENIA) indicates bone marrow depression, that may result from metastatic carcinoma, lymphoma or toxic reaction to substance, such as antineoplastic agents. The TWBCs also include white blood cell differential that comprises granulocytes and non-granulocytes.

Total red blood cells reports the number of RBCs in a cubic millimeter of whole blood, provides an indirect estimate of the blood's Hb content. Normal values are: 4.3-5.9million/$mm^3$of blood for men, and 3.5-5.0million/$mm^3$ of blood for women. It comprises Hb, Hct, MCV and

erythrocyte sedimentation rate (ESR), RBC indices, mean cell Hb (MCH), RBC distribution width (RDW ) and Reticulocytes counts.

Mean cell volume (MCV) is the ratio of the Hct to the RBC count. It essentially assesses average RBC size and reflects any anisocytosis.

Platelet count, expresses the number of platelets (thrombocytes) per liter, the normal values are $150,000/mm^3 — 300,000mm^3$. A decreased platelet count is referred to as Thrombocytopenia and increased platelet count as Thrombocytosis .

## 2.9 OTHER RESEARCH STUDIES

According to Ram 2001, the *Ocimum sanctum* alcoholic leaf extract shows significant hepatoprotective activity and synergism with silymarin.

The leaves of Tulsi (*Ocimum sanctum*) have highly significant ($P < 0.01$) hepatoprotective activity. When concurrently administered, *Ocimum sanctum* leaves and silymarin have a highly significant ($P < 0.01$) synergistic hepatoprotective activity. The *Ocimum sanctum* group shows better hepatoprotection than the *Ocimum sanctum* and silymarin combination group. However, in the given doses, the *Ocimum sanctum* leaf extract alone and in combination with silymarin showed lesser hepatoprotective effect than silymarin alone. Silymarin is a well-known standard hepatoprotective, whereas presence of impurities in the *Ocimum sanctum* extract may have caused a lower hepatoprotective effect. Moreover, we used lower doses of *Ocimum sanctum* (100 mg/kg) and standard hepatoprotective silymarin (50 mg/kg) in the combination group (*Ocimum sanctum* extract and silymarin) than in the silymarin group alone (Chattopadhyay *et al*, 1992).

***Ocimum sanctum* leaf extracts stimulate insulin secretion from perfused pancreas, isolated islets and clonal pancreatic β-cells**

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. Current drugs used for diabetes therapy are not free from side effects and do not restore normal glucose homeostasis ( Rang *et al.* 1991).

Leaves of *O. sanctum* have been shown to possess hypoglycaemic effects in experimental animals ( Joglekar *et al.* 1959, Dhar *et al.* 1968, Chattopadhyay 1993, Rai 1997).

*O. sanctum* leaf extracts exert prominent stimulatory effects on insulin secretion from the β-cells via physiological pathways. *In vivo* studies also indicate that the ethanol extract decreased blood glucose and increased plasma insulin in type 2 diabetic rats (Hannan *et al.* 2003).

## 2.10 STRUCTURE AND FUNCTIONS OF ORGANS

### 2.10.1 LIVER

The liver, which is the largest organ in the body, lies mainly in the upper right section of the abdominal cavity, just inferior to the diaphragm. The liver has two main lobes, the right lobe and the smaller left lobe, separated by a ligament. Each lobe is divided into many hepatic lobules that serve as its structural and functional units. Phagocytic *Kupffer cells* remove pathogens and debris that may have entered the hepatic portal vein at the small intestine (Sylvia 2004).

### 2.10.2 KIDNEYS

A kidney has three regions. The renal cortex is an outer, granulated layer that dips down in between a radially striated inner layer called the renal medulla. The renal medulla consists of cone shaped tissue masses called renal pyramids. The renal pelvis is a central space, or cavity, that is continuous with the ureter. Kidney is composed of over one million nephrons. Each nephron has its own blood supply, including two capillary regions - afferent arteriole leading to the glomerulus, and the efferent arteriole taking blood to the peritubular capillary network which surrounds the rest of the nephron. Each nephron is made up of several parts - the closed end of the nephron, Bowman's capsule composed of podocytes and a proximal convoluted tubule (Sylvia 2004).

### 2.10.3 LUNGS

The lungs are paired, cone-shaped organs that occupy the thoracic cavity except for the mediastinum, a central area that contains the primary bronchi, the heart, and other organs. The right lung has three lobes, and the left lung has two lobes, allowing room for the heart whose apex points left. A lobe is further divided into lobules, and each lobule has a bronchiole serving many alveoli. Each lung is enclosed by a double layer of serous membrane called the pleura. The pleura produce a lubricating serous fluid that allows its two layers to slide against one another (Sylvia 2004).

### 2.10.4 TESTES

The testes, which produce sperm and also the male sex hormones, lie outside the abdominal cavity of the male within the scrotum. The testes begin their development inside the abdominal cavity but descend into the scrotal sacs during the last two months of fetal development. A sagittal section of a testis shows that it is enclosed by a tough, fibrous capsule. The connective tissue of the capsule extends into the testis, forming septa that divide the testis into compartments called lobules. Each lobule contains one to three tightly coiled seminiferous tubules.

A microscopic cross section of a seminiferous tubule reveals that it is packed with cells undergoing spermatogenesis, the production of sperm. Delicate connective tissue surrounds the seminiferous tubules. Cells that secrete the male sex hormones, the androgens, are located here between the seminiferous tubules (Sylvia 2004].

### 2.10.5 STOMACH

The stomach is a thick-walled, J-shaped organ that lies on the left side of the abdominal cavity deep to the liver and diaphragm. The stomach is continuous with the esophagus above and the duodenum of the small intestine below. The length of the stomach remains at about 25 cm (10 in.). The stomach has four regions. The cardiac region, which is near the heart, surrounds the lower esophageal sphincter where food enters the stomach. The fundic region, which holds food temporarily, is an expanded portion superior to the cardiac region. The body region, which comes next, is the main part. The pyloric region narrows to become the pyloric canal leading to the pyloric sphincter through which food enters the duodenum, the first part of the small intestine.

The columnar epithelial lining of the stomach has millions of *gastric pits*, which lead into gastric glands. The gastric glands produce gastric juice, which contains pepsinogen, HCl, and mucus. *Chief cells* secrete pepsinogen, which becomes the enzyme pepsin when exposed to hydrochloric acid (HCl) released by *parietal cells*. The HCl causes the stomach to have a high acidity with a pH of about 2, and this is beneficial because it kills most of the bacteria present in food (Sylvia 2004).

# CHAPTER THREE

## 3.0 Methodology

### 3.1 Study design

The design of the study was experimental that involved laboratory rats.

### 3.2 Setting of the study

The study was carried out in the Laboratory of Kampala International University-Western Campus, Ishaka, Bushenyi District, Uganda.

### 3.3 Selection of study population

The study was conducted on laboratory rats. They were mature male rats .

### 3.4 Determination of sample size

Given that this was an experimental study which involved inflicting pain on the laboratory live animals, the smaller the sample the better given that the animals have to be protected. The sample size was 18 laboratory rats per experiment according to the standard methods to be used in the studies.

### 3.5 selection criteria

#### 3.5.1 Inclusion criteria

- Mature male rats were used
- good health mature male were used

### 3.6 Exclusion criteria

- Sick rats
- Immature rats
- Those weighing less than 100g and pregnant animals were excluded from the study.

### 3.7Sampling Techniques

Given that the study was to cover a small number of animals, all the available 18 rats were used for the study.

### 3.8 Data Collection Methods

Data was collected by the use of data collection form which had different characteristics of the study.

### 3.9 Plant material identification

The *ocimum suave* plant was taxonomically identified by a botanist from Kampala International University-Western Campus and a voucher specimen prepared and deposited in the herbarium of the School of Pharmacy.

### 3.10 Extract preparation

The leaves of *Ocimum suave* plant were collected and dried under shade, then ground into powder. Extraction was boiled in water (decoction) and this is how it was used traditionally. The extract was filtered then the filtrate evaporated and dried in water bath.

### 3.11 Phytochemical screening

Conventional protocol (Trease and Evans, 1983) for detecting the presence of different chemical constituents in the plant extract would be employed. Secondary metabolites tested included; tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, reducing sugars.

- **Tannins**

  Small quantity of the extract was mixed with water and heated on water bath then filtered. A few drops of 0.1% ferric chloride were added to the filtrate and observed for the presence of brownish green or blue-black coloration which was considered as positive.

- **Phlobatannins**

  0.5g of the extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCL solution. Observation of a red precipitate was considered as positive.

- **Saponins**

  About 0.2g of the extract was shaken with 5ml distilled water and then heated to boil. Absence of frothing indicated no Saponins.

- **Flavonoids**

  About 0.2g of the extract was dissolved in dilute NaOH. HCL was added to the mixture and observation of a yellow solution that didn't turn colorless was considered as negative.

- **Steroids**

  0.5g of the extract was mixed with 2ml sulphuric acid then 2mls acetic anhydride added to the mixture. Observation of color change from violet to blue or green indicated the presence of steroids.

- **Terpenoids**

  0.5g of the extract was dissolved in 1ml chloroform then 1ml concentrated sulphuric acid carefully added to form a layer. Observation of a reddish brown or yellow coloration of the interphase indicated presence of terpenoids.

- **Cardiac Glycosides**

  A drop of ferric chloride solution was added to 2ml glacial acetic acid. This solution was used to treat 0.5g of the extract. The mixture was under laid with 1 ml concentrated sulphuric acid. A brown ring of the interphase indicated a deoxy-sugar characteristic of cardenolides. A violet ring also appeared below the brown ring while in the acetic acid layer, a greenish ring formed just gradually throughout the thin layer which indicated the presence of cardiac glycosides.

- **Reducing Sugars**

  The extract was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solutions A and B for two minutes. An orange red precipitation was formed indicating the presence of reducing sugar.

### 3.12 Laboratory animal acquisition and maintenance

Male Wistar rats(weighing not less than 100g) were used for this study. The animals were bred and housed in the Animal Facility Centre of the School of Pharmacy, Kampala International University-Western Campus. The animals were then kept in a cage lined with sawdust, at room temperature with adequate ventilation, under a naturally illuminated environment with 12 hours

of light and 12 hours of darkness. They were fed with standard diet (Nuvita (R) Animal Feed Ltd, Jinja Uganda) and had access to clean drinking water ad libitum.

The animal experiment was conducted according to the National Institute of Health Guide for the care and use of laboratory animals (NIH, 1996) and ethical guidelines for investigation of experimental pain in conscious animals (Zimmerman, 1993).

### 3.13 Toxicity Evaluation (Acute toxicity)

This involved observation for signs of acute toxicity and other effects of the extract following administration.

Acute toxicity refered to the effects on the whole body of a single dose of a chemical (or several doses within a 24- hour period), which was manifested over a period of 14 days. Determination of acute oral toxicity was an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. Acute toxicity data were used to provide a rough guide for dose selection among many other factors.

*Phase 1*

Three groups of three rats per group randomly picked and each group was given one dose level orally. The treated animals were observed for at least three hours post administration for the signs of toxicity and after 24 hours, they were scored for mortality and general behavior.

*Phase 2*

After 24 hours, three (3) groups of one rat each were given geometrically increasing doses based on the findings of phase 1 (if no death was recorded) orally. The observation was done similarly as in phase 1 above.

The geographic mean of the least dose that did not kill the rats and the highest dose that killed the rats was taken as the median lethal dose.

***Signs recorded during acute toxicity studies***:

These included, increased motor activity, anaesthesia, tremors, arching and rolling, clonic convulsions, ptosis, tonic extension, lacrimation, Straub reaction, exolphthalmos, pilo-erection, salivation, muscle spasm, opisthotonus , writhing, hyperesthesia , loss of righting reflex, depression, ataxia, stimulation, sedation, blanching, hypnosis, intestinal distension, diarrhoea, lethargy, nasal or anal bleeding, coma and death.

The $LD_{50}$ test was used to determine the therapeutic index, i.e. ratio between the lethal dose and the pharmacologically effective dose in the same strain and species ($LD_{50}/ED_{50}$). The greater the index, the safer the compound was. $LD_{50}$ with confidence limits was to be established on one common laboratory species such as mice using the standard method. Lorke`s method proceeded in phases.

