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Evaluation of in vivo toxicity properties of commiphora campestris

Nyunza, Furaha Godfrey

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EVALUATION OF *IN VIVO* TOXICITY PROPERTIES OF *Commiphora campestris*

Furaha Godfrey Nyunza

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Commiphora campestris leaves and stem bark are used by Pare society for management of infectious diseases, therefore this research evaluated *in vivo* toxicity of *C. campestris*. The leaves and stem bark were pulverized, sequentially extracted using chloroform, ethyl acetate and methanol and stored at -20°C. Three groups of mice (n=5/group/sex) were given oral dose of *C. campestris* extracts at 300, 600 and 1200 mg/kg. Clinical changes, body weight, mortality, relative organ weight, hematology and histopathological changes were recorded after 14 days. LD_{50} (424 mg/kg) was calculated by Lorke method. In acute tests mice showed behavioral changes, mortalities in both sexes given leaf methanolic, stem chloroform, stem ethyl acetate and stem methanol extract. Significant increase in hematological parameters ($p>0.05$) for males given leaf chloroform, stem ethyl acetate, stem chloroform extract and decrease in leaf methanolic, stem bark ethyl acetate and stem bark methanolic extract ($p<0.05$). Female mice that were given leaf chloroform and leaf ethyl acetate extract showed significant increase in hematology and decrease to those received leaf methanol, stem chloroform, stem ethyl acetate and stem methanol extract ($p>0.05$). In histopathology, kidneys showed increased glomerural space and distended liver sinusoids. In sub acute toxicity test, rats were given *C. campestris* extracts at doses 150, 200 and 250 mg/kg for 28 days. Body weight, relative organ weight, biochemical, hematological, biochemical and histopathological changes were determined. No mortality was recorded. Biochemical tests showed significant increase ($p<0.05$) in liver enzymes, hematological parameters and histological changes were distended liver sinusoids and kidney glomerural space. This study concludes that in acute toxicity study the extracts caused mortalities while in sub-acute toxicity study no mortalities were recorded and there was reduction in cholesterol levels indicating extracts antilipidemic effect, however more research to validate safety doses in humans is still needed.

DECLARATION

I, Furaha Godfrey Nyunza do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

29/3/2019

Furaha Godfrey Nyunza **Date**

Name and signature of candidate

The above declaration is confirmed

29/03/19

Dr. Musa Chacha **Date**

Name and signature of supervisor

29.03.2019

Dr. Edna Makule **Date**

Name and signature of supervisor

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CERTIFICATION

The undersigned certify that they have read the dissertation titled "Evaluation of *In vivo* Toxicity Properties of *Commiphora campestris*" and recommend for examination in fulfillment of the requirements for the degree of Master of Life Science and Bio-engineering of the Nelson Mandela African Institution of Science and Technology.

29/03/2019

Dr. Musa Chacha **Date**

Name and signature of supervisor

29.03.2019

Dr. Edna Makule **Date**

Name and signature of supervisor

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In first place I praise to Almighty God for giving me life and good health throughout to time I conducted this research. Deep thanks are directed to my parents, Godfrey Marco Nyunza (Late) and my mother Anastasia Nyunza for their uncountable parenthood to me and also keeping me understand God feelings that kept me in right track to this achievement.

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DEDICATION

I humbly dedicate this work to my beloved parents Mr. Godfrey Nyunza and Mrs. Godfrey Nyunza, my family and people of Kisiwani for sharing their knowledge and ability to serve forests that will serve the future.

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LIST OF ABREVIATIONS

DMSO	Dimethyl Sulfoxide
LCE	Leaf Chloroform Extract
LEA	Leaf Ethyl Acetate Extract
LME	Leaf Methanol Extract
NM-AIST	Nelson Mandela African Institution of Science and Technology
SCE	Stem Chloroform Extract
SEAE	Stem Ethyl Acetate Extract
SME	Stem Methanol Extract
TFDA	Tanzania Food and Drugs Authority
WHO	World Health Organization
LD_{50}	Lethal dose 50

CHAPTER ONE

1.0 Introduction

This chapter gives general information on the uses of *Commiphora campestris* in treatment of humaninfectious diseases. The acute and sub acute in vivo toxicity results are discussed.

1.1 Background information

This chapter explains the medicinal use of *C. campestris* in treatment human infections. Medicinal plants prepared using African knowledge has played a significant role in provision of health care services for many years even before the introduction of orthodox conventional medicines in African countries. Traditionally medicinal plants are used as decoction, powder form added in soft drinks or chewed as raw plant. Medicinal plants have been used as source of drug templates for pharmaceuticals development (Katiyar *et al*., 2012). Due to challenges facing the pharmaceutical industry including drug resistance and adverse drug reactions, the demand for plant-based drugs which are referred as African medicines and complementary alternative medicine in Africa and developed countries respectively has increased over sudden (WHO, 2002). The number of herbal products imported in the country has increased in recent years for instance from 2005 to 2014, TFDA registered 34 products (TFDA, 2006). The grown demand for herbal products necessitates validation of its efficacy, safety and quality (WHO, 2002). Godfrey and Coworkers (2016) has established that the latex from the chopped stem bark inhibits the growth of various pathogenic microbes such as *Klebsiella oxytoca Klebsiella pneumoniae Salmonella kisarawe Proteus mirabilis Salmonella typhi, Pseudomonas aeruginosa Escherichia coli*, *Cryptococcus neuformans* and *Candida albicans*. It is therefore likely that *C. campestris* is a potential source of drug template for the management of Gram negative bacteria and fungi. This discovery necessitates toxicological studies on the *C. campestris*.

1.2 Research Background

1.2.1 Research problem

In Tanzania, medicinal plants have been extensively exploited for management of microbial infections by the rural communities (Godfrey *et al*., 2016). This is also a concern with Tanzania Food and Drugs Authority (TFDA), a regulatory organ that is responsible for registration and authorization of the use of herbal medicines in Tanzania. This study therefore evaluates the *in vivo* toxicity of *C. campestris* leaves and stem barks extracts using White

Albino Swiss Mice. Leaves and stem barks of *C. campestris* are traditionally used by Pare communities for management of cough, wounds, diarrhea and other gastrointestinal infections.

1.2.2 Research Justification

This study contributes to WHO and TFDA on the safe use of herbal medicines in the management of diseases. Currently drug resistance is a crisis worldwide amplified by overuse, misuse and lack of new drugs development in pharmaceutical industry (Ventola, 2015). It is in this view that, the research on *C. campestris* paves the way for development of new drug templates.

1.3 Research Objectives

1.3.1 Main objective

To establish *in vivo* toxicity properties of leaves and stem bark exracts of *C. campestris*

1.3.2 Specific Objectives

(i) To evaluate acute toxicity in mice administered with leaves and stem bark extracts of *C. campestris*

(ii) To evaluate sub-acute pathological changes in mice induced with leaves and stem bark extracts of *C. campestris*

1.4 Research questions

(i) What are the toxic effects of leaves and stem bark extracts of *C. campestris* to mice and rats.

(ii) What are the pathological changes in body cells of mice and rats *induced with* leaves and stem bark extracts of *C. campestris*?

1.5 Significance of the Research

Use of traditional medicine has expanded in the world. People consume herbal medicines for their health care. Herbal medicines have resulted to several reported adverse events that cause negative impression on use of these products, this attracted World Health Organisation to call on urgent monitoring of the herbal medicine in the member states by creating analytical laboratories for herbal products (WHO, 2004). Different countries have been allowing the herbal products into the market for use by considering long standing use of the products, no detailed analysis for toxicity is done to get evidence of safety of such products, this pose risk

to users (Trampisch *et al*., 2017). Herbal products are used without efficacy analysis, this is based on long standing use of the products hence no toxilogical studies are conducted and most of the herbal products are also suspected to be carcinogenic with/or hepatotoxicity effects (Moreira *et al.*, 2014). Therefore this study intended to provide scientific evidence of *in vivo* toxicity in animal models to substantiate the risks that the public is exposed to when are using *C. campestris* for treatment of various diseases.

CHAPTER TWO

Acute toxicity of leaves and stem bark extracts of *Commiphora campestris* in White Albino Swiss Mice

Abstract

The genus *Commiphora* is one of the most diverse Genera of the family Burseraceae. *C. campestris* is used by Pare communities for the management of gastrointestinal, skin and wounds infections. This study therefore evaluated the in *vivo* acute toxicity of the *C. campestris* using mice as model. Four groups of mice (n=5/group/sex) were orally treated with doses of 300 mg/kg, 600 mg/kg and 1200 mg/kg and clinical manifestation, body weight, mortality, relative organ weight, hematological and histopathological changes in vital organs were determined and recorded for 14 days. Results showed behavioral changes in mice and resumed to bright status. Mortalities were recorded at a dose of 600 mg/kg and 1200 mg/kg. Males showed no significant hematological changes in leaf chloroform extract, leaf ethyl extract, stem chloroform extract ($p>0.05$) and significant decrease in leaf methanolic extract, stem ethyl acetate extract and stem bark methanolic extract ($p<0.05$). Females that received leaf chloroform extract showed increase in hematological parameters, those which received leaf ethyl acetate extract, leaf methanolic extract, stem chloroform extract, stem ethyl acetate extract and stem bark methanolic extract showed significant decrease in hematological parameters ($p>0.05$). In histopathology kidneys showed increased glomerural space.

Conclusion: High doses of all extracts are toxic to mice causing mortalities, relative organ weight and histopathological changes in vital organs. The most affected organs are liver and kidneys, this attracted sub chronic exposure to identify more toxic effects.

Key words: *Commiphora campestris*, acute toxicity, sub acute toxicity, Dimethyl Sulphoxide (DSMO), Lethal Dose 50 (LD_{50}), Mortality, Hematology, Histopathology

2.0 Introduction

2.1 Background information

The genus *Commiphora* is one of the most diverse Genera of the family Burseraceae. It is largely represented in Africa, where it is confined to arid and semi-arid areas. The plant is native of the Horn and East Africa, the plant grow well in between 1 - 2100 M above sea level (Soromessa, 2013). In Tanzania it flourishes in the slopes of Pare mountains, *Commiphora campestris* is used by Pare communities for the management of diarrhea, cough, wounds and gastrointestinal infections. So far, there is one study that has validated the ethnomedical information pertaining to the use of this plant (Godfrey *et al.,* 2016). It has been found that *C. campestris* had antibacterial effects against *Klebsiela oxytoca, Klebsiela. pneumoniae and Pseudomonas aeroginosa* and antifungal effects against *Candida albicans* and *Cryptococus neuformans* (Godfrey *et al.,* 2016). The plant was further evaluated for cytotoxicity activity against *Artemia species (*brine shrimp larvae) in order to establish the safety use of the herbal products formulated from this species. Extracts with good antimicrobial activity and low cytotoxicity activity against *Artemia species* reported by Godfrey and Co-workers (2016) warrants further toxicological studies using animal models as per World Health Organisation (WHO, 2004). Therefore this chapter reports on the in vivo toxic effects of *C. campestris* in tissues of laboratory animals.

2.2 Materials and Methodology

2.2.1 Plant materials collection and identification.

Commiphora campestris leaves and stems were collected from Pare mountains at Kisiwani Village. Identification of the plant before collection was done by skilled botanist Mr. Emmanuel Mboya from Tropical Pesticides Research Institute (TPRI) and the voucher specimens coded CCEL-21 were deposited at Nelson Mandela African Institution of Science and Technology.

2.2.2 Preparation of plant extracts

Fresh collected plant materials were first washed and dried under the shade at room temperature until completely dried to avoid fungal growth.

Plate 1: *Commiphora campestris* plant

Plate 2: Filtration of *C. campestris* extract prior to evaporation

Dried leaves and stem were pulverized using Swinging Traditional Chinese Medicine Pulverizer, Diaxiang Electronic Equipment (DXF-20D) machine, followed by sequential extraction in increasing polarity using chloroform, ethyl acetate and methanol. Leaves and stem bark weighing 500 g were soaked in a 1 litre flat bottomed flask for 72 hours then sieved and filtered using Whatman filter size 40 before solvent removal by evaporation in Heidolph Rota Evaporator set at pressure 875 mbar, temperature 42 $^{\circ}$C and rotation of 113 rpm. The yield per 500 g plant material was leaf in chloroform 20 g, ethyl acetate 15 g and methanol 35 g for stem in chloroform 11.27 g, ethyl acetate 14.58 g and methanol 77 g. Solvents were evaporated using Heidolph Rota Evaporator, German and dried crude extracts were stored at -20°C until further use.

2.2.3 Grouping of mice

The sample size of the mice was calculated using the Resource Equation Method for Determining Sample Size (Law of Diminishing Returns), where the total number of animals per group was calculated as response of value E. Experiment was conducted on 170 healthy White Albino Swiss mice weighing 25 to 35 g aged 8 to 10 weeks bred at NM-AIST laboratory were used for testing 6 plant extracts. Three dose groups per extract and one control group were used, where each dose group used 5 mice (n=5/group/sex). All groups were marked depending on dose they receive, those received 300 mg/kg, 600 mg/kg and 1200 mg/kg were marked with one mark, two marks and three marks on tail respectively using green, red and black ink depending on extract they were given and control group was not marked and were administered with Di-Methyl Sulfoxide (DMSO) which was used as solvent for dissolving plant extracts prior to administration. The protocol and ethical handling of animals was approved by National Institute Medical Research (NIMR). Male and female (non pregnant) animals were used in this research. The experimental procedures developed by Organization for Economic Co-operation and Development (OECD) version 423 (2001) were adopted (OECD, 2001) with some modifications.

2.2.4 Caging and feeding

Animals were kept in a meshed cage size 10 cm x 10 cm x 10 cm (1000 cm^3) for 5 mice, covered with sawdust beddings at room temperature 25°C - 30°C. Same sex mice were put together during experiment to avoid physiological interaction like mating and different litter mice were not mixed to avoid fighting. Light was set such that there is 12 hour light supply and 12 hour darkness for a 24 hour period. Mice were fed with chicken growers mash throughout the study period. Each cage was identified with a card showing extract given, number of animals in a group, weight of each animal, route of administration and dose level.

Plate 3: A cage with mice during experiment.

2.2.5 Dose (in volume) calculation and extract administration

The *C. campestris* extract samples were weighed using sensitive Citizen Scale CY 204 and dissolved in 10% Dimethyl Sulfoxide (DMSO) in distilled water to make a stock administration solution.

Plate 4: Administration of mice with *C. campestris* extracts

Plate 5: Preparations of *C. campestris* extracts

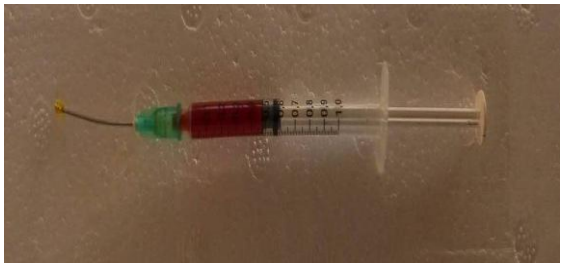


Plate 6: Administration gavage for *C. campestris* extracts

The mice were administered per oral route as a single dose using stomach gavage. The dose volume (ml) was calculated depending on the weight of the animal (kg), pre determined concentration of extract (mg/ml) and dose (mg/kg Bwt), the volume to be administered to each mouse was calibrated not to exceed 10 ml/kg. The dose in volume was calculated as:

$$\text{Dose in volume (ml)} = \frac{\text{dose (mg/kg) x body weight (kg)}}{\text{Concentration (mg/ml)}}$$

After oral administration animals were fasted for 3 hours but water was given ad-libitum. Following fasting, animals were measured their weight, dose volume calculated and extract was administered. After administration of the extract, food was withheld for at least 2 hours.

2.2.6 Physical observation time

Animals administered with the plant extract were intensively monitored and observed for behavioral changes in the first 30 minutes up to 4 hours. After the first observation, clinical manifestation of ill health or behavioral changes were recorded daily for a period of 14 days

2.2.7 Body weight and relative organ weight (row)

Body weight of each animal and their differences were recorded on start (day 0), mid (Day 7) and at the end of experiment (day14) and after sacrifice of the mice vital organs were weighed using Citizen CY 204 sensitive balance scale.

2.2.8 Hematology and histopathology

In hematology, blood samples were drawn from the test animal on day 14 and were analyzed for different hematological blood parameters using *NS4s* automated analyzer (Germany), The hematological parameters analyzed included: Packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count (Lymphocytes and Monocytes), and hemoglobin concentration [Hb] concentration. Others were; Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT), red cell distribution width (RDW) and recorded as mean ± standard error. In histopathology, mice subjected for acute toxicity were sacrificed, vital organ tissues were fixed in 10% formalin then embedded in the paraffin wax and stained by hematoxilin and eosin (H&E). The tissue slides were examined under light microscope 40x magnification and micrograph taken.

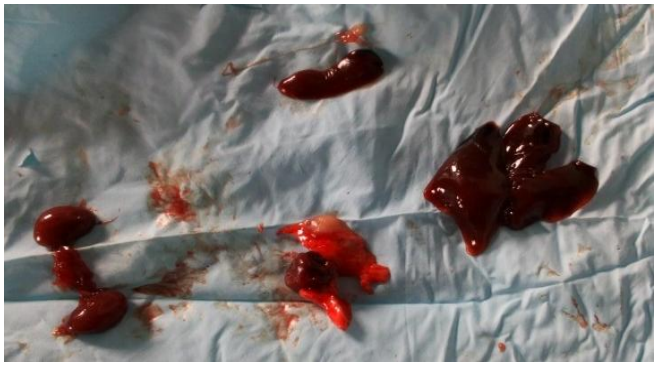


Plate 7: Vital organs (liver, lung, heart, kidney and spleen) taken from mice for weighing

2.3 Results

2.3.1 Physical observation time

After administration of the extract all animals in all groups showed signs of rough hair coat, breathing difficulty, sleep and closure of the eyelid and huddling in the first thirty minutes then all animals resumed normal condition and continued to eat and drink. Mortalities were recorded as seen in Table 1.

Table 1: Mortality in acute toxicity after administration of extracts.

Extract	Sex	Dose (mg/kg)	Number of Death
Leaf Chloroform	Male and females	300-1200	No mortality
Leaf Chloroform	Male and Female	5000	Survived the dose
Leaf Ethyl Acetate	Male and female	300-1200	No mortality
Leaf Ethyl Acetate	Male and Female	5000	Killed a mice immediately
Leaf Methanol	Male	600	1
	Male	1200	1
	Females	300-1200	No mortality
Stem Chloroform	Males	1200	5
	Females	300-1200	No mortality
Stem Ethyl Acetate	Males	1200	1
	Females	300-1200	No mortality
Stem Methanol	Males		5
	Females		2
	Females		3

For the extracts that did not produce mortality, mice were administered with a 5000 mg/kg to establish extracts toxicity at 5000 mg/kg which is the highest recommended dose for determination of extract safety (OECD,2001). The results are as indicated in Table 2.

Male mortalities were recorded in the first day at a dose of 1200 mg/kg where all five mice died. After a week three mice died at a dose of 600 mg/kg. No mortality at 300 mg/kg, the remaining mice resumed to normal until sacrificed. The highest dose that didn't kill the mice was 300 mg/kg and the minimum dose that killed mice was 600 mg/kg. The Lethal Dose Fifty (LD_{50}) was 424 mg/kg. The method of Lorke (Lorke 1983) was adopted for calculating the lethal dose fifty using the formular below:

$$\mathbf{LD_{50}=\sqrt{D_{hl}D_{lm}}}$$

Where: $\boldsymbol{D_{hl}}$ is the highest dose without mortality

$\boldsymbol{D_{lm}}$ is the lowest dose with mortality

$\boldsymbol{LD_{50}}$ is the Lethal Dose 50

2.3.2 Body weight (Bwt) changes

Body weights of tested rats were measured in grammes (g) using the sensitive balance and recorded. Male mice that received leaf chloroform extract their body weight significantly increased in all three doses ($p < 0.05$) except on day 14 there was no statistically significant change in weight ($p>0.05$) as compared to males control group, however the weight shows to be increased. In females, there was significant increase in mean body weights of mice ($p<0.05$) as compared to controls. Male mice that received leaf ethyl acetate extract showed increase in body weight ($p<0.05$) compared to controls, however females showed no significant statistical changes in body weights ($p<0.05$) as compared to control, however figures in female weights shows increase. Male mice that were given leaf methanol extract shows significant increase of body weight ($P>0.05$). Females shows that there is significant increase in body weight ($p>0.05$).

Male and female mice that received stem chloroform extract showed increase in body weight ($p<0.05$). All male mice that received stem chloroform extract at 1200 mg/kg died. Male and female mice that received stem ethyl acetate extract increased in body weight ($p< 0.05$). Male mice that received stem bark methanolic extract showed increase in body weight ($p< 0.05$) at 300 – 600 mg/kg, but all mice that received 1200 mg/kg died. Females showed significant increase in body weight ($p<0.05$) as indicated in Table 2.