**Materials:**

Materials used included mature male rats which were weighing above 100g.Test compound used was aqueous leaf extract of *Ocimum suave.* 1 ml syringes and normal saline were also used.

**Procedure:**

Freshly prepared solution of normal saline, test compound was provided.

1. The animals were weighed and divided into three groups of 3 animals each.

2. The volume of drugs to be administered to each animal was calculated based on the body weight and low, medium and high dose range (e.g. 100, 500 and 1000mg/kg)

3 The drug was administered and the animal placed in individual transparent cages.

4. Observation and recording of the signs of toxicity was carried out in each rat.

*Calculation:*

The $LD_{50}$ value of the test compound was calculated as explained above. At the end, a conclusion was drawn which showed that the LD50 value of the test compound [aqueous leaf extract of *Ocimum suave*] was above 10,000mg/kg.

### 3.13.1 Biochemical assay

Serum and liver alanine (ALT) and aspartate amino (AST) transferases were assayed by the method described by Reitman and Frankel of 1957. Gamma glutamyl transpeptidase (GGT), LDH and CK,were analyzed.

### 3.13.2 Hematological studies

Blood samples were collected into heparinized tubes and used for the estimation of Complete Blood Count, Erythrocyte Count, WBC Count, Lymphocytes and Monocytes, Differential Leukocyte Count, Hemoglobin, Mean Cell Volume, Mean Cell Hemoglobin, Mean Cell Hemoglobin Concentration, Hematocrit Value, Reticulocyte Count and Blood Platelet Count.

All data generated was presented as Mean ± Standard Error of the Mean (SEM) and statistical comparisons were performed using descriptive statistics and ANOVA-repeated measures $p<0.05$ was considered as statistically significant.

### 3.13.3 Preparation of tissue processing

The tissues were obtained from wistar rats and were fixed in 10% formal saline immediately to prevent tissue degradation. They were fixed for 24hours before processing. Chemical fixatives were used to preserve tissue from degradation, and to maintain the structure of the cell and of sub-cellular components such as cell organelles (e.g., nucleus, endoplasmic reticulum, mitochondria) (Clark G 1981). They were trimmed using a surgical blade. Each sample/tissue

was loaded in an individual cassette with respective label, which was made using indelible pencil. They were labeled from number 1-18.

### 3.14 Outcome measures

At the end of the study it was ascertained that *ocimum suave* has acute toxic effects. It also ascertained that *Ocimum suave* had biochemical, hematological, histological effects.

Phytochemical screening was also evaluated to identify the constituents responsible for the biochemical and hematological effects of *Ocimum suave*.

### 3.15 Data analysis

The data collected from the study was analyzed by using the Statistical Package for Social Scientists (SPSS) version 8.0 for windows to obtain descriptive statistical correlation and the results were presented in form of tables and figures. Statistician was consulted during data analysis.

### 3.16 Ethical considerations

(i) The study was carried out after approval of the proposal by research and postgraduate committee

(ii) Letter of introduction was obtained from the dean of school of pharmacy.

### 3.17 Dissemination of findings

A copy of the research was submitted to the office of the dean of school of pharmacy.

Another copy was left in the library of KIU-WC.

### 3.18 Study limitations

1. Lack of equipment; for example personal computer to be used as required.
2. Frequent power black outs; this hampered the undertaking of the study.
3. Limited university facilities, hence transporting laboratory rats to Mbarara university of science and technology for tissue, blood and enzyme analysis.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Acute Toxicity.

*Ocimum suave* leaf extract administered orally up to 10,000 mg/kg to rats was found to cause no death in the two phases of the test. Thus, the $LD_{50}$ of *Ocimum suave* extract in rats was estimated to be greater than 10,000 mg/kg.

### 4.2 Phytochemical Analysis.

Phytochemical analysis of *Ocimum suave* aqueous leaf extract gave positive reactions to phlobatannins, steroids, terpenoids, cardiac glycosides, reducing sugars and tannins.

**Table 1**

| *PHYTOCHEMICAL CONSTITUENTS* | *RELATIVE PRESENCE* |
|---|---|
| Tannins | Positive |
| Phlobatannins | Positive |
| Saponins | Negative |
| Flavonoids | Negative |
| Steroids | Positive |
| Terpenoids | Positive |
| Cardiac glycosides | Positive |
| Reducing sugars | Positive |

### 4.3 Biochemical parameters.

**Results expressed as Mean±SEM. Statistical significance tested with student's t-test, $p<0.05$.**

ALP levels were found to be higher in the 800mg/kg OSE treated group as compared to the control group with a decrease in 200 and 400mg/kg OSE treated groups. However, the increase was not statistically significant at $p<0.05$ .CK levels were found to be higher in the OSE treated

groups as compared to the control group with a decrease in 400mg/kg OSE treated group. The increase of CK levels appeared to be dose dependent. The increase was not statistically significant at $p<0.05$. ASAT levels were higher in the 800mg/kg OSE treated group as compared to the control group with a decrease in 200 and 400mg/kg OSE treated groups.GGT levels were found to be high in all OSE treated groups as compared to the control group. The increase of GGT levels appeared to be dose dependent. The increase was not statistically significant at $p<0.05$. ALAT levels were found to have decreased in all OSE treated groups as compared to the control group.

**Table 2**

| | **TREATMENT** | | | |
|---|---|---|---|---|
| **BIOCHEMICAL PARAMETERS.** | **10ml/kg (control)** | **200mg/kg** | **400mg/kg** | **800mg/kg** |
| **ALP DGKC U/L** | 252.2±72.652 | 158.6±70.84 | 208.8±112.495 | 365±105.754 |
| **CK 2R U/L** | 2287.2±600.45 | 3864.6±2570.29 | 1060±528.708 | 3388±1248.286 |
| **GOT-ASAT U/L** | 128.60±114.583 | 94.4±37.098 | 92.20±43.929 | 141.8±55.674 |
| **GGT U/L** | 26.2±8.224 | 46.80±18.148 | 69.4±43.929 | 55.6±22.065 |
| **GPT- ALAT U/L** | 189.8±22.758 | 92.2±37.090 | 101.2±29.006 | 136.00±27.395 |

**4.4 Hematological values.**

**Results expressed as Mean±SEM. Statistical significance tested with student's t-test, $p<0.05$.**

WBC increased in the 800mg/kg OSE treated group as compared to the control group with a decrease in 200 and 400mg/kg OSE treated groups. RBC decreased in all OSE treated groups as compared to the control group. Hemoglobin levels were found to be higher in the 800mg/kg OSE treated group as compared to the control group with a decrease in 200 and 400mg/kg OSE treated groups .Hematocrit percentage was found to be higher in the 800mg/kg OSE treated group as compared to the control group with a decrease in 200 and 400mg/kg SOE treated groups. Mean cell volume increased in the 800mg/kg OSE treated group as compared to the

control group with a decrease in 200 and 400mg/kg OSE treated groups .Mean cell hemoglobin decreased in the 200 and 400mg/kg OSE treated groups as compared to the control group with no slight increase in 800mg/kg OSE treated group as compared to the control group .Mean cell hemoglobin concentration decreased in all OSE treated groups as compared to the control group. Platelet count was found to be higher in the 800mg/kg OSE treated group as compared to the control group with a decrease in 200 and 400mg/kg OSE treated groups. Neutrophil percentage was found to be higher in 400 and 800mg/kg OSE treated groups as compared to the control group with a decrease in 200mg/kg group. Lymphocyte percentage was higher in the 800mg/kg as compared to the control group with a decrease in 200 and 400mg/kg OSE treated group. Monocyte percentage was found to be lower in 200 and 800mg/kg groups as compared to the control group . Eosinophil percentage decreased in all OSE treated groups as compared to the control group. Basophil percentage increased in all OSE treated groups as compared to the control group.

**Table 3**

| **Hematological values** | **TREATMENT** | | | |
|---|---|---|---|---|
| **Haematograms** | **10ML/KG (control)** | **200MG/KG** | **400MG/KG** | **800MG/KG** |
| **WBC $10^3\mu L$** | 9.74±1.697 | 9.70±3.112 | 7.42±2.352 | 12.62±1.705 |
| **RBC $10^6\mu L$** | 8.69±0.328 | 6.79±1.733 | 6.89±1.759 | 8.65±0.327 |
| **HGB g/dl** | 14.8±0.586 | 11.72±2.974 | 11.72±2.961 | 15.06±0.453 |
| **HCT%** | 46.18±1.893 | 36.98±9.370 | 36.90±9.365 | 47.62±1.271 |
| **MCV Fl** | 53.2±1.393 | 43.8±10.901 | 42.4±1.656 | 54.40±2.074 |
| **MCH pg** | 17.42±0.493 | 13.82±3.465 | 13.62±3.414 | 17.46±0.256 |
| **MCHC g/dl** | 32.22 ±0.073 | 25.38+6.358 | 25.46±6.377 | 32.02±0.136 |
| **RDW %** | 11.56±0.282 | 8.98±2.292 | 9.38±2.414 | 11.48±0.538 |
| **PLT$10^{3\mu L}$** | 583.8±128.2575 | 535.6±182.704 | 527.2±144.527 | 616.6±115.289 |
| **MPV fl** | 7.61±0.938 | 5.50±1.401 | 7.02±1.771 | 8.48±0.381 |
| **NE%** | 11.72±1.976 | 10.42±3.025 | 14.88±5.068 | 14.34±3.057 |
| **LY%** | 65.34±5.464 | 53.22±13.682 | 44.92±11.647 | 68.78±5.556 |

| | | | | |
|---|---|---|---|---|
| **MO%** | 17.9±3.851 | 13.72±4.078 | 17.90±4.506 | 14.72±2.426 |
| **EO %** | 4.74±0.946 | 1.82±0.681 | 1.66±0.797 | 2.32±1.063 |
| **BA %** | 0.68±0.37 | 0.82±0.256 | 0.84±0.254 | 1.04±0.268 |

**Figure 1:Graph showing enzyme levels of CK and GGT against the Dose**

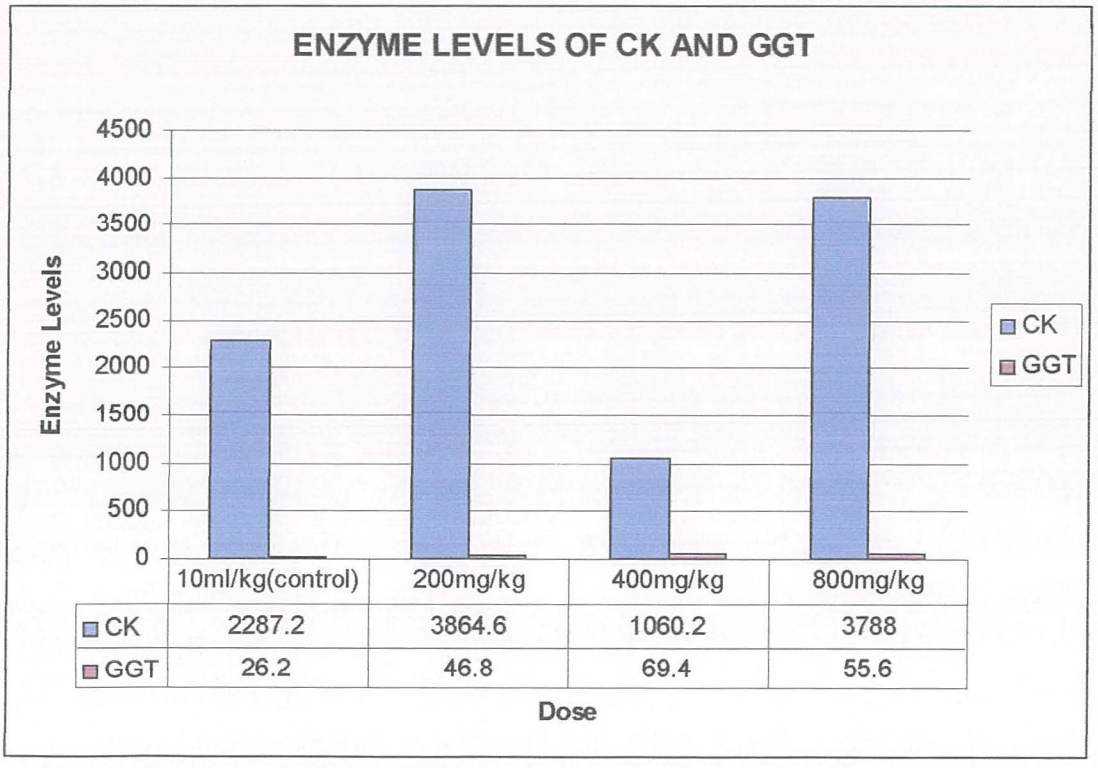


**Figure 2:Graph showing enzyme levels of Ck and AST against the Dose**

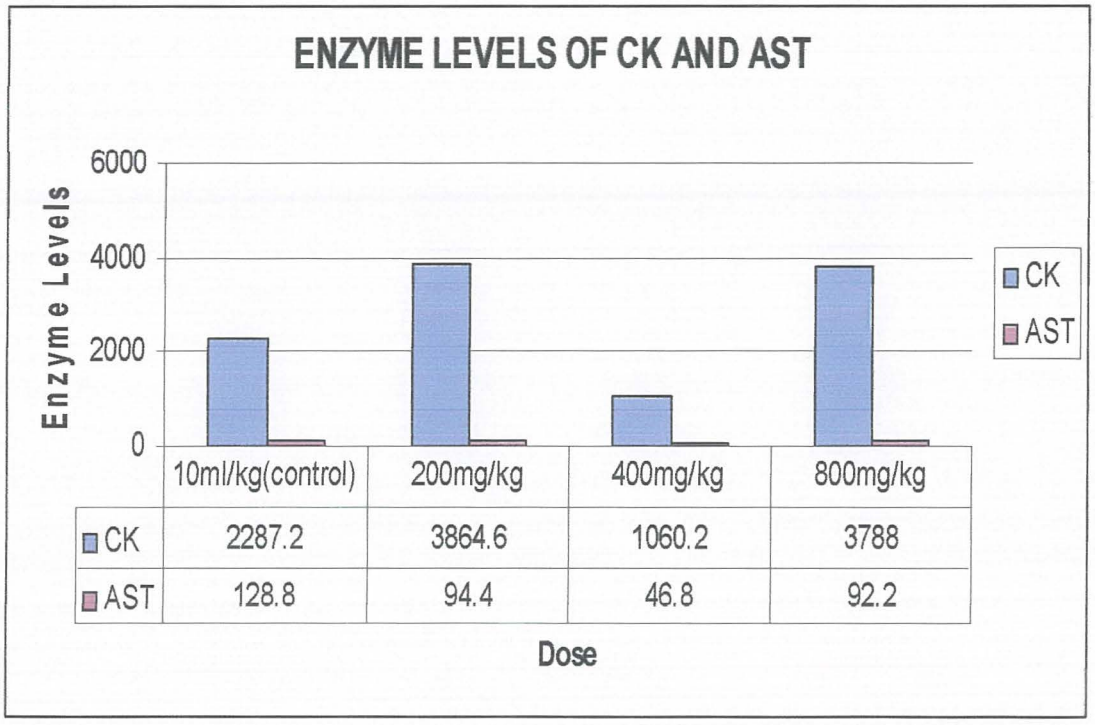


**Figure 3 :Graph showing enzyme levels of AST and ALT against the Dose**

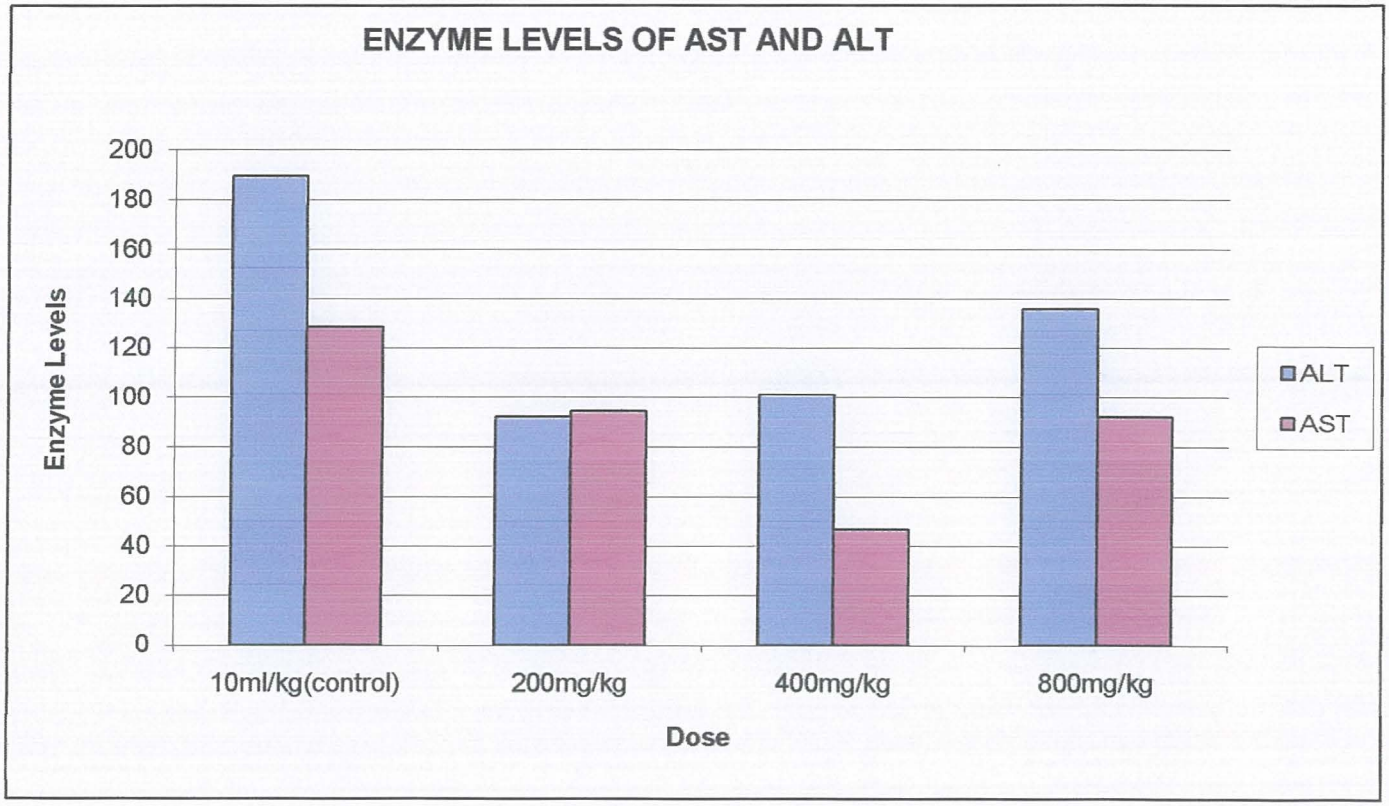


**Figure 4:Graph showing enzyme levels of ALP and ALT against the Dose**

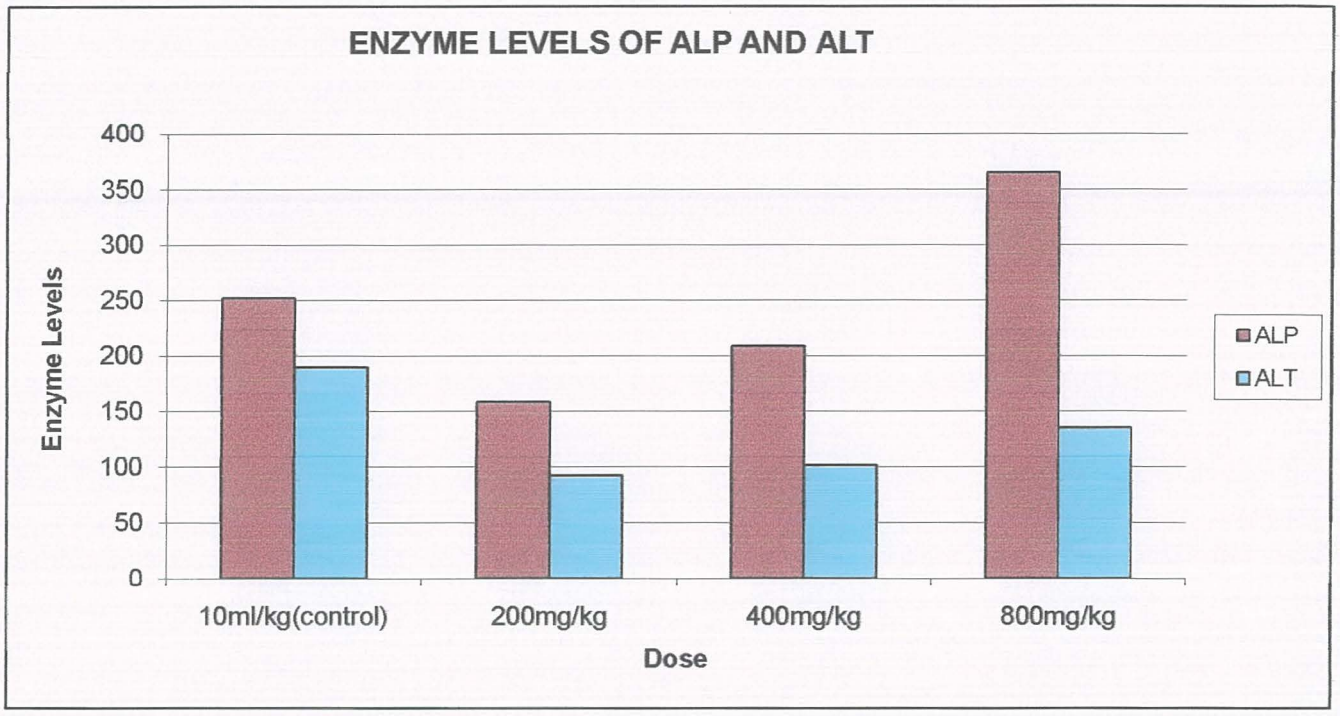


**Figure 5:Graph Showing enzyme levels of GGT against the Dose**

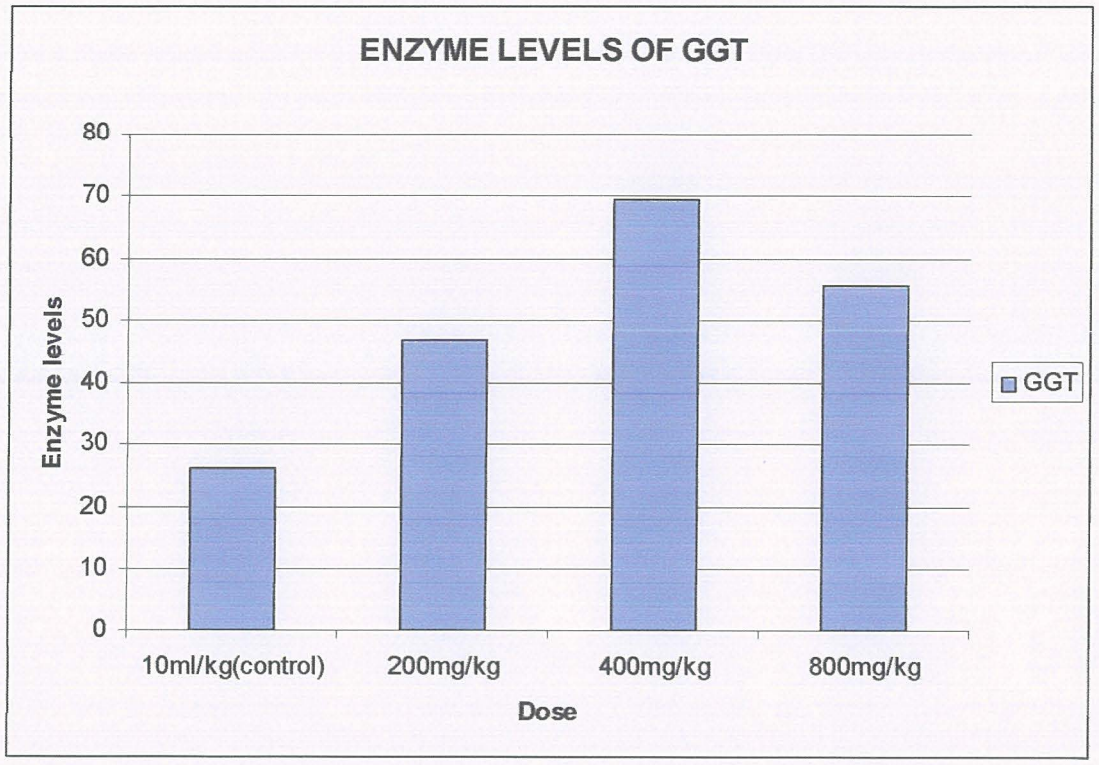


**Figure 6:Graph Showing the WBC count against the Dose**

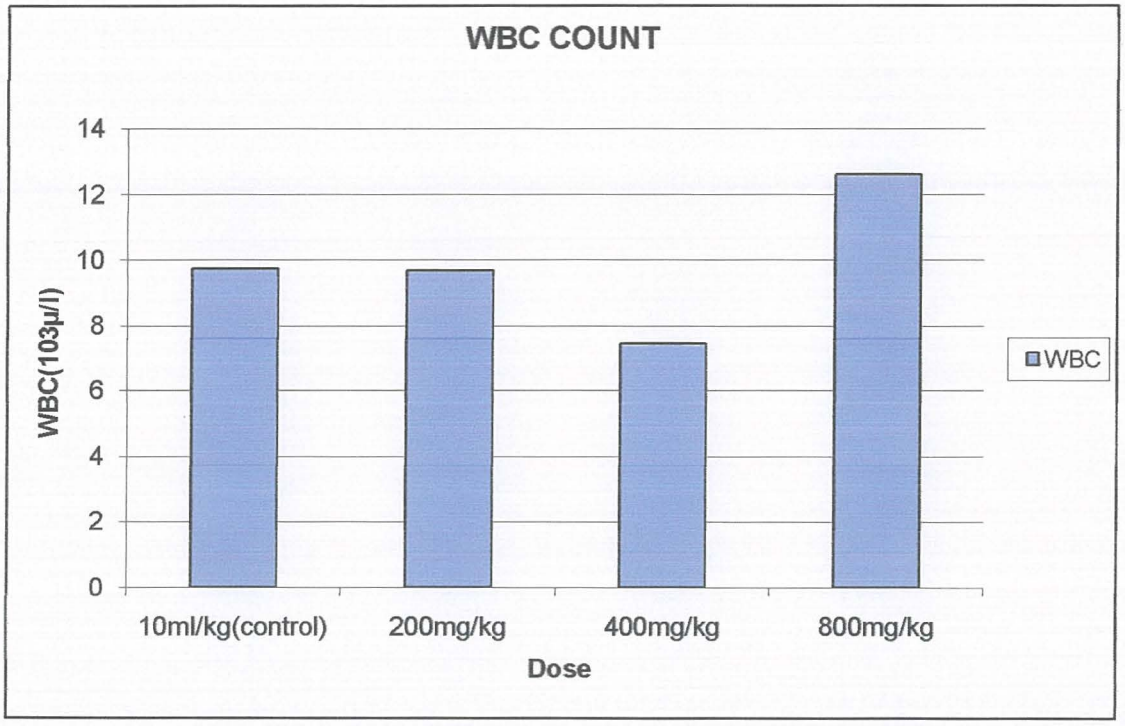
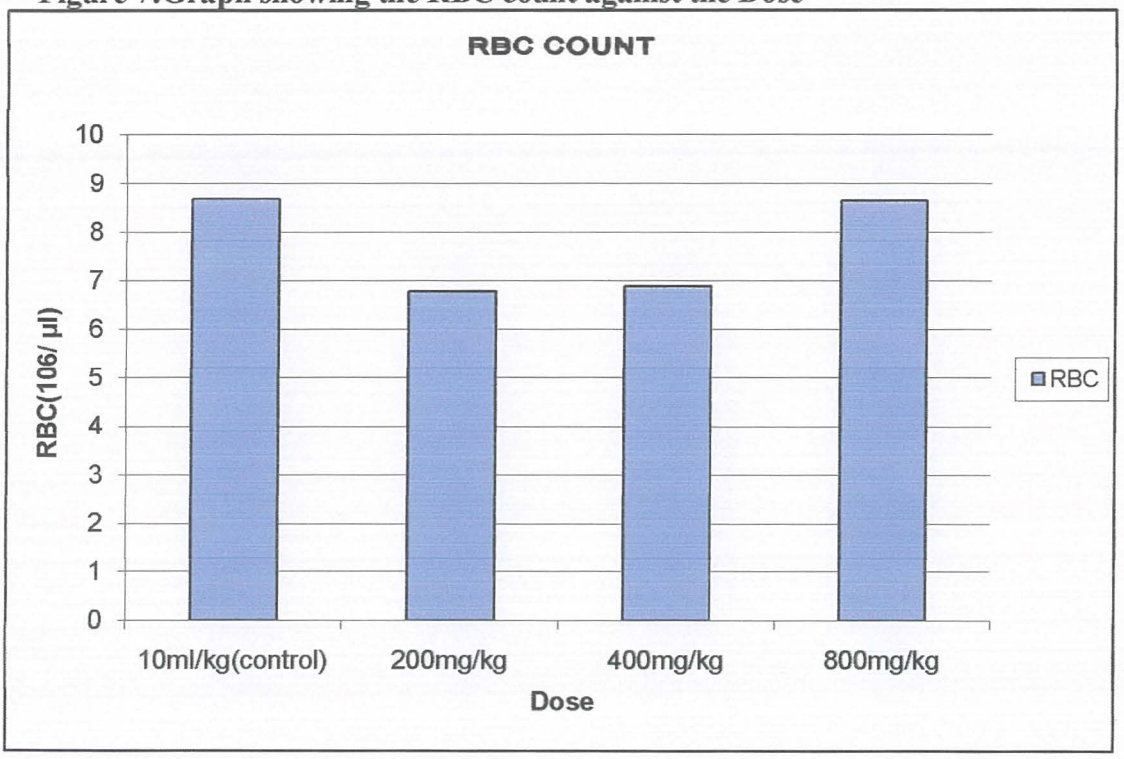


**Figure 7:Graph showing the RBC count against the Dose**



## 4.5 HISTOLOGICAL STUDY RESULTS.

**Table 1: shows mean body weight of rats**
**Results expressed as Mean±SEM. Statistical significance tested with student's t-**

**Test ,p<0.05.**

There was weight gain between day1 and day7 in 200mg/kg and 400mg/kg OSE-treated groups compared to the control group with a decrease in day1 800mg/kg OSE- treated group. Between day7 and day 14 weight gain increased in the OSE-treated groups compared to the control group. Between day14 and day 21 weight gain also increased in the OSE-treated groups compared to the control group. There was weight gain between day 21 and day28 in 200mg/kg and 400mg/kg OSE-treated groups compared to the control group with a decrease in day28 800mg/kg OSE-treated group. Between day28 and day 31 weight gain increased in the 200mg/kg and 400mg/kg OSE-treated groups compared to the control group, with a decrease in day28.

| MEAN BODY WEIGHT OF RATS | | | | |
|---|---|---|---|---|
| | Treatment | | | |
| **DURATION** | **10ml/kg** | **200mg/kg** | **400mg/kg** | **800mg/kg** |