Table 2: Body weight (g) of male and female mice during acute toxicity study after administration of leaf chloroform extract

			Dose (mg/kg Bwt)				
Extract	**Sex**	**Parameter**	**control**	**300**	**600**	**1200**	**P – values**
LCE	Male	Bwt Day 0	19.4±0.24	23.2±1.16	23.2±1.16	26±0.45	0.046
		Bwt Day 7	22.2±0.49	24.8±0.92	24±0.89	28±0.55	0.000
		Bwt Day 14	27.8±0.49	29±1.34	30±0.45	30.8±1.56	0.269
	Females	Bwt Day 0	19.4±0.24	23.52±0.21	17.64±0.16	17.44±0.17	0.001
		Bwt Day 7	22.2±0.49	26.45±0.24	19.6±0.18	20.6±1.11	0.000
		Bwt Day 14	27.8±0.49	29.39±0.27	23.52±0.21	24.32±0.94	0.002
LEA	Males	Bwt Day 0	19.4±0.24	24.6±0.25	20.8±0.92	21.2±1.16	0.019
		Bwt Day 7	22.2±0.49	23±1	23.4±0.6	19.8±0.8	0.016
		Bwt Day 14	27.8±0.49	27.8±0.49	31±0.89	29.4±1.08	0.000
	Female	Bwt Day 0	19.4±0.24	16.66±0.15	24.5±0.22	17.64±0.16	0.001
		Bwt Day 7	22.2±0.49	19.6±0.18	24.5±0.22	19.6±0.18	0.073
		Bwt Day 14	27.8±0.49	23.52±0.21	27.43±0.25	25.47±0.23	0.057
LME	Males	Bwt Day 0	19.4±0.24	28.41±0.26	46.4±0.26	29.62±0.17	0.001
		Bwt Day 7	22.2±0.49	24.5±0.22	27.64±0.16	36.53±0.21	0.001
		Bwt Day 14	27.8±0.49	31.35±0.28	35.54±0.2	35.54±0.2	0.002
	Females	Bwt Day 0	19.4±0.24	28.41±0.26	46.4±0.26	29.62±0.17	0.001
		Bwt Day 7	22.2±0.49	24.5±0.22	27.64±0.16	35.54±0.2	0.001
		Bwt Day 14	27.8±0.49	31.35±0.28	35.54±0.2	36.53±0.21	0.000
SCE	Male	Bwt Day 0	19.4±0.24	22.4±1.12	24.8±0.92	-	0.003
		Bwt Day 7	22.2±0.49	24.6±1.4	22.8±1.2	-	0.312
		Bwt Day 14	27.8±0.49	30.2±0.97	28.6±2.09	-	0.468
	Females	Bwt Day 0	19.4±0.24	23.52±0.21	14.7±0.13	16.66±0.15	0.001
		Bwt Day 7	22.2±0.49	26.45±0.24	18.62±0.17	19.6±0.18	0.001
		Bwt Day 14	27.8±0.49	30.37±0.28	23.52±0.21	23.52±0.21	0.001

Extract	Sex	Parameter	Dose (mg/kg Bwt)				P – values
			control	300	600	1200	
SEA	Males	Bwt Day 0	19.4±0.24	16.66±0.15	25.47±0.23	29.62±0.17	0.001
		Bwt Day 7	22.2±0.49	21.6±0.21	29.39±0.27	29.62±0.17	0.003
		Bwt Day 14	27.8±0.49	26.45±0.24	33.31±0.3	34.55±0.2	0.001
	Female	Bwt Day 0	19.4±0.24	17.64±0.16	17.64±0.16	21.56±0.2	0.002
		Bwt Day 7	22.2±0.49	19.6±0.18	20.58±0.19	25.47±0.23	0.001
		Bwt Day 14	27.8±0.49	25.47±0.23	23.52±0.21	30.37±0.28	0.000
SME	Males	Bwt Day 0	19.4±0.24	24.5±0.22	26.66±0.15	-	1.000
		BwtDay 7	22.2±0.49	25.47±0.23	33.57±0.19	-	1.000
		Bwt Day 14	27.8±0.49	29.39±0.27	36.53±0.21	-	1.000
	Females	Bwt Day 0	19.4±0.24	11.76±0.11	14.9±0.04	24.88±0.08	0.000
		Bwt Day 7	22.2±0.49	16.66±0.15	21.85±0.06	29.85±0.09	0.010
		Bwt Day 14	27.8±0.49	22.54±0.2	24.83±0.07	31.84±0.1	0.000

Values are expressed as mean ± sem, Bwt = Body weight, LCE = leaf chloroform extract, LEA = leaf ethyl acetate extract, LME = leaf methanolic extract, SCE = stem bark chloroform extract, SEA = stem ethyl acetate extract, SME = stem bark methanolic extract.

2.3.3 Relative organ weights (row) changes

The relative organ weight of each mouse was measured using sensitive weighing balance and record taken in grammes (g). Male mice that received leaf chloroform extract showed there is no significant change in weight of the liver and heart ($p>0.05$) with significant decrease in weight of lung and spleen ($p<0.05$) and significant increase of kidney. Females showed significant decrease in weight in liver and spleen ($p<0.05$), kidney and lung showed significant increase in weight ($p>0.05$). Heart showed no significant change in weight ($p>0.05$).

Male mice that received leaf ethyl acetate extract showed that there is significant decrease in size of liver, kidney, lung (except at 300 mg/kg there is increase in weight might be caused by individual group), significant increase in heart weight ($p<0.05$) and significant decrease in liver size ($p<0.05$) and for lungs the there is abnormal deviation at 600 mg/kg (weight was 0.4 g) this might have been contributed by individual weakness of the mice.

Female mice showed there is significant increase in size of kidney (at 1200 mg/kg), spleen, heart ($p<0.05$) where as for liver there is significant decrease in size ($p<0.05$) and for lungs the there is abnormal deviation at 600 mg/kg (weight was 0.4 g) although data at this might have been contributed by individual weakness of the mice.

Male mice given leaf methanol extract showed no significant change in size of the heart ($P>0.05$) and significant decrease in size of liver, kidneys, lung and spleen ($P<0.05$). Females showed that there is significant no significant change in heart size ($p>0.05$), there is significant decrease in weight of liver, kidney, lung and significant increase of the spleen ($p<0.05$).

Male Mice that were administered with stem chloroform extract showed significant increase in weight of liver ($p<0.05$), for spleen there is no significant increase in size ($p>0.05$) although data showed an obvious increase in size of the organ. There is significant decrease in weight of the kidney at (300 mg/kg) and for heart, lung ($p>0.05$) no significant decrease of the organs but data showed an obvious decrease in weight. Females showed there is no significant change in heart weight ($p>0.05$), with significant decrease in liver and kidneys ($p<0.05$). There is increase in weight of spleen at (1200 mg/kg) and of lung ($p<0.05$).

Male mice administered with stem ethyl acetate extract showed significant decrease in weight of the liver ($p<0.05$), statistical significant increase in weight of the kidney spleen, heart, lung (except at 600 mg/kg for lungs) ($p<0.05$). Female mice showed there is significant decrease

in weight of the liver ($p<0.05$), significant increase in weight of the kidney spleen, heart, lung (except at 600 mg/kg for lungs) ($p<0.05$).

Male mice that were administered with stem bark methanolic extract showed decrease in weight of the liver, kidney, heart ($p<0.05$) and significant increase in weight of lungs and spleen ($p<0.05$). Females showed significant decrease in weight of the liver, kidney, heart, lung ($p<0.05$) and significant increase of the spleen at 1200 mg/kg. The results are summaried in Table 3.

Table 3: Changes in row (g) of males mice vital organs during acute toxicity study after administration of leaf chloroform extract

			Dose (mg/kg Bwt)				
	Sex	**Organ**	**Control**	**300**	**600**	**1200**	**P – values**
LCE	Males	Liver	1.45±0.07	1.36±0.08	1.39±0.04	1.43±0.06	0.741
		Kidney	0.24±0.02	0.25±0.01	0.27±0.03	0.28±0.01	0.0046
		Heart	0.08±0.01	0.07±0.01	0.07±0.00	0.08±0.01	0.563
		Lung	0.17±0.00	0.10±0.00	0.08±0.01	0.12±0.00	0.000
		Spleen	0.07±0.01	0.06±0.00	0.02±0.00	0.04±0.01	0.0036
	Females	Liver	1.56±0.07	1.23±0.04	0.86±0.03	1.1±0.03	0.000
		Kidney	0.27±0.01	0.34±0.01	0.28±0.01	0.27±0.01	0.00002
		Heart	0.08±0.01	0.07±0.01	0.07±0.00	0.08±0.01	0.5899
		Lung	0.07±0.01	0.12±0.00	0.07±0.00	0.01±0.00	0.011
		Spleen	0.17±0.00	0.35±0.01	0.21±0.01	0.24±0.01	0.011
LEA	Males	Liver	1.45±0.07	1.32±0.05	1.37±0.02	1.96±0.07	0.0074
		Kidney	0.24±0.02	0.26±0.02	0.21±0.02	0.16±0.01	0.0055
		Heart	0.08±0.01	0.15±0.01	0.11±0.00	0.11±0.01	0.0095
		Lung	0.17±0.00	0.20±0.00	0.13±0.02	0.14±0.01	0.0074
		Spleen	0.07±0.01	0.14±0.01	0.11±0.00	0.16±0.01	0.000057
	Females	Liver	1.56±0.07	1.03±0.03	1.09±0.03	1.05±0.03	0.066
		Kidney	0.27±0.01	0.21±0.01	0.26±0.01	0.31±0.01	0.00002
		Heart	0.08±0.01	0.15±0.01	0.11±0.00	0.11±0.01	0.0095
		Lung	0.07±0.01	0.07±0.00	0.15±0.00	0.08±0.00	0.004
		Spleen	0.17±0.00	0.17±0.01	0.40±0.01	0.15±0.00	0.0026

			Dose (mg/kg Bwt)				
	Sex	**Organ**	**Control**	**300**	**600**	**1200**	**P – values**
LME	Males	Liver	1.45±0.07	1.32±0.04	1.41±0.04	1.41±0.04	0.382
		Kidney	0.24±0.02	0.35±0.01	0.39±0.01	0.40±0.01	0.004
		Heart	0.08±0.01	0.09±0.00	0.01±0.00	0.08±0.00	0.059
		Lung	0.17±0.00	0.37±0.01	0.21±0.01	0.35±0.01	0.004
		Spleen	0.07±0.01	0.20±0.01	0.41±0.01	0.11±0.00	0.001
	Females	Liver	1.56±0.07	0.98±0.03	1.43±0.04	1.22±0.04	0.000
		Kidney	0.27±0.01	0.24±0.01	0.29±0.01	0.20±0.01	0.000
		Heart	0.08±0.01	0.09±0.00	0.09±0.00	0.07±0.00	0.059
		Lung	0.07±0.01	0.23±0.01	0.07±0.00	0.30±0.01	0.011
		Spleen	0.17±0.00	0.11±0.00	0.16±0.00	0.17±0.01	0.041
SCE	Males	Liver	1.45±0.073	1.77±0.070	1.20±0.13	-	0.004
		Kidney	0.24±0.018	0.13±0.008	0.24±0.04	-	0.017
		Heart	0.08±0.006	0.07±0.006	0.06±0.008	-	0.149
		Lung	0.17±0.004	0.12±0.021	0.14±0.02	-	0.183
		Spleen	0.07±0.006	0.09±0.002	0.10±0.016	-	0.129
	Females	Liver	1.56±0.07	0.98±0.03	1.43±0.04	1.22±0.04	0.000
		Kidney	0.27±0.01	0.24±0.01	0.29±0.01	0.20±0.01	0.000
		Heart	0.08±0.01	0.09±0.00	0.09±0.00	0.07±0.00	0.059
		Lung	0.07±0.01	0.23±0.01	0.07±0.00	0.30±0.01	0.011
		Spleen	0.17±0.00	0.11±0.00	0.16±0.00	0.17±0.01	0.041