| **Day 1** | 191.53±1.545 | 225.1±16.883 | 219.77±1.048 | 202.7±1.572 |
| **Day 7** | 210.9±6.31 | 268.87±7.85 | 257.87±2.392 | 234.93±4.129 |
| **Day 14** | 220.2±4.187 | 288.4±7.275 | 264.53±5.716 | 235.7±3.74 |
| **Day 21** | 225.8±0.275 | 291.57±15.306 | 280.37±4.715 | 252.23±7.464 |
| **Day 28** | 243.3±5.285 | 307.53±16.36 | 289.37±3.077 | 239±5.903 |
| **Day 31** | 248.7±3.044 | 319.2±24.652 | 289.37±1.184 | 258.97±6.939 |

**Table 2: Shows the relative organ weight**
**Results expressed as Mean±SEM. Statistical significance tested with student's t-**

**Test ,p<0.05.**

There was decrease in relative organ weight of liver in 200mg/kg and 400mg/kg OSE treated groups compared to the control group, with slight increase in 800mg/kg OSE-treated group .Relative organ weight of lungs decreased in 200mg/kg and 400mg/kg OSE-treated groups compared to the control group. There was decrease in relative organ weight of kidney in 200mg/kg and 400mg/kg OSE- treated groups compared to the control group, with an increase in 800mg/kg OSE- treated group. Relative organ weight of testes and stomach decreased in the OSE-treated groups compared to the control group.

| | **RELATIVE ORGAN WEIGHT** | | | |
|---|---|---|---|---|
| | Mean organ weight ±S.E.M (Values are in grams) | | | |
| **Relative body organ** | **10ml/kg** | **200mg/kg** | **400mg/kg** | **800mg/kg** |
| Liver | 2.37±0.67 | 2.01±0.070 | 2.13±0.033 | 2.4±0.058 |
| Lungs | 0.47±0.033 | 0.44±0.031 | 0.37±0.033 | 0.47±0.033 |
| Kidney | 0.47±0.033 | 0.41±0.007 | 0.43±0.033 | 0.57±0.038 |
| Testes | 1.83±0.033 | 1.26±0.057 | 1.43±0.033 | 1.58±0.033 |
| Stomach | 1.6±0.0 | 0.67±0.033 | 0.67±0.033 | 0.6±0.0 |

Values presented as mean ± standard error of mean (S.E.M.)

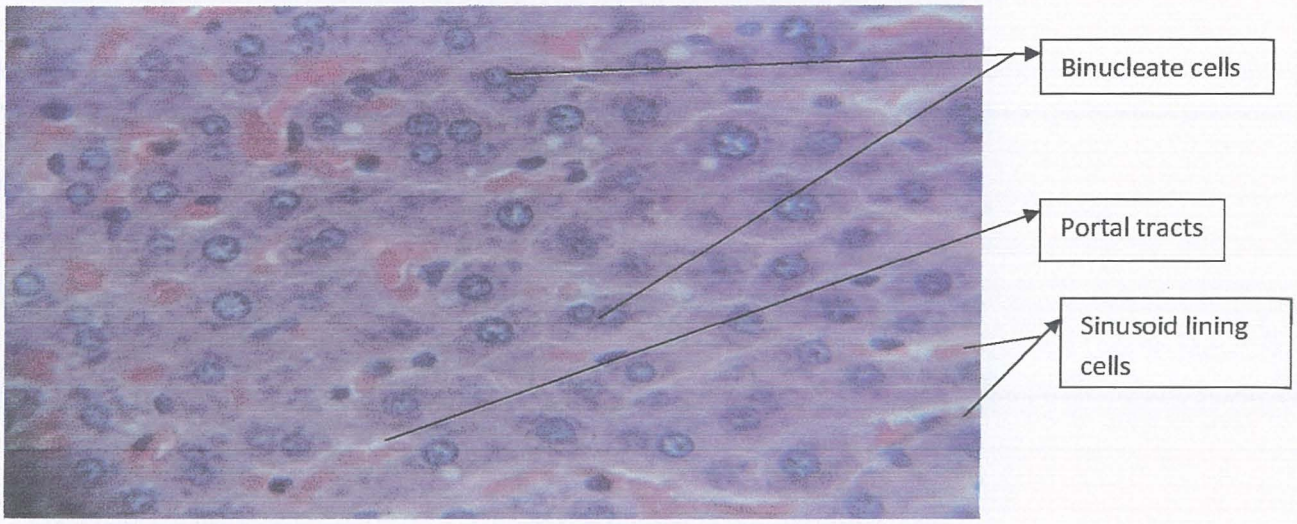
Values are in grams.

Data is deemed significant at $p<0.05$.

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrificed day (g)}} \times 100$$

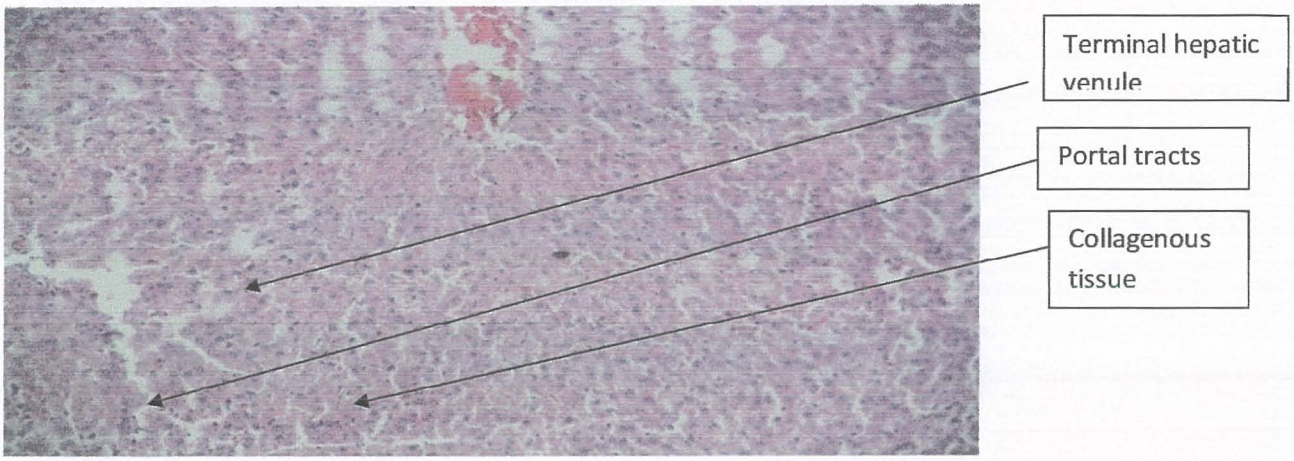
**LIVER (TREATED GROUP)**

Figure 1




Light micrograph of liver x 400

Figure 2




Light micrograph of liver x100

Liver appear normal with no changes versus the control.

**LIVER (CONTROL GROUP)**

Figure 3: Light micrograph of liver x400

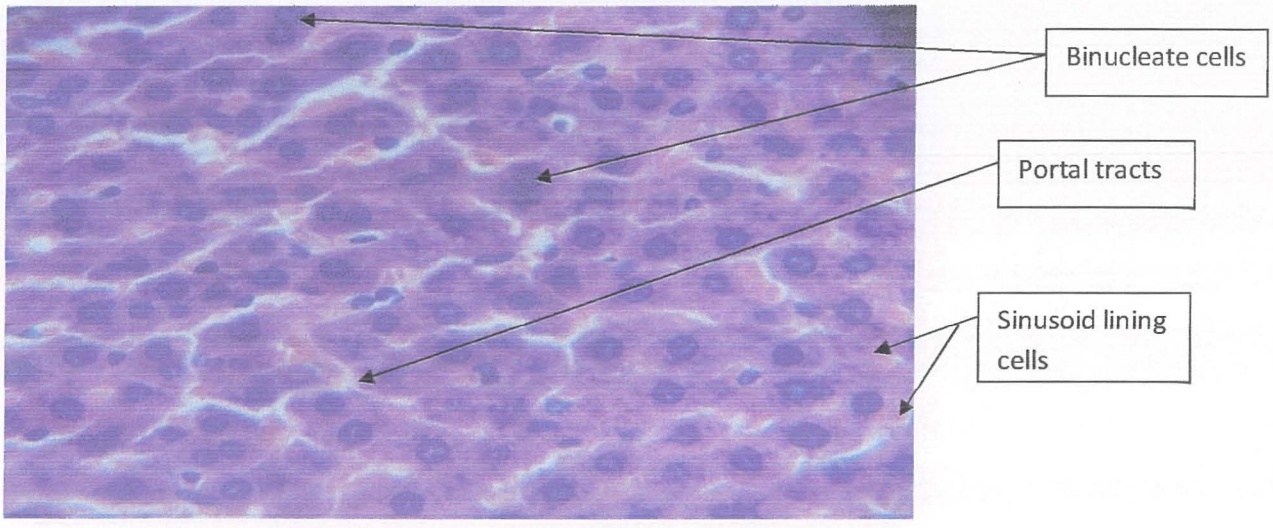
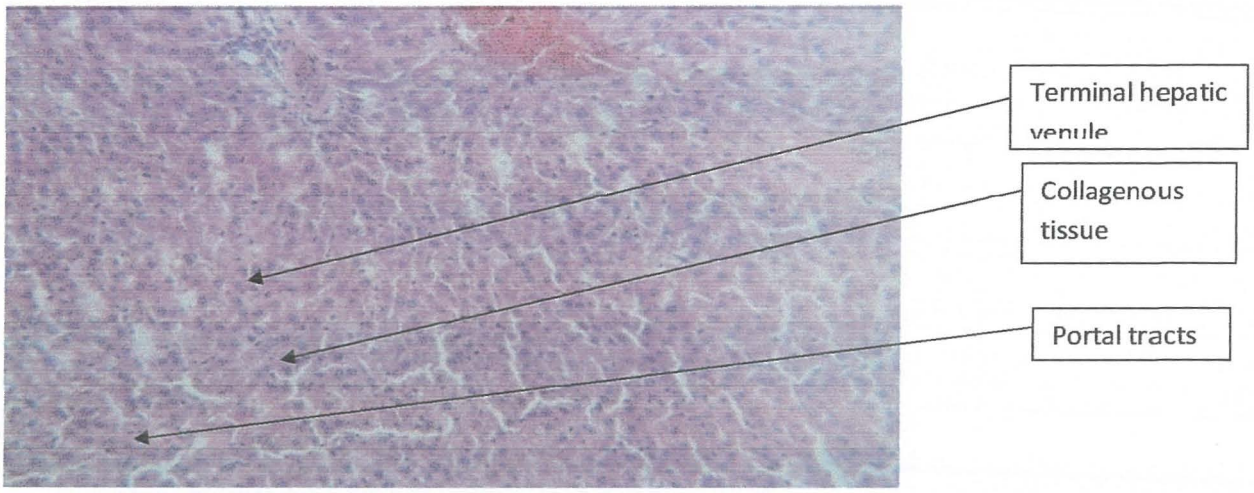


Figure 4: Light micrograph of liver x 100



**KIDNEYS**

**KIDNEYS (TREATED GROUP)**

Figure 5: Light micrograph of Kidney x400

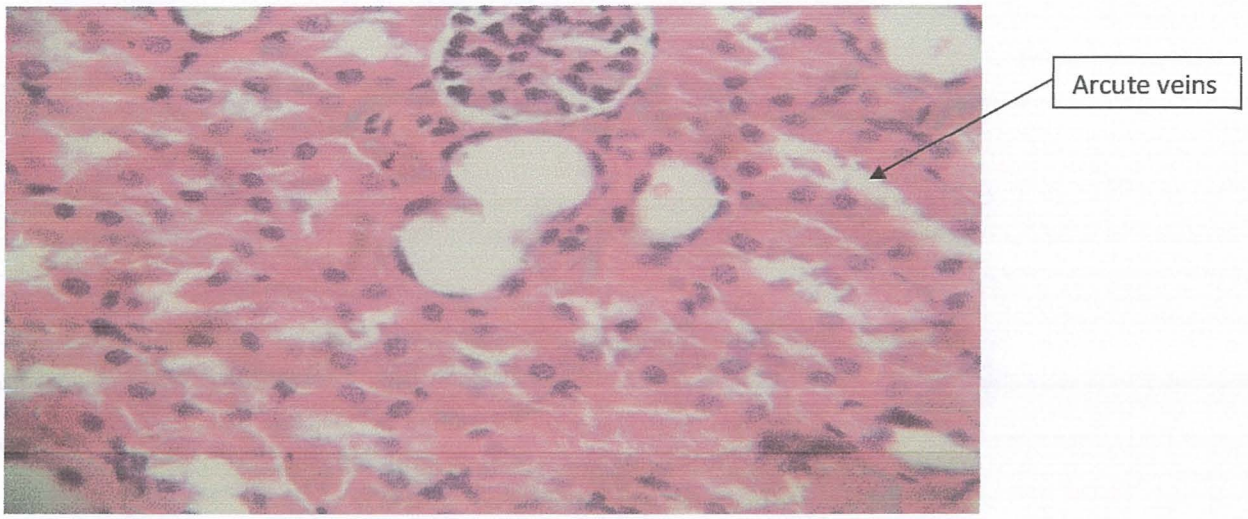
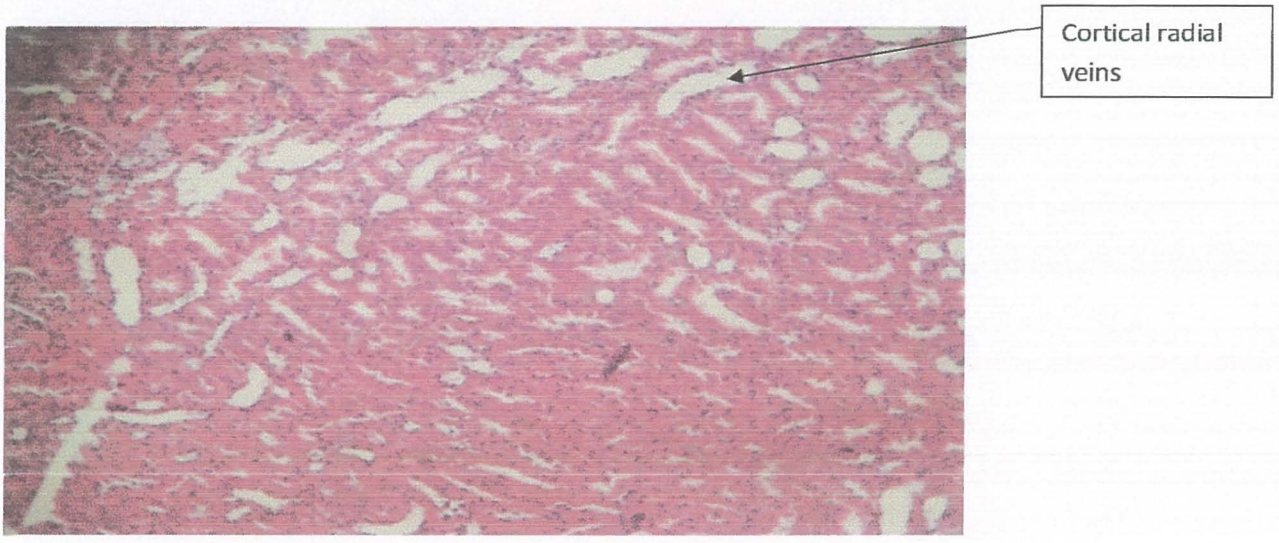



Figure 6: Light micrograph of Kidney x100




Kidneys appear normal with no changes versus the control.