			Dose (mg/kg Bwt)				
	Sex	**Organ**	**Control**	**300**	**600**	**1200**	**P – values**
SEA	Males	Liver	1.45±0.07	1.34±0.04	1.58±0.05	1.44±0.04	0.044
		Kidney	0.24±0.02	0.57±0.02	0.40±0.01	0.45±0.01	0.000
		Heart	0.08±0.01	0.09±0.00	0.11±0.00	0.09±0.00	0.000
		Lung	0.17±0.00	0.19±0.01	0.11±0.00	0.35±0.01	0.001
		Spleen	0.07±0.01	0.11±0.00	0.23±0.01	0.1±0.00	0.001
	Females	Liver	1.56±0.07	1.27±0.04	0.64±0.02	1.22±0.04	0.001
		Kidney	0.27±0.01	0.29±0.01	0.15±0.00	0.28±0.01	0.007
		Heart	0.08±0.01	0.09±0.00	0.11±0.00	0.08±0.00	0.000
		Lung	0.07±0.01	0.12±0.00	0.10±0.00	0.08±0.00	0.000
		Spleen	0.17±0.00	0.24±0.01	0.13±0.00	0.27±0.01	0.001
SME	Males	Liver	1.45±0.07	0.86±0.03	0.70±0.02	-	0.002
		Kidney	0.24±0.02	0.20±0.01	0.22±0.01	-	0.265
		Heart	0.08±0.01	0.06±0.00	0.06±0.00	-	0.006
		Lung	0.17±0.00	0.06±0.00	0.14±0.00	-	0.002
		Spleen	0.07±0.01	0.11±0.00	0.12±0.00	-	0.000
	Females	Liver	1.56±0.07	1.56±0.07	0.89±0.03	0.70±0.02	0.005
		Kidney	0.27±0.01	0.27±0.01	0.21±0.01	0.24±0.01	0.021
		Heart	0.08±0.01	0.08±0.01	0.06±0.00	0.06±0.00	0.014
		Lung	0.07±0.01	0.07±0.01	0.07±0.00	0.14±0.00	0.009
		Spleen	0.17±0.00	0.17±0.00	0.12±0.00	0.11±0.00	0.020

Values are expressed as Mean ± Sem, Bwt = Body Weight, LCE = leaf chloroform extract, LEA = leaf ethyl acetate extract, LME = leaf methanolic extract, SCE = stem bark chloroform extract, SEA = stem ethyl acetate extract, SME = stem bark methanolic extract.

2.3.4 Hematology

In hematology blood samples were drawn from the test mice on day 14 and were analyzed for different hematological blood parameters using *NS4s* automated analyzer (Germany), the hematological parameters analyzed included: PCV, RBC count, WBC count and [Hb]. Others were; MCV, MCH, MCHC, HCT and RDW. In male mice that received leaf chloroform extract shows that there is no significant changes in all hematology parameters ($p>0.05$), while females shows significant changes ($p<0.05$) in hematological parameters as compared to control as indicated in Table 4.

Table 4: Hematological parameters of males and female mice during acute toxicity study after administration of leaf chloroform extract

		Dose mg/kgBwt				
Sex	**Parameter**	**Control**	**300**	**600**	**1200**	**P – value**
Males	WBC (m/mm3)	19.62±3.04	27.4±6.15	26.89±0.55	22.02±0.65	0.369
	Lymph (#)	16.38±4.07	23.8±4.88	19.36±0.39	18.43±0.97	0.463
	Mon (#)	1.91±0.51	2.08±1.95	4.27±0.4	2.33±0.95	0.467
	RBC (m/mm3)	7.44±0.34	7.13±1.13	5.51±1	6.52±0.77	0.452
	MCV (fl)	51.93±0.52	57.17±2.44	54.13±0.84	57.9±2.76	0.050
	HCT (%)	38.53±1.4	41.07±7.49	29.7±5.05	37.6±4.33	0.464
	MCH (pg)	22.23±0.72	22.43±2.71	36.77±10.42	22.27±1.45	0.424
	MCHC (g/dl)	42.93±1.07	39.73±6.34	67±17.66	38.87±4.05	0.347
	RDW	12.17±0.43	13.33±0.91	11.97±0.48	12.33±0.54	0.459
	[Hb] (g/dl)	16.53±0.32	15.4±0.86	18.63±3.19	14.4±1.22	0.392
Females	WBC (m/mm3)	19.62±3.04	34.7±0.06	25.83±0.05	22.81±0.04	0.016
	Lymph (#)	16.38±4.07	24.19±0.09	19.94±0.07	17.88±0.06	0.045
	Mon (#)	1.91±0.51	5.95±0.02	3.66±0.01	3.21±0.01	0.016
	RBC (m/mm3)	7.36±0.01	8.61±0.02	4.42±0.01	8.04±0.01	0.003
	MCV (fl)	51.56±0.09	56.55±0.1	55.65±0.1	56.75±0.1	0.016
	HCT (%)	38.02±0.07	48.77±0.09	24.63±0.04	45.68±0.08	0.022
	MCH (pg)	22.69±0.04	19.05±0.03	56.25±0.1	20.25±0.04	0.016
	MCHC (g/dl)	43.99±0.08	33.61±0.06	99.63±0.18	35.7±0.06	0.016
	RDW	12.14±0.02	13.66±0.02	10.97±0.02	13.36±0.02	0.016
	[Hb] (g/dl)	16.82±0.03	16.46±0.03	24.93±0.04	16.36±0.03	0.016

Values are expressed as Mean ± Sem, Bwt = Body Weight, WBC = White blood cells, Lymph = Lymphocytes, Mon = Monocytes, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, HCT = Hematocrit, MCH = Mean Cell Hemoglobin, MCHC = Mean Cell Hemoglobin Concetration, RDW = Red Cell Distribution Width, [HB] = Hemoglobin concentration, # = Number of cells.

Male mice that received leaf ethyl acetate extract shows no significant changes in WBC counts and RBC Counts (P<0.05), but there is decreased [HB] (P<0.05) and no significant change in other RBC indices such as MCV, HCT, MCH and RDW (P>0.05). In females there is significant decrease in WBC counts, RBC counts, MCV, HCT and other RBC indices (P<0.05), significant increase in MCH, MCHC, RDW, (P<0.05) and significant decrease in [HB] (P<0.05) as indicated in Table 5.

Table 5: Hematological parameters of male mice during acute toxicity study after administration of leaf ethyl acetate extract.

		Dose in mg/kg Bwt				
Sex	**Parameter**	**Control**	**300**	**600**	**1200**	**P-value**
Males	WBC (m/mm3)	19.62±3.04	14.57±4.87	8.8±2.04	26.79±4.52	0.053
	Lymph (#)	16.38±4.07	13.39±3.96	8.71±2.05	24.6±3.49	0.063
	Mon (#)	1.91±0.51	0.79±0.63	0.08±0.07	1.59±0.74	0.084
	RBC (m/mm3)	7.44±0.34	6.52±0.23	6.97±0.27	7.23±0.25	0.191
	MCV (fl)	51.93±0.52	51.97±0.86	52.23±1.95	54.27±1.37	0.552
	HCT (%)	38.53±1.4	33.83±0.67	36.23±0.37	39.2±1.9	0.056
	MCH (pg)	22.23±0.72	21.5±0.96	21.17±0.99	20.6±0.66	0.605
	MCHC (g/dl)	42.93±1.07	41.37±1.16	40.63±0.98	38.03±0.24	0.035
	RDW	12.17±0.43	13.7±1.05	12.83±0.62	13.13±0.47	0.500
	[Hb] (g/dl)	16.53±0.32	14±0.12	14.73±0.20	14.93±0.8	0.022
Females	WBC (m/mm3)	19.62±3.04	6.55±0.01	8.24±0.01	9.49±0.02	0.016
	Lymph (#)	16.38±4.07	6.52±0.02	8.13±0.03	9.35±0.03	0.237
	Mon (#)	1.91±0.51	0.01±0	0.08±0	0.11±0	0.016
	RBC (m/mm3)	7.36±0.01	1.19±0	1.63±0	1.29±0	0.016
	MCV (fl)	51.56±0.09	46.08±0.08	46.48±0.08	45.28±0.08	0.016
	HCT (%)	38.02±0.07	5.39±0.01	7.48±0.01	5.88±0.01	0.016
	MCH (pg)	22.69±0.04	40.99±0.07	37.3±0.07	45.98±0.08	0.016
	MCHC (g/dl)	43.99±0.08	90.46±0.16	81.08±0.14	99.63±0.18	0.016
	RDW	12.14±0.02	15.16±0.03	13.66±0.02	13.66±0.02	0.024
	[Hb] (g/dl)	16.82±0.03	4.89±0.01	6.08±0.01	5.98±0.01	0.016

Values are expressed as Mean ± Sem, Bwt = Body weight, WBC = White blood cells, Lymph = Lymphocytes, Mon = Monocytes, RBC = Red blood cell, MCV = Mean corpuscular volume, HCT = Hematocrit, MCH = Mean cell hemoglobin, MCHC = Mean cell hemoglobin concetration, RDW = Red cell distribution width, [HB] = Hemoglobin concentration, # = Number of cells.

The results of male mice that received leaf methanolic extract shows significant decrease in WBC Counts, RBC Counts and RBC indices (P>0.05). Female mice that received LME shows significant decrease in WBC Counts, RBC Counts and RBC indices (P>0.05) as summarized I Table 6.

Table 6: Hematological parameters of male and female mice during acute toxicity study after administration of leaf methanolic extract.

		Dose in mg/kg Bwt				
Sex	**Parameter**	**Control**	**300**	**600**	**1200**	**P – value**
Males	WBC (m/mm3)	19.62±3.04	6.85±0.01	8.45±0.01	9.35±0.02	0.016
	Lymph (#)	16.38±4.07	6.74±0.02	8.32±0.03	9.2±0.03	0.024
	Mon (#)	1.91±0.51	0.08±0	0.1±0	0.11±0	0.016
	RBC (m/mm3)	7.44±0.34	1.94±0	1.59±0	1.21±0	0.016
	MCV (fl)	51.93±0.52	50.27±0.09	49.77±0.09	44.68±0.08	0.016
	HCT (%)	38.53±1.4	7.98±0.01	7.88±0.01	5.39±0.01	0.016
	MCH (pg)	22.23±0.72	28.82±0.05	23.14±0.04	43.68±0.08	0.019
	MCHC (g/dl)	42.93±1.07	57.35±0.1	46.68±0.08	97.84±0.17	0.016
	RDW	12.17±0.43	17.55±0.03	19.55±0.03	15.06±0.03	0.016
	[Hb] (g/dl)	16.53±0.32	4.59±0.01	3.69±0.01	5.29±0.01	0.016
Females	WBC (m/mm3)	19.62±3.04	5.56±0.01	6.24±0.01	9.84±0.02	0.016
	Lymph (#)	16.38±4.07	5.53±0.02	6.16±0.02	9.69±0.03	0.024
	Mon (#)	1.91±0.51	0.01±0	0.06±0	0.12±0	0.016
	RBC (m/mm3)	7.36±0.01	1.93±0	1.59±0	0.8±0	0.016
	MCV (fl)	51.56±0.09	49.96±0.09	49.77±0.09	45.98±0.08	0.022
	HCT (%)	38.02±0.07	7.93±0.01	7.88±0.01	3.59±0.01	0.016
	MCH (pg)	22.69±0.04	28.65±0.05	23.14±0.04	52.36±0.09	0.016
	MCHC (g/dl)	43.99±0.08	57±0.1	46.68±0.08	99.63±0.18	0.016
	RDW	12.14±0.02	17.45±0.03	19.55±0.03	13.46±0.02	0.016
	[Hb] (g/dl)	16.82±0.03	4.56±0.01	3.69±0.01	4.19±0.01	0.016

Values are expressed as Mean ± Sem, Bwt = Body Weight, WBC = White blood cells, Lymph = Lymphocytes, Mon = Monocytes, RBC = Red blood cell, MCV = Mean corpuscular volume, HCT = Hematocrit, MCH = Mean cell hemoglobin, MCHC = Mean cell hemoglobin concetration, RDW = Red cell distribution width, [HB] = Hemoglobin concentration, # = number of cells.