**KIDNEYS (CONTROL GROUP)**

Figure 7: Light micrograph of kidney x400

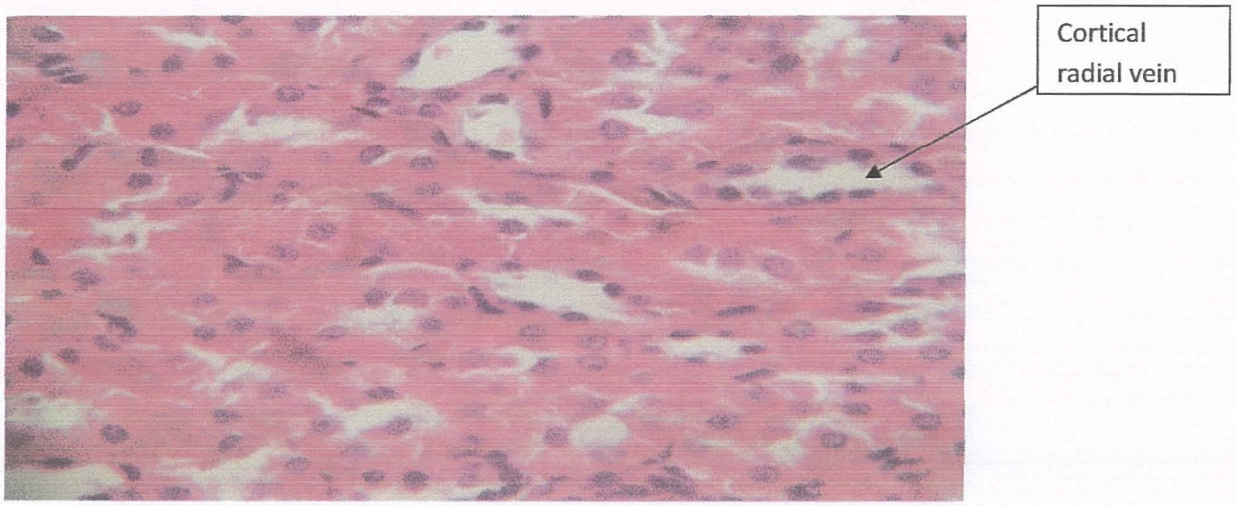
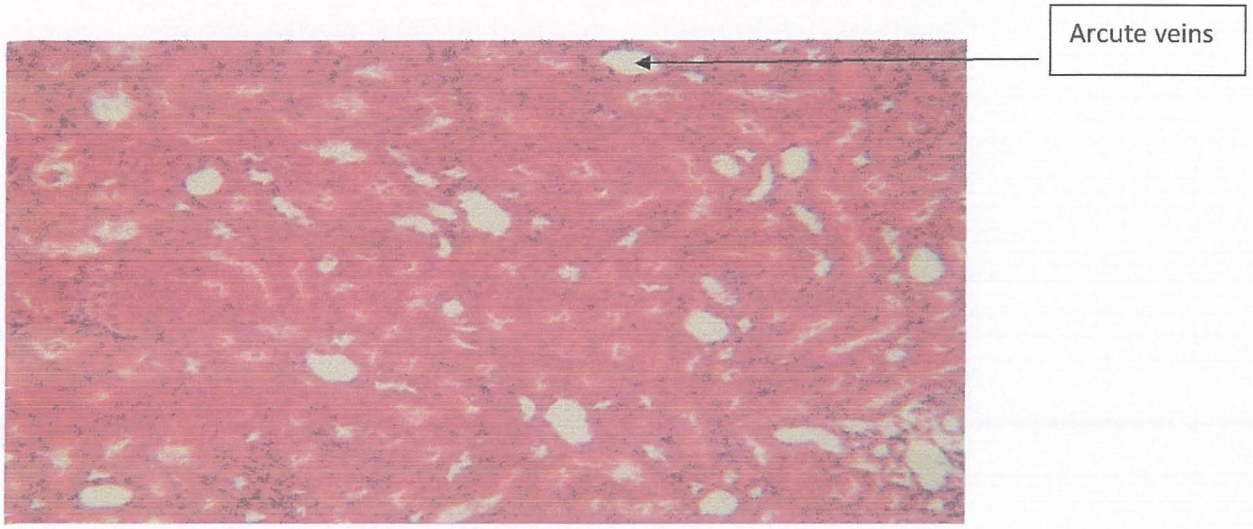


Figure 8: Light micrograph of kidney x 100



**LUNGS**

**LUNGS (TREATED GROUP)**

Figure 9: Light micrograph of lung x400

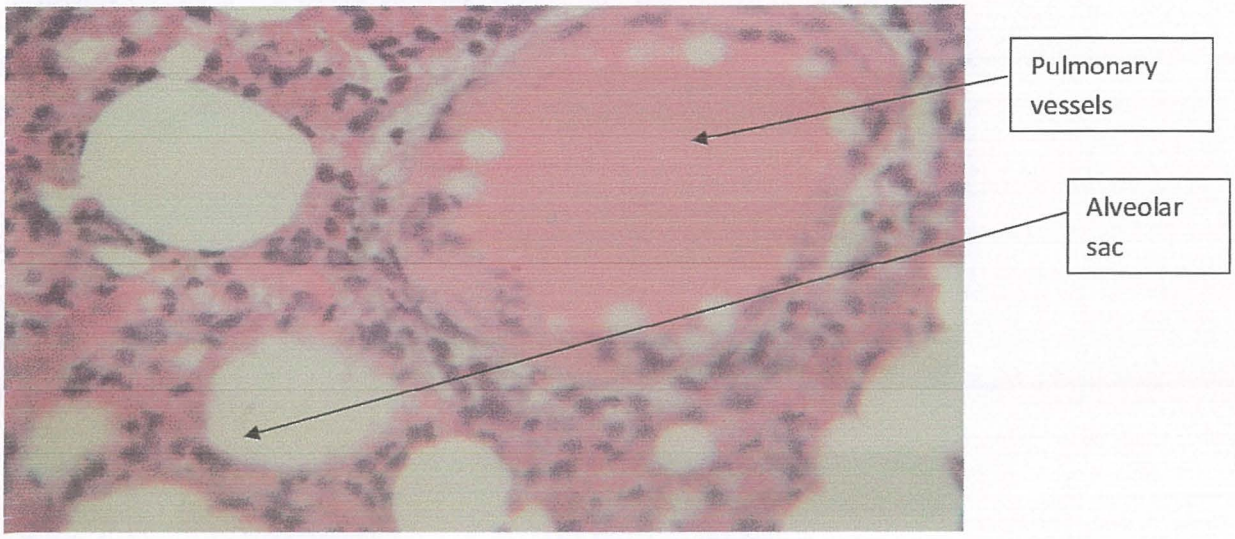
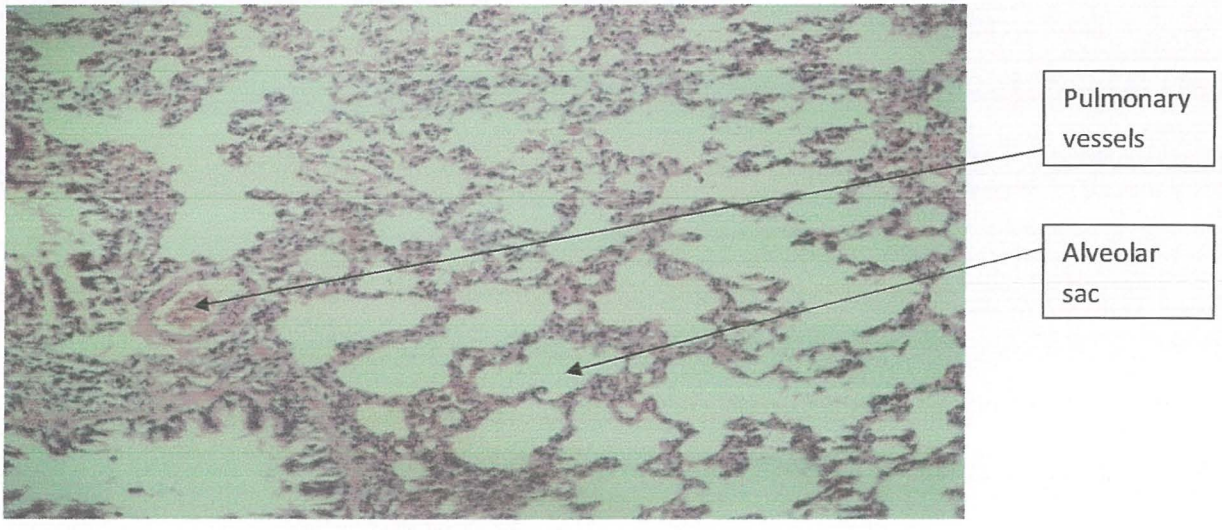



Figure 10: Light micrograph of Lung x100




Lungs appear normal with no changes versus the control.

**LUNGS (CONTROL GROUP)**

Figure 11: Light micrograph of lungs x400

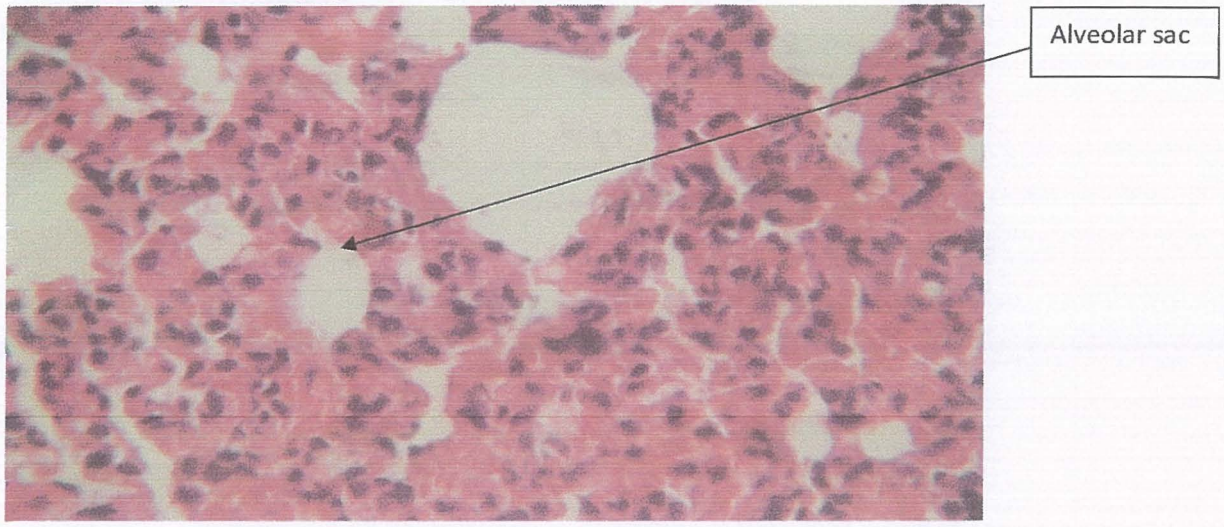
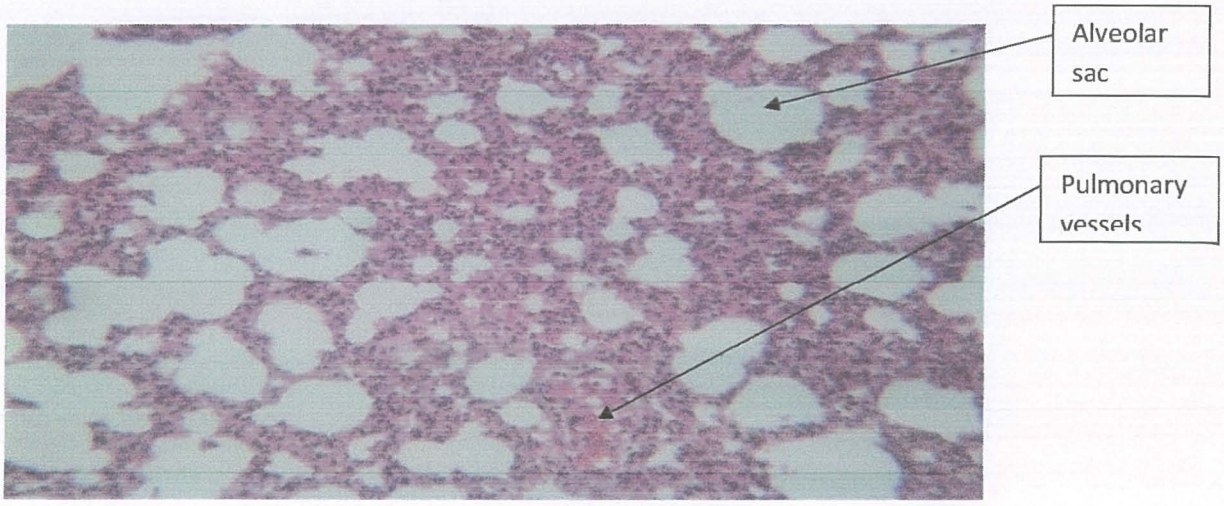



Figure 12: Light micrograph of lungs x100



**TESTES**

**TESTES (TREATED GROUP)**

Figure 13: Light micrograph of Testis x400

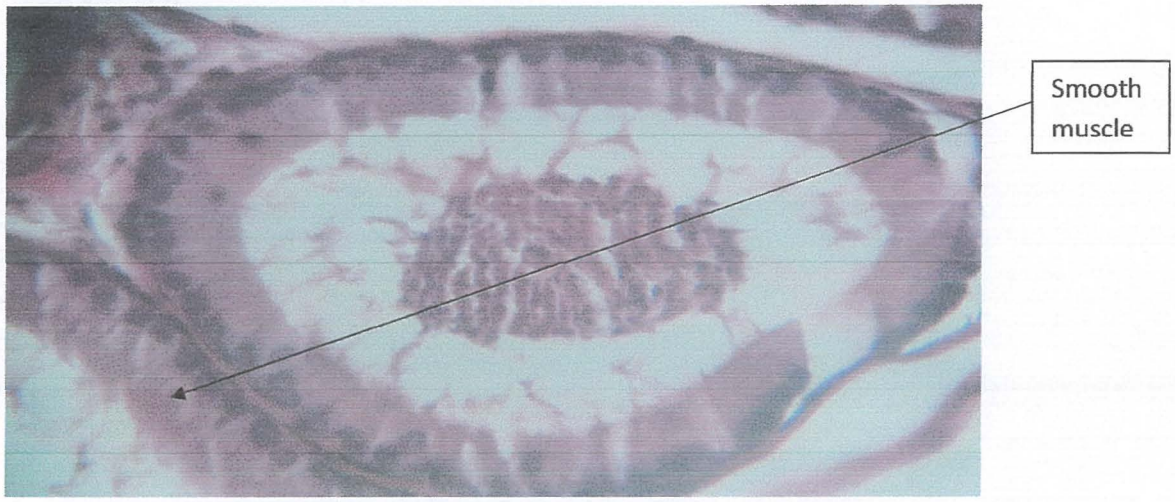
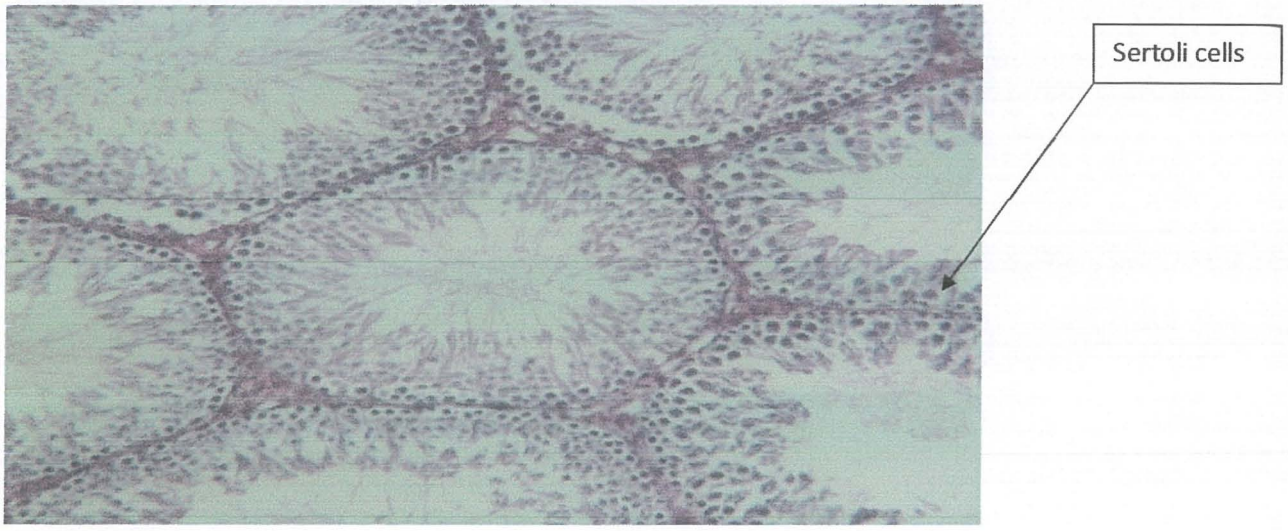



Figure 14: Light micrograph of Testis x100




Testis appear normal with no changes versus the control.

## TESTIS (CONTROL GROUP)

Figure 15: Light micrograph of testis x400

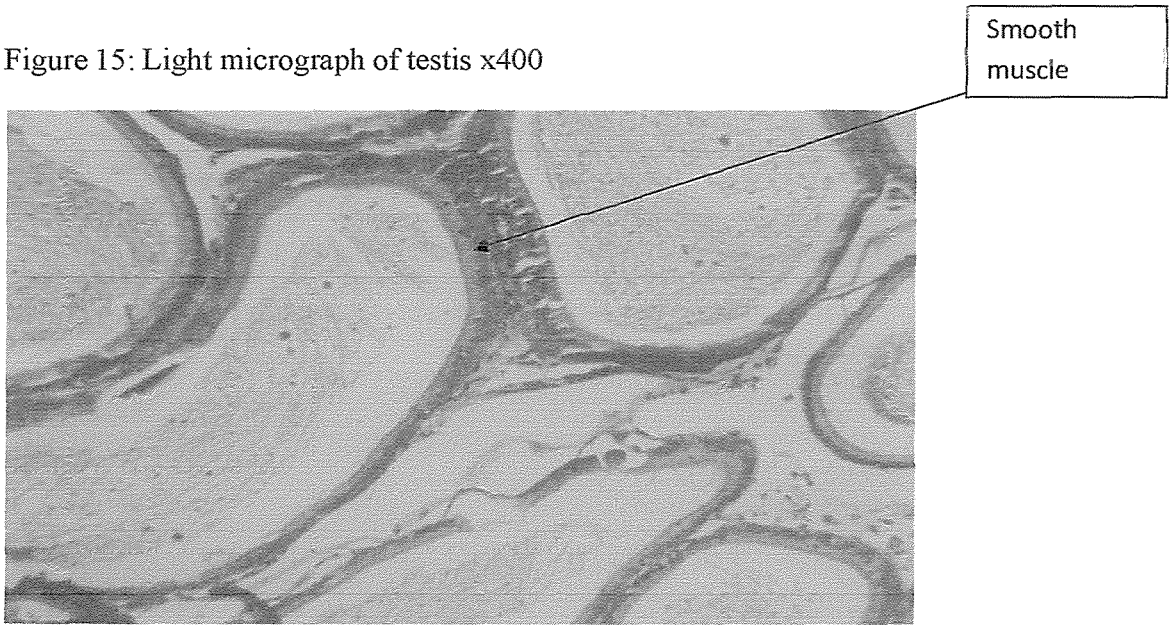
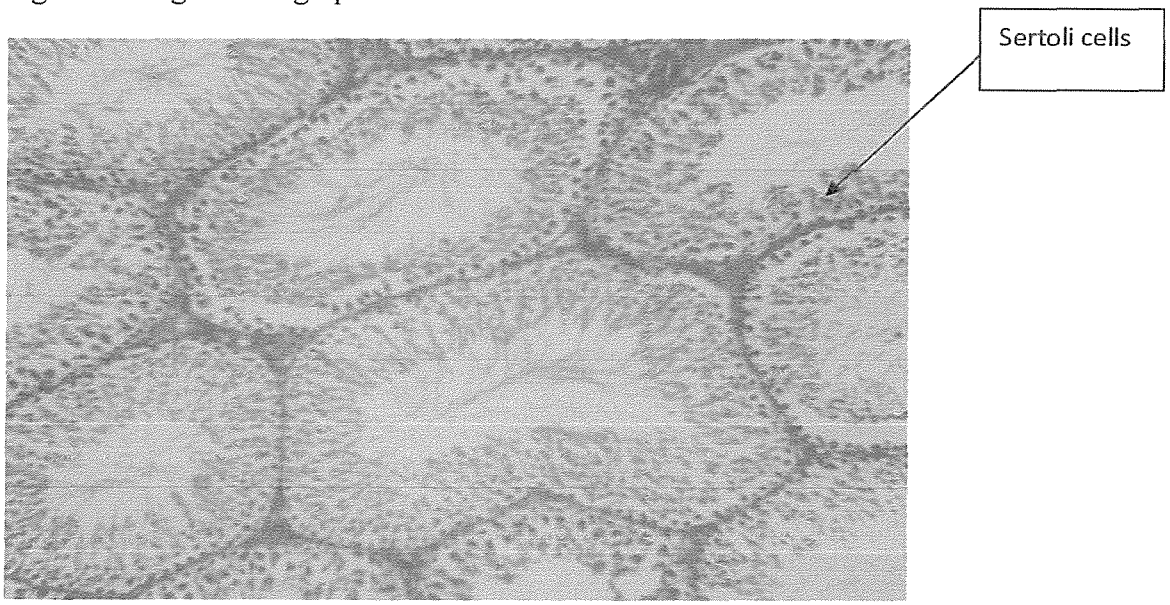



Figure 16: Light micrograph of testis x 100



**STOMACH**

**STOMACH (TREATED GROUP)**

Figure 17: Light micrograph of stomach x 400

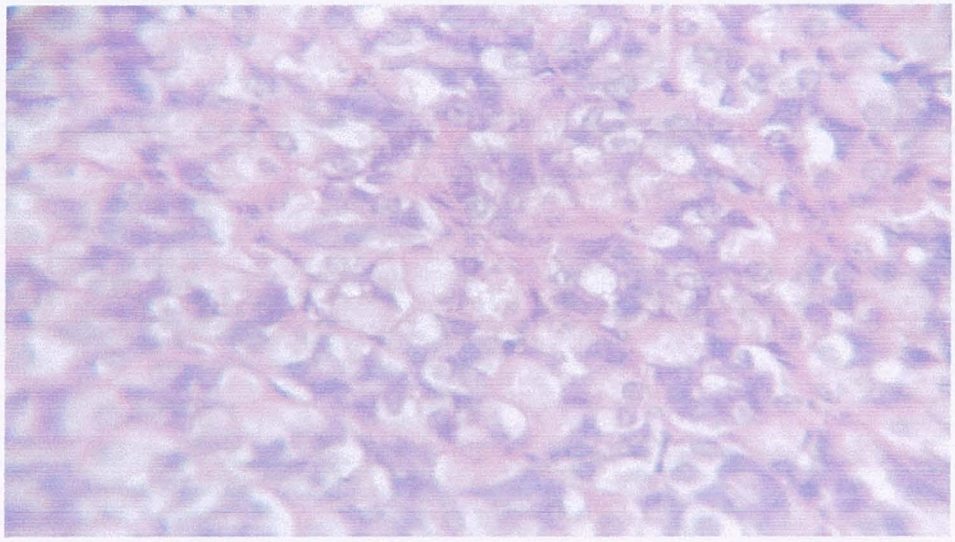
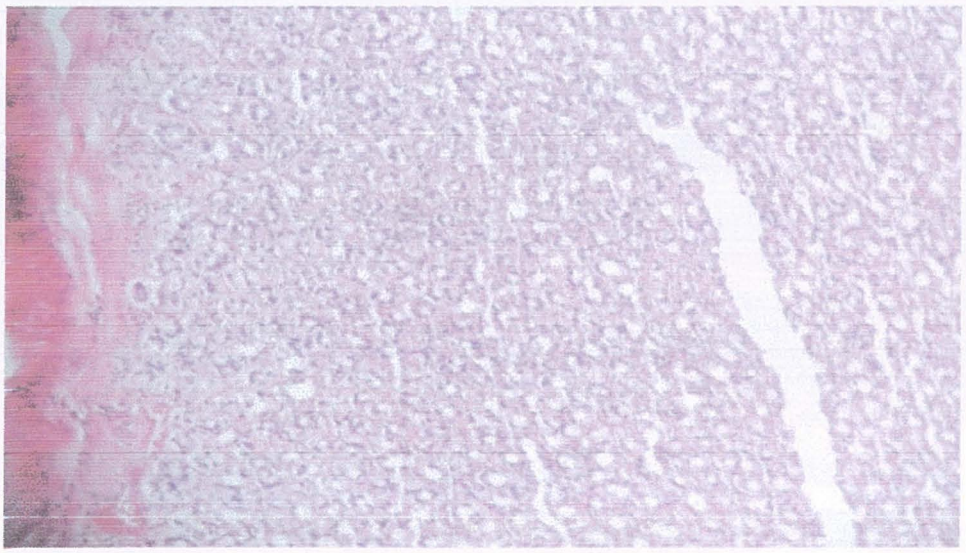