In males mice that received stem chloroform extract no significant change in WBC counts, RBC and RBC indices ($P>0.05$) and decrease in [HB] concentration ($P<0.05$). In females the results shows there is no significant change in WBC counts and significant decrease in RBC and RBC indices, [HB] concentration ($P<0.05$) as indicated in Table 7.

Table 7: Hematological parameters of male mice during acute toxicity study after administration of stem chloroform extract.

		Dose in mg/kg Bwt				
Sex	**Parameter**	**Control**	**300**	**600**	**1200**	**P – value**
Males	WBC (m/mm3)	19.62±3.04	19.56±0.48	15.29±0.55	-	0.000
	Lymph (#)	16.38±4.07	18.01±0.93	14.54±0.52	-	0.624
	Mon (#)	1.91±0.51	1.2±0.51	0.61±0.25	-	0.192
	RBC (m/mm3)	7.44±0.34	7.8±0.17	7.13±0.14	-	0.211
	MCV (fl)	51.93±0.52	57.1±1.12	54.23±0.57	-	0.010
	HCT (%)	38.53±1.4	44.53±1.39	38.6±0.36	-	0.016
	MCH (pg)	22.23±0.72	21.27±0.83	19.6±0.67	-	0.113
	MCHC (g/dl)	42.93±1.07	37.3±0.85	36.27±1.54	-	0.015
	RDW	12.17±0.43	12.13±0.52	13.1±0.12	-	0.229
	[Hb] (g/dl)	16.53±0.32	16.63±0.66	14.03±0.71	-	0.035
Females	WBC (m/mm3)	19.62±3.04	25.16±0.04	20.2±0.04	19.32±0.03	0.080
	Lymph (#)	16.38±4.07	24.87±0.09	17.95±0.06	18.11±0.06	0.066
	Mon (#)	1.91±0.51	0.20±0	1.59±0.01	0.96±0	0.007
	RBC (m/mm3)	7.36±0.01	7.80±0.01	1.29±0	0.64±0	0.016
	MCV (fl)	51.56±0.09	54.42±0.1	45.21±0.08	48.97±0.09	0.000
	HCT (%)	38.02±0.07	42.84±0.08	5.85±0.01	3.09±0.01	0.016
	MCH (pg)	22.69±0.04	19.39±0.03	45.7±0.08	80.98±0.14	0.016
	MCHC (g/dl)	43.99±0.08	35.32±0.06	99.04±0.18	99.63±0.18	0.017
	RDW	12.14±0.02	12.96±0.02	13.58±0.02	17.45±0.03	0.016
	[HB] (g/dl)	16.82±0.03	15.34±0.03	5.95±0.01	5.19±0.01	0.016

Values are expressed as Mean ± Sem, Bwt = Body weight, WBC = White blood cells, Lymph = Lymphocytes, Mon = Monocytes, RBC = Red blood Cell, MCV = Mean corpuscular volume, HCT = Hematocrit, MCH = Mean cell hemoglobin, MCHC = Mean cell hemoglobin concetration, RDW = Red cell distribution width, [HB] = Hemoglobin concentration, # = number of cells.

Male mice that received stem ethyl acetate extract, the results shows there is significant decrease in WBC count and RBC, MCV, HCT, [HB] ($p<0.05$) and significant increase in MCH, MCHC, RDW, ($p>0.05$). In females showed significant decrease in WBC counts RBC, MCV, HCT, [HB] ($p<0.05$) however there is significant increase in MCH, MCHC and RDW ($p<0.05$) as indicated in Table 8.

Table 8: Hematological parameters of male and female mice during acute toxicity study after administration of stem bark ethyl acetate extract

		Dose in mg/kg Bwt				
Sex	**Parameter**	**Control**	**300**	**600**	**1200**	**P – value**
Males	WBC (m/mm3)	19.62±3.04	4.55±0.01	7.84±0.01	10.24±0.02	0.001
	Lymph (#)	16.38±4.07	4.53±0.02	7.61±0.03	9.93±0.04	0.018
	Mon (#)	1.91±0.51	0±0.00	0.20±0.00	0.27±0.00	0.024
	RBC (m/mm3)	7.44±0.34	1.87±0.00	1.3±0.00	0.6±0.00	0.000
	MCV (fl)	51.93±0.52	49.27±0.09	45.48±0.08	45.98±0.08	0.000
	HCT (%)	38.53±1.40	9.18±0.02	5.88±0.01	2.69±0.00	0.000
	MCH (pg)	22.23±0.72	34.61±0.06	45.98±0.08	63.13±0.11	0.016
	MCHC (g/dl)	42.93±1.07	70.41±0.12	99.63±0.18	99.63±0.18	0.024
	RDW	12.17±0.43	17.95±0.03	13.66±0.02	14.06±0.02	0.000
	[Hb] (g/dl)	16.53±0.32	6.48±0.01	5.98±0.01	3.79±0.01	0.000
Females	WBC (m/mm3)	19.62±3.04	5.55±0.01	6.84±0.01	8.25±0.01	0.016
	Lymph (#)	16.38±4.07	5.52±0.02	6.64±0.02	8±0.03	0.016
	Mon (#)	1.91±0.51	0±0.00	0.17±0.00	0.21±0.00	0.015
	RBC (m/mm3)	7.36±0.01	1.85±0.00	1.14±0.00	0.6±0.00	0.016
	MCV (fl)	51.56±0.09	48.97±0.09	44.88±0.08	45.89±0.08	0.016
	HCT (%)	38.02±0.07	9.12±0.02	5.09±0.01	2.69±0.00	0.016
	MCH (pg)	22.69±0.04	34.4±0.06	49.87±0.09	63±0.11	0.000
	MCHC (g/dl)	43.99±0.08	69.99±0.12	99.63±0.18	99.43±0.18	0.022
	RDW	12.14±0.02	17.84±0.03	15.16±0.03	14.03±0.02	0.000
	[Hb] (g/dl)	16.82±0.03	6.44±0.01	5.68±0.01	3.78±0.01	0.016

Values are expressed as Mean ± Sem, Bwt = Body Weight, WBC = White blood cells, Lymph = Lymphocytes, Mon = Monocytes, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, HCT = Hematocrit, MCH = Mean Cell Hemoglobin, MCHC = Mean Cell Hemoglobin Concetration, RDW = Red Cell Distribution Width, [HB] = Hemoglobin concentration, # = number of cells.

Male mice that received stem bark methanolic extract, shows significant decrease in WBC counts, RBC and RBC indices such as MCV, HCT and [HB] ($p<0.05$) and significant increase seen in MCH, MCHC and RDW ($p<0.05$). In female mice there is significant decrease in WBC counts, RBC, RBC indices, MCV, HCT and [HB] ($p<0.05$) and significant increase seen in MCH, MCHC, and RDW ($p<0.05$) as summarised in Table 9.

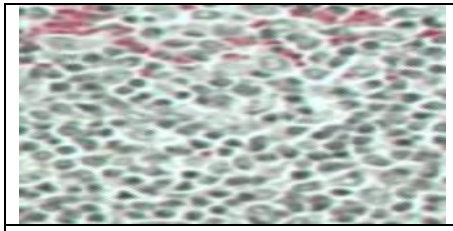
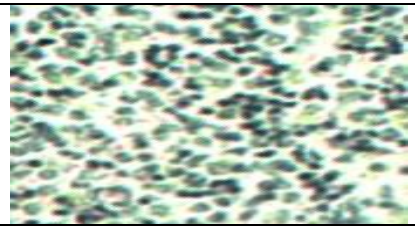
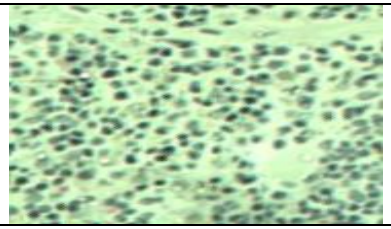
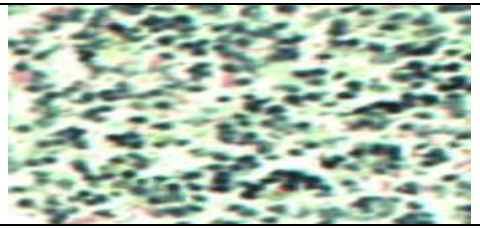
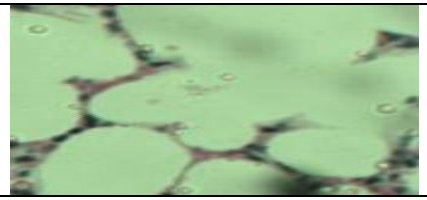
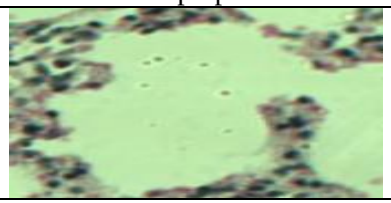
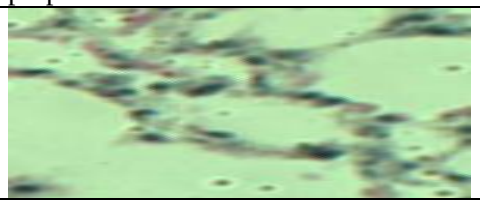
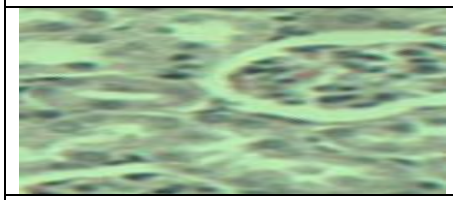
Table 9: Hematological parameters of male and female mice during acute toxicity study after administration of stem bark methanolic extract

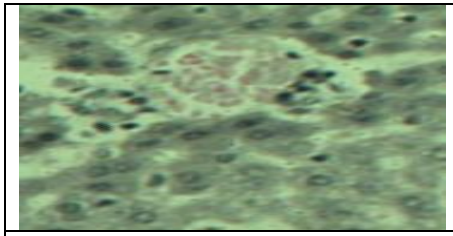
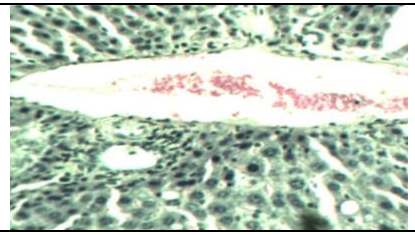
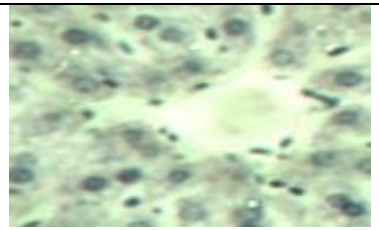
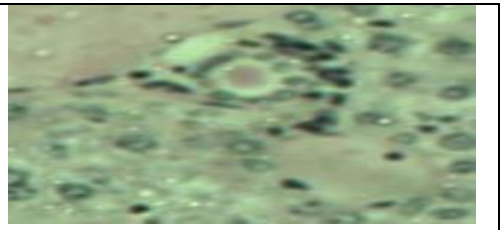
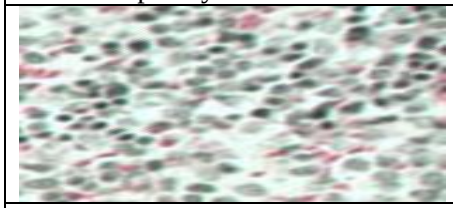
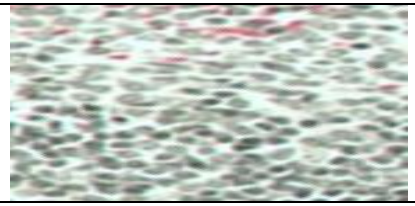
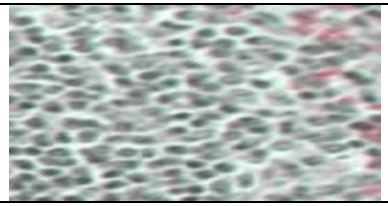
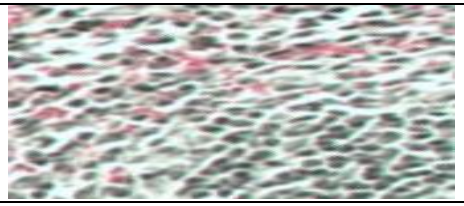
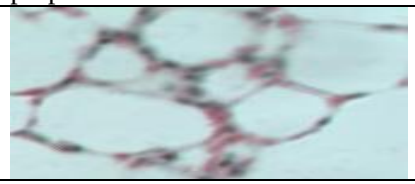
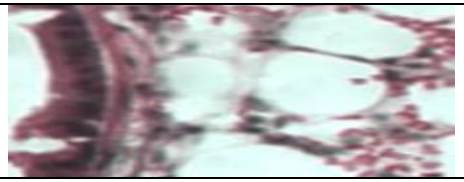
		Dose in mg/kg Bwt				
Sex	**Parameter**	**Control**	**300**	**600**	**1200**	**P – value**
Males	WBC (m/mm3)	19.62±3.04	6.93±0.01	9.72±0.02	-	0.0270
	Lymph (#)	16.38±4.07	6.39±0.31	8.19±1.21	-	0.1290
	Mon (#)	1.91±0.51	0.16±0.01	0.21±0.03	-	0.0550
	RBC (m/mm3)	7.44±0.34	0.59±0.00	0.59±0.00	-	0.0270
	MCV (fl)	51.93±0.52	45.34±0.08	45.61±0.08	-	0.0321
	HCT (%)	38.53±1.4	2.66±0.00	2.67±0.00	-	0.0321
	MCH (pg)	22.23±0.72	62.25±0.11	62.63±0.11	-	0.0321
	MCHC (g/dl)	42.93±1.07	98.24±0.17	98.84±0.00	-	0.0320
	RDW	12.17±0.43	13.87±0.02	13.95±0.02	-	0.0320
	[Hb] (g/dl)	16.53±0.32	3.74±0.01	3.76±0.01	-	0.0320
Females	WBC (m/mm3)	19.62±3.04	3.55±0.01	5.94±0.01	6.95±0.01	0.0240
	Lymph (#)	16.38±4.07	3.54±0.01	5.77±0.02	6.76±0.01	0.0240
	Mon (#)	1.91±0.51	0.00±0.00	0.15±0.00	0.17±0.00	0.0230
	RBC (m/mm3)	7.36±0.01	0.60±0.00	0.6±0.00	1.3±0.00	0.0400
	MCV (fl)	51.56±0.09	45.48±0.08	45.98±0.08	45.55±0.04	0.0310
	HCT (%)	38.02±0.07	2.69±0.00	2.69±0.00	5.89±0.00	0.0400
	MCH (pg)	22.69±0.04	71.41±0.13	63.13±0.11	46.05±0.04	0.0310
	MCHC (g/dl)	43.99±0.08	99.63±0.18	99.63±0.18	99.8±0.08	0.0890
	RDW	12.14±0.02	13.46±0.02	14.06±0.02	13.69±0.01	0.0240
	[Hb] (g/dl)	16.82±0.03	4.29±0.01	3.79±0.01	5.99±0.00	0.0240

Values are expressed as Mean ± Sem, Bwt = Body Weight, WBC = White blood cells, Lymph = Lymphocytes, Mon = Monocytes, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, HCT = Hematocrit, MCH = Mean Cell Hemoglobin, MCHC = Mean Cell Hemoglobin Concetration, RDW = Red Cell Distribution Width, [HB] = Hemoglobin concentration. # = number of cells

2.3.5 Histopathology

The micrograph of the vital organ tissues that were taken from mice subjected to acute toxicity showed increased glumerural space. In some liver micrograph shows distended sinusoids. The lungs appears to contain scattered RBC, Spleen showed normal red and white pulp as seen in (Micrographs 1 – 171) below.

			
Micrograph 1: Female mouse control spleen administered with DMSO showing normal distribution red pulp and white pulp.	Micrograph 2 Female mouse spleen administered with 300 mg/kg of LCE with normal structure.	Micrograph 3: Spleen of female mouse administered with 600 mg/kg of LCE showing normal red and white pulp.	Micrograph 4: Spleen of a female mouse administered with 1200 mg/kg of LCE showing normal red and white pulp.
			
Micrograph 5: Lung of the female mouse control mouse after administration of DMSO	Micrograph 6: Lung of the female mouse administered with 300 mg/kg of LCE showing normal alveolar sac.	Micrograph 7: Lung of female mouse administered with 600 mg/kg of LCE showing thickened alveolar walls.	Micrograph 8: lung of female mouse administered with 1200 mg/kg of LCE showing thickened alveolar wall.
			
Micrograph 9: Kidney of control female mouse administered with DMSO showing normal proximal convoluted tubules and glomeruli.	Micrograph 10: Kidney of a female mouse administered with 300 mg/kg of LCE showing widened glomeruli space.	Micrograph 11: Kidney of a female mouse administered with 600 mg/kg of LCE showing disintegrated glomeruli.	Micrograph 12: Kidney of female mouse administered with 1200 mg/kg of LCE showing widened glomerular space.

			
Micrograph 13: Liver of control female mouse showing normal bile duct (brown arrow), central vein and normal hepatocytes.	Micrograph 14: Liver of female mouse administered with 300 mg/kg of LCE showing normal bile duct.	Micrograph 15: Liver of the female mouse administered with 600 mg/kg of LCE showing normal central canal.	Micrograph 16: Liver of a female mouse administered with 1200 mg/kg of LCE showing normal central canal.
			
Micrograph 17: Spleen of male mouse control mouse administered with DMSO showing normal spleen red pulp.	Micrograph 18: Spleen of male mouse administered with 300 mg/kg of LCE showing normal red pulp.	Micrograph 19: Spleen of male mouse administered with 600 mg/kg of LCE showing normal red and white pulp.	Micrograph 20: Spleen of female mouse administered with 1200 mg/kg of LCE showing normal red and white pulp.
			
Micrograph 21: Lung of male control mouse administered with DMSO showing normal alveolar wall of the lung with scattered RBC.	Micrograph 22: Lung of the male mouse administered with 300 mg/kg of LCE showing normal alveolar sac with scattered RBC.	Micrograph 23: Lung of male mouse administered with 600 mg/kg of LCE showing normal alveolar wall and scattered RBC.	Micrograph 24: Lung of the male mouse administered with 1200 mg/kg of LCE showing normal alveolar wall with scattered RBC.