Figure 18: Light micrograph of stomach x 100



Stomach appear normal with no changes versus control

**STOMACH (CONTROL GROUP)**

Figure 19: Light micrograph of stomach x 400

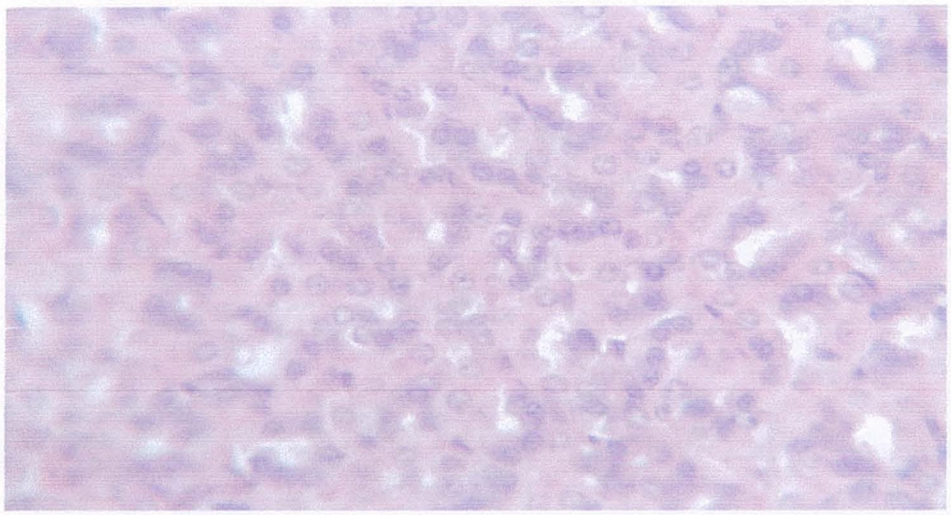
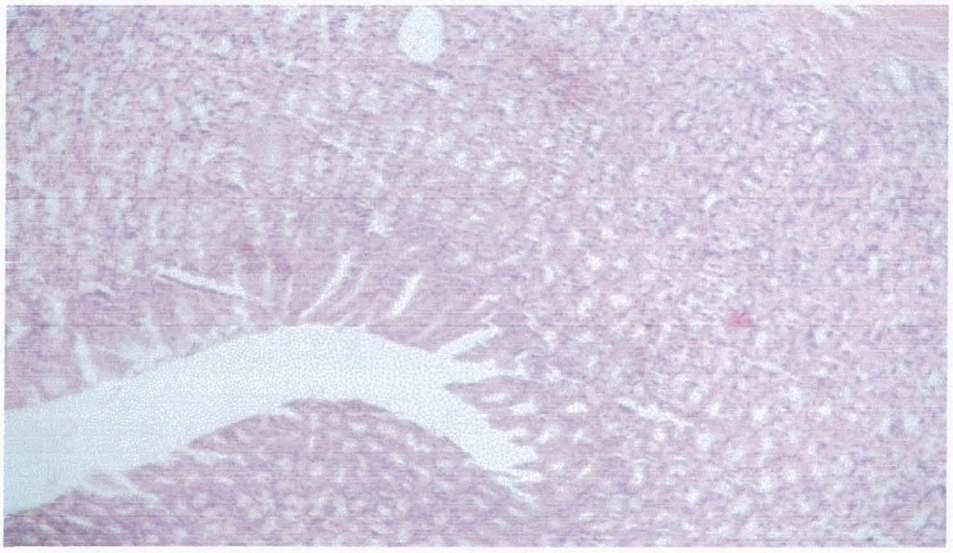


Figure 20: Light micrograph of stomach x 100



# CHAPTER FIVE

## 5.1 DISCUSSION

The safety of drugs and plant products for human use can be determined using toxicological evaluation which is usually carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a safe dose in humans. The acute toxicity studies showed that the aqueous leaf extract of *Ocimum suave* has a high safety profile when given orally with an $LD_{50}$ value greater than 10000mg/kg body weight. Therefore, the medicinal plant in its local formulation can be categorized as relatively non-toxic based on the method proposed by Lorke (Lorke 1983). Analysis of blood parameters is relevant in risk evaluation as changes in the hematological system have higher predictive value for studies (Olson *et al.*, 2000). WBC increased in the 800mg/kg OSE treated group as compared to the control group. The increase in WBC was due to the increase of the dose. From the observed values of WBC, it is clear that an increase in the number of WBC is a normal reaction of rats to foreign substances, which alter their normal physiological process . RBC decreased in all OSE treated groups as compared to the control group .The decreased levels of RBC may be due to the low rate of erythropoiesis occurring in the bone marrow of the rats. Hemoglobin levels were found to be higher in the 800mg/kg OSE treated group as compared to the control group and the increase was due to the increase of the dose. Increase in hemoglobin [polycythemia] mainly occurs as a result of high altitudes and sickle cell disease. Hematocrit percentage was found to be higher in the 800mg/kg OSE treated group as compared to the control group and the increase was due to the increase of the dose. This also resulted from polycythemia. Mean cell volume increased in 800mg/kg OSE treated group which was as a result of dose increment . This could have indicated the possibility of liver damage or disease.

Platelet count was found to be higher in the 800mg/kg OSE treated group as compared to the control group and this indicated platelet aggregates or macroplatelet. Neutrophil percentage increased in the treated groups [400 and 800mg/kg] and this indicated neutrophillia. Neutrophillia occurs as a result of bacterial infections where the neutrophils are involved to fight the infection. Lymphocyte percentage was higher in the 800mg/kg OSE treated group as compared to the control group and this was as a result of dose increment. This increment of lymphocytes indicated lymphocytosis and lymphocytosis occurs mainly when viral infections are present. Monocyte percentage was found to be lower in the treated groups [200 and 800mg/kg] and this indicated monocytopenia . Eosinophil percentage decreased in all OSE treated groups as compared to the control group and this indicated eosinopenia which showed that allergic reactions or parasitic infections were not manifested in the rats. . Basophil percentage increased in all OSE treated groups as compared to the control group. This increment of basophil percentage indicated basophillia . Basophillia occurs mainly when allergic reactions are manifested.

ALP levels were found to be higher in the 800mg/kg OSE treated group as compared to the control group. . Increase in ALP levels by administration of aqueous leaf extract of *Ocimum suave* showed possible cholestasis which occurred at the dose levels tested since a rise in plasma ALP level is usually a characteristic finding in cholestatic liver disease. ). CK levels were found to have increased in the treated groups of rats and this indicated kidney damage. Therefore, the excretory system of animals [rats] exposed to the Aqueous leaf extract of *Ocimum suave* may have been slightly affected. AST levels were higher in the treated group [800mg/kg] as compared to the control group . AST is a marker of skeletal muscles damage (Laterza *et al.*, 2008); Rasekh *et al.*, 2008; Rosalki *et al.*, 2004).

Decreased ALT levels in the treated groups indicated that there was no hepatocellular injury or damage. ALT is a cytoplasmic enzyme found in very high concentration in the liver (Aliyu *et al., 2007*), and indicates hepatocellular damage, while AST is less specific than ALT as an indicator of liver function.

Increased or decreased organ weight has been observed as a sensitive indicator of organ toxicity. The aqueous leaf extract of *Ocimum suave* did not produce any demonstrable toxic effects to the relative organ weights of liver, lungs, kidney, testes and stomach. The 200mg/kg dose decreased the relative organ weights of liver, lungs, kidney, testes and stomach while 800mg/kg dose increased the relative organ weights of liver and kidney.

## 5.2 CONCLUSION

In conclusion, these results provide evidence for the safety profile of the aqueous leaf extract of *Ocimum suave* in the treatment of the various ailments because the aqueous extract did not produce any significant toxic effects in the organs tested and some haematological and biochemical indices in rats but caution needs to be taken on the appropriate dosage as higher concentration could induce renal toxicity. It can also be concluded that:

- Aqueous leaf extract of *Ocimum suave* contains the following secondary metabolites: tannins, phlobatannins, terpenoids, steroids, reducing sugars and cardiac glycosides.

## 5.3 Recommendations

Chronic toxicity of aqueous leaf extract of *ocimum suave* should be done for histological, haematological and biochemical evaluation.

There is a need to study the effect of the extract on female wistar rats so that toxic effects can be established in both sexes.

## 6.0 REFERENCES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# APPENDICES

## 7.1 APPENDIX 1

### 7.1.1 HISTOLOGICAL STUDY

### 7.1.2 PREPARATION BEFORE PROCESSING

The tissues obtained from wistar rats were fixed in 10% formal saline immediately to prevent tissue degradation. They were fixed for 24hours before processing. Chemical fixatives are used to preserve tissue from degradation and to maintain the structure of the cell and of sub-cellular components such as cell organelles (e.g., nucleus, endoplasmic reticulum, mitochondria) (Clark G 1981). They were trimmed using a surgical blade. Each sample/tissue was loaded in an individual cassette with respective label, which was made using indelible pencil. They were labeled from number 1-18.

### 7.1.3 PROCESSING

Tissues were processed in automatic tissue processor/histokinet (Autotechnicum machine). Processing involved three stages: 1. Dehydration- where tissues are passed through different concentrations of isopropyl alcohol in increasing order 80%, 90% and 100%. It took between 18-24 hours. 2. Clearing- where the tissues are treated with xylene. 3. Infiltration/impregnation- where the tissues are put into molten wax at a temperature of 56-60$^0$C. It takes six hours. After dehydration and clearing, the tissue losses its component (water) and become weak.

Impregnation fills the spaces left open during clearing so as to support the tissue. The machine used is automatic and stops after impregnation is complete.

The aim of Tissue Processing is to remove water from tissues and replace with a medium that solidifies to allow thin sections to be cut. Biological tissue must be supported in a hard matrix to allow sufficiently thin sections to be cut. For light microscopy, paraffin wax is most frequently used. Since it is immiscible with water, the main constituent of biological tissue, water must first be removed in the process of dehydration. Samples are transferred through baths of progressively more concentrated ethanol to remove the water. This is followed by a hydrophobic clearing agent (such as xylene) to remove the alcohol, and finally molten paraffin wax, the infiltration agent, which replaces the xylene (Clark G 1981).

### 7.1.4 EMBEDDING

The tissues were embedded using molten paraffin wax, which use pieces of wood or cassettes. After the tissues have been dehydrated, cleared, and infiltrated with the embedding material, they are ready for external embedding. During this process the tissue samples are placed into molds along with liquid embedding material (such as agar, gelatine or wax) which is then hardened. This is achieved by cooling in the case of paraffin wax (Clark G 1981).

### 7.1.5 SECTIONING

After drying, they were transferred to microtome and sectioned to 5microns in width so as to make them thin and easier to reveal what was to be observed. They were then floated out on water bath at $37^0$C in order to spread them and allow recognition of specific tissue. Sections are

put on glass slides and dried in incubator at $37^0$C (GALLENKAM HOTBOX OVEN SIZE 1) overnight. They are ready for staining.

Sectioning can be done in limited ways. Vertical sectioning perpendicular to the surface of the tissue is the usual method. Horizontal sectioning is often done in the evaluation of the hair follicles and pilosebaceous units (Clark G 1981).

### 7.1.6 STAINING TECHNIQUE

Wax in the samples was first removed from the slide by dipping them in staining jars containing xylene, alcohol (isopropyl alcohol) and then water. They were then stained starting from water-isopropyl alcohol-xylene-eosin and then they were mounted using DPX (Dibutylpthalate Plasticizer Xylene).

Biological tissue has little inherent contrast in either the light or electron microscope. Staining is employed to give both contrasts to the tissue as well as highlighting particular features of interest (Clark G 1981).

## 7.2 APPENDIX II

### AVERAGE BODY WEIGHT OF RATS

| DAYS | CONTROL | 10mlS/Kg | 200mg/Kg | 400mg/Kg | 800mg/Kg |
|---|---|---|---|---|---|
| Day 1 | 191.5 | 238.4 | 219.8 | 202.7 | |
| Day 7 | 209.2 | 278.2 | 257.9 | 234.9 | |
| Day 14 | 227.1 | 288.4 | 264.5 | 235.7 | |
| Day 21 | 231.8 | 291.6 | 280.4 | 252.2 | |
| Day 28 | 243.3 | 307.5 | 289.7 | 239 | |
| Day 31 | 248.7 | 307.2 | 289.4 | 258.9 | |

## 7.3 APPENDIX III

*OCIMUM SUAVE [Lamiceae].*