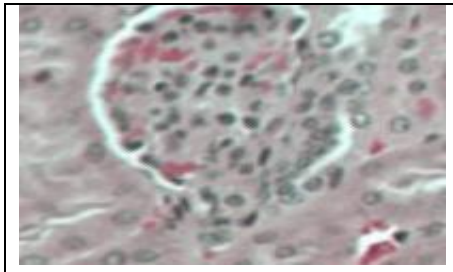
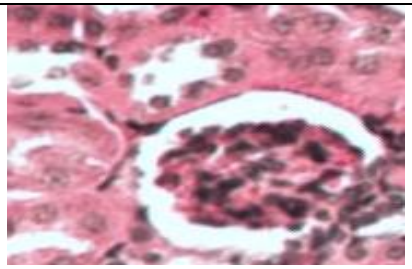
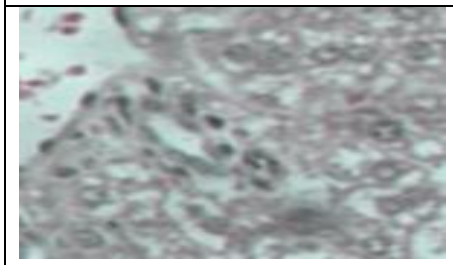
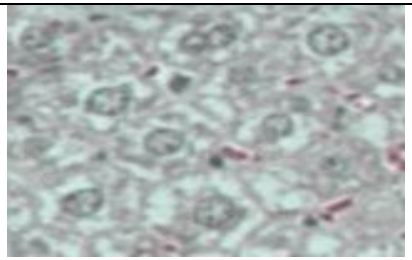
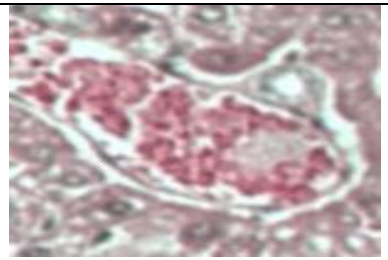
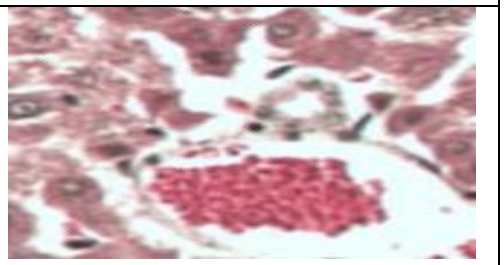
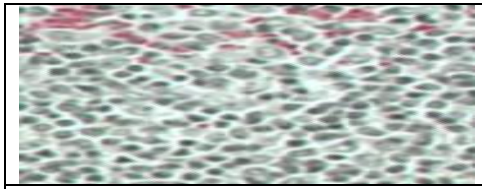
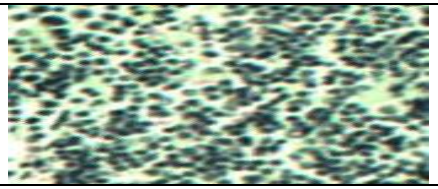
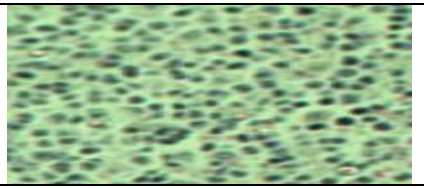
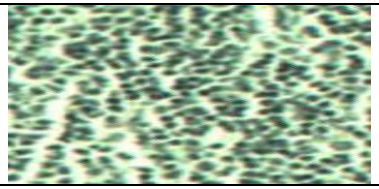
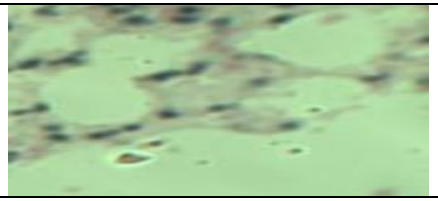
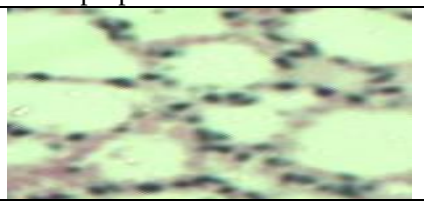
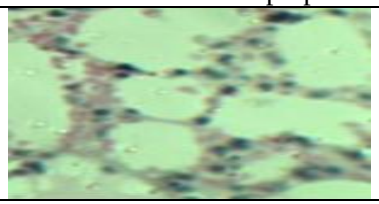
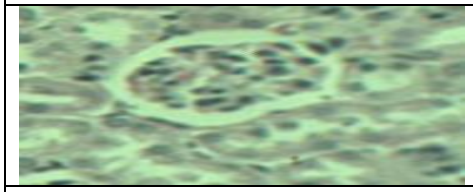
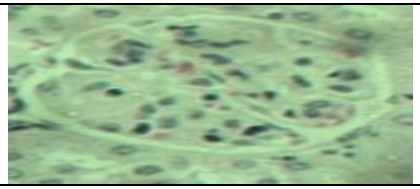
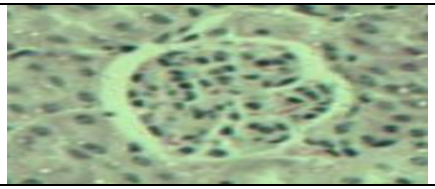
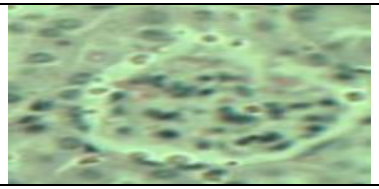
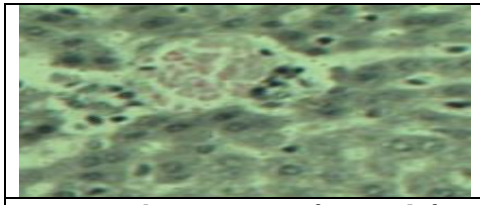
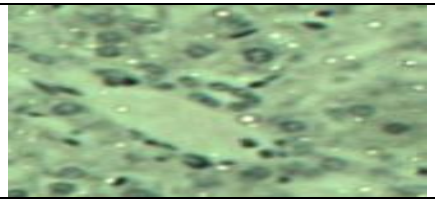
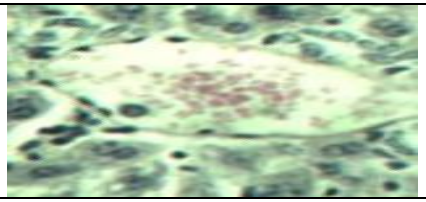
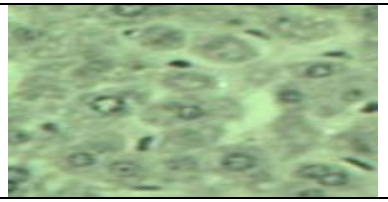
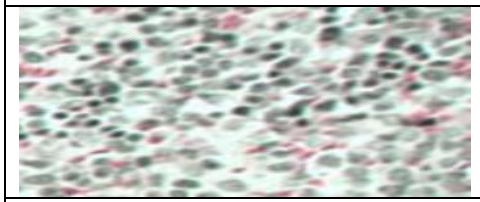
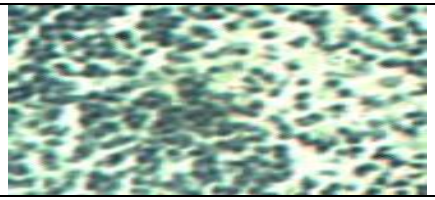
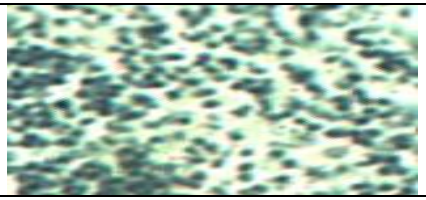
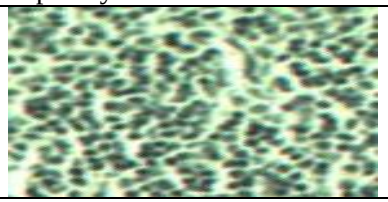
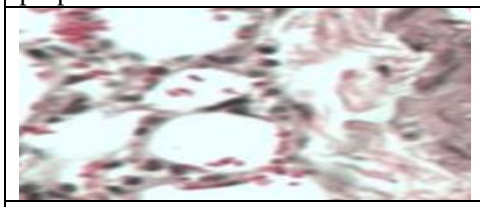
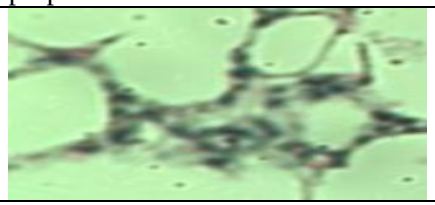
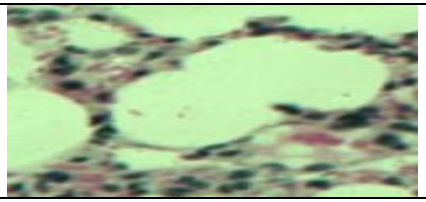
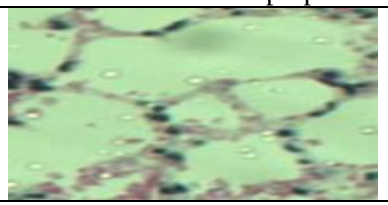
			
Micrograph 25: Kidney of control Male mouse administered with DMSO showing normal and proximal convoluted tubules	Micrograph 26: Kidney of male mouse administered with 300 mg/kg of LCE showing increased glomerula space.	Micrograph 27: Kidney of male mouse administered with 600 mg/kg of LCE showing widened glomerular space.	Micrograph 28: Kidney of male mouse administered with 1200 mg/kg of LCE showing widened glomerular space.
			
Micrograph 29: Liver of control male mouse administered with DMSO showing normal bile duct (normal hepatocytes).	Micrograph 30: Liver of male mouse administered with 300 mg/kg of LCE showing normal hepatocytes.	Micrograph 31: liver of male mouse administered with 600 mg/kg of LCE showing normal bile duct and central vein	Micrograph 32: Liver of male mouse administered with 1200 mg/kg of LCE showing distended sinusoids, normal bile duct

Figure 1: Mice vital organs micrographs after acute exposure to leaf chloroform extract (LCE) and DMSO as control (H&E stain and magnification 40x).

			
Micrograph 33: Female control spleen administered with DMSO showing normal distribution red and white pulp.	Micrograph 34: Female mouse spleen administered with 300 mg/kg LEAE showing normal red and white pulp.	Micrograph 35: Spleen of female mouse administered with 600 mg/kg LEAE showing normal red and white pulp.	Micrograph 36: Spleen of a female mouse administered with 1200 mg/kg LEAE showing normal red and white pulp .
			
Micrograph 37: Lung of the female mouse control mouse after administration of DMSO	Micrograph 38: Lung of the female mouse given a dose of 300mg/kg LEAE showing thickened alveolar walls scattered RBC	Micrograph 39: Lung of female mouse given LEAE at 600 mg/kg showing thickened alveolar walls with scattered RBC	Micrograph 40: lung of female mouse given LEAE at a dose of 1200 mg/kg showing thickened alveolar with RBC
			
Micrograph 41**:** Kidney of control female mouse administered with DMSO showing normal proximal convoluted tubules and glomeruli	Micrograph 42: Kidney of a female mouse administered with LEAE at 300 mg/kg showing widened glomeruli space	Micrograph 43: Kidney of a female mouse administered with LEAE at 600 mg/kg showing disintegrated glomeruli, widened Bowman's space	Micrograph 44: Kidney of female mouse administered with LEAE at 1200 mg/kg showing disintegrated glomeruli.

			
Micrograph 45: Liver of control female mouse showing normal bile duct, central vein and normal hepatocytes	Micrograph 46: Liver of female mouse administered with LEAE at 300 mg/kg showing normal bile duct	Micrograph 47: Liver of the female mouse administered with LEAE at 600 mg/kg showing central canal normal bile duct	Micrograph 48: Liver of a female mouse administered with LEAE at 1200 mg/kg normal hepatocytes
			
Micrograph 49: Spleen of male control mouse administered with DMSO showing normal spleen red and white pulp.	Micrograph 50: Spleen of male mouse administered with LEAE 300 mg/kg showing normal red and white pulp.	Micrograph 51: Spleen of male mouse administered with LEAE 600 mg/kg showing red and white pulp.	Micrograph 52: Spleen of female mouse administered with LEAE 1200 mg/kg showing normal red and white pulp.
			
Micrograph 53: Lung of male control mouse administered with DMSO showing normal alveolar) of the lung with scattered RBC.	Micrograph 54: Lung of the male mouse administered with LEAE at 300 mg/kg showing alveolar wall with scattered RBC	Micrograph 55: Lung of male mouse administered with LEAE at 600 mg/kg showing normal alveolar wall with scattered RBC	Micrograph 56: Lung of the male mouse administered with LEAE at 1200 mg/kg showing normal alveolar wall with scattered RBC

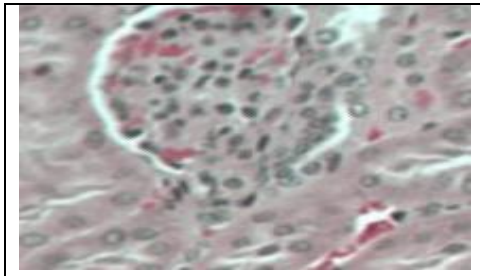
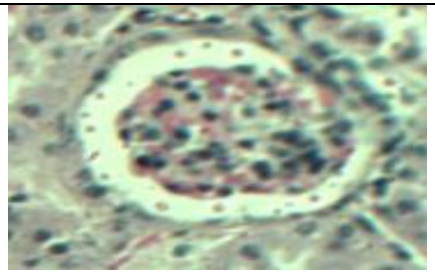
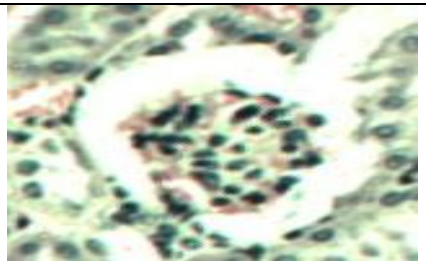
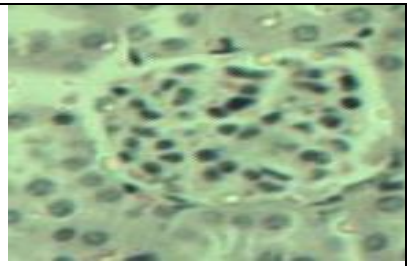
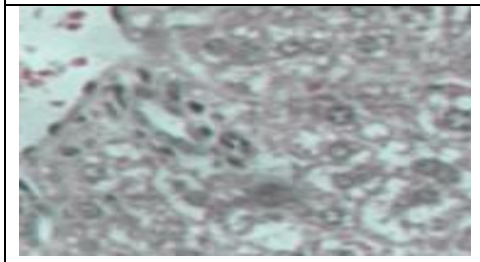
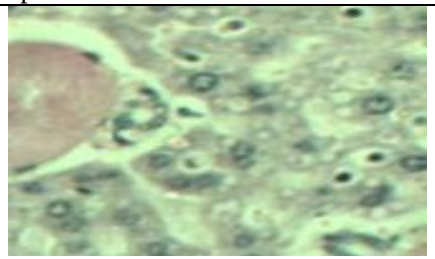
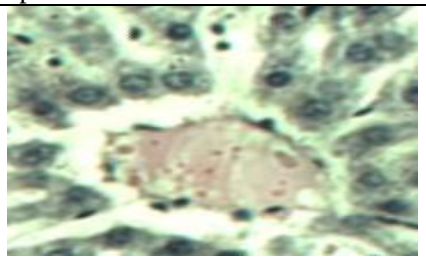
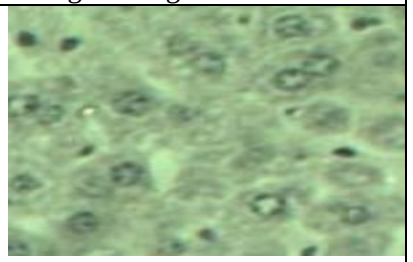
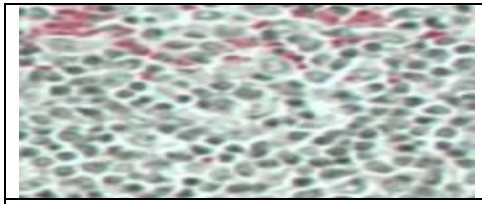
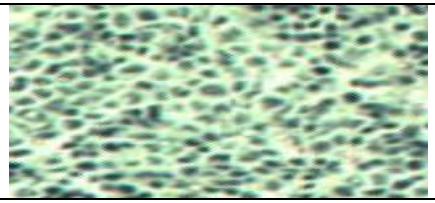
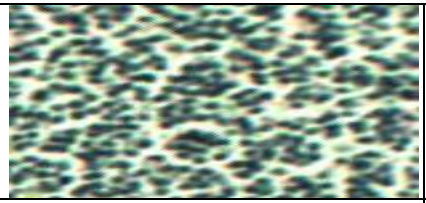
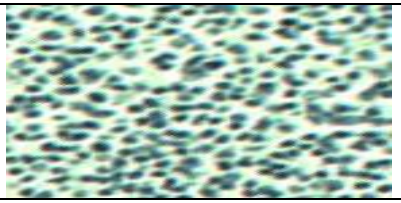
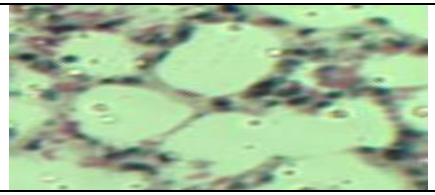
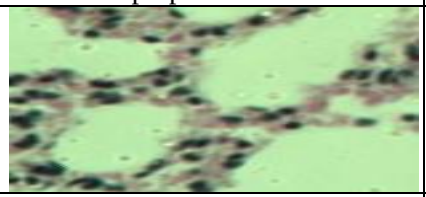
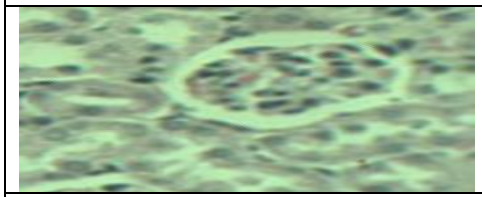
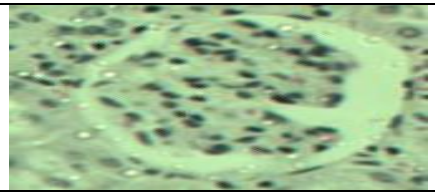
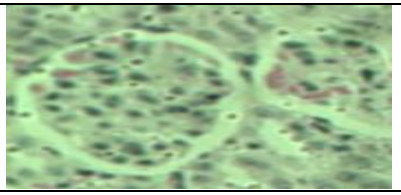
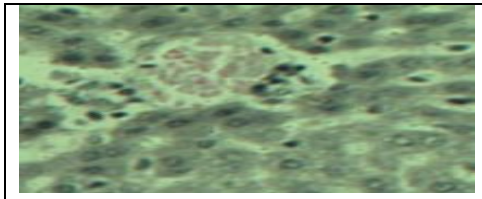
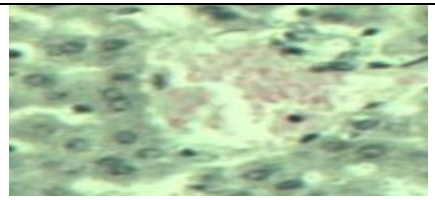
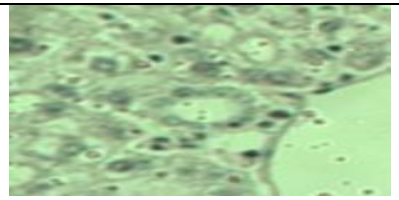
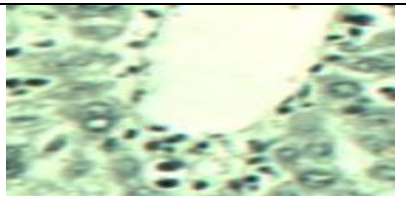
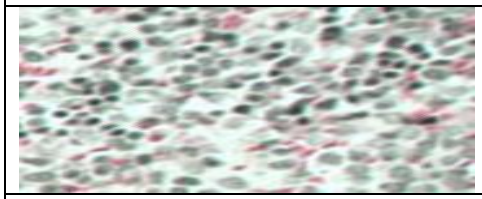
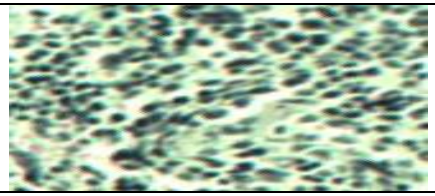
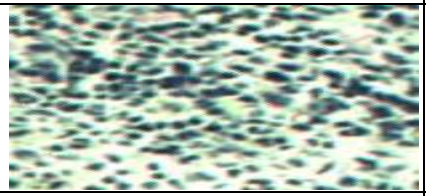
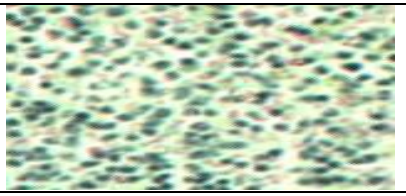
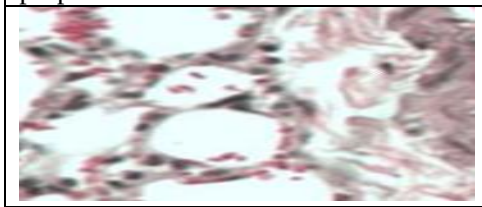
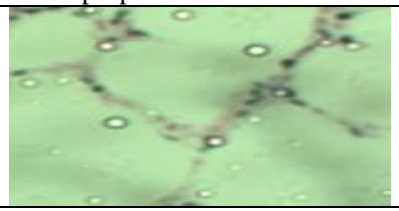
			
Micrograph 57: kidney of control male mouse administered with DMSO showing normal glomeruli and proximal convoluted tubules.	Micrograph 58: Male mouse kidney administered with LEAE at 300 mg/kg showing increased glomerula space	Micrograph 59: Male mouse kidney administered with LEAE at 600 mg/kg showing widened glomerular space	Micrograph 60: Kidney of male mouse administered with LEAE 1200 mg/kg showing disorganized glomeruli
			
Micrograph 61: Liver of control male mouse administered with DMSO showing normal bile duct and hepatocytes.	Micrograph 62: Liver of male mouse administered with LEAE extract at 300mg/kg showing normal central vein and hepatocytes.	Micrograph 63: liver of male mouse administered with LEAE at 600 mg/kg wide central vein and distended sinusoids.	Micrograph 64: Liver of male mouse administered with LEAE at 1200mg/kg showing pyknosis disorganization of the hepatocytes.

Figure 2: Mice vital organs micrographs after acute exposure to leaf ethyl acetate extract (LEAE) and DMSO as control (H&E stain and magnification 40x).

			
Micrograph 65: Female control spleen administered with DMSO showing normal distribution red and white pulp.	Micrograph 66: Female mouse spleen administered with 300 mg/kg LME showing normal red and white pulp	Micrograph 67: Spleen of female mouse administered with 600 mg/kg LME showing normal red and white pulp	Micrograph 68: Spleen of a female mouse administered with 1200 mg/kg LME showing normal distribution of red and white pulp
			
Micrograph 69: Lung of the female mouse control mouse after administration of DMSO	Micrograph 70: Lung of the female mouse given a dose of 300 mg/kg LME showing thickened alveolar walls with scattered RBC	Micrograph 71: Lung of female mouse given LME at 600 mg/kg showing thickened alveolar walls with scattered RBC	Micrograph 72: Lung of female mouse given LME at a dose of 1200 mg/kg showing thickened alveolar wall and disorganised alveolar wall
			
Micrograph 73: Kidney of control female mouse administered with DMSO showing normal proximal convoluted and glomeruli.	Micrograph 74: Kidney of a female mouse administered with LME at 300 mg/kg with widened glomeruli space.	Micrograph 75: Kidney of a female mouse administered with LME at 600 mg/kg with widened Bowman's space	Micrograph 76: Kidney of female mouse administered with LME at 1200 mg/kg with widened glomerular space

			
Micrograph 77: Liver of control female mouse showing normal bile duct, central vein and normal hepatocytes.	Micrograph 78: Liver of female mouse administered with LME at 300 mg/kg showing normal central vein.	Micrograph 79: Liver of the female mouse administered with LME at 600 mg/kg showing normal central canal.	Micrograph 80: Liver of a female mouse administered with LME at 1200 mg/kg normal central canal.
			
Micrograph 81: Spleen of male control mouse administered with DMSO showing normal spleen red and white pulp.	Micrograph 82: Spleen of male mouse administered with LME 300 mg/kg showing normal red and white pulp.	Micrograph 83: Spleen of male mouse administered with LME 600 mg/kg showing red and white pulp.	Micrograph 84: Spleen of female mouse administered with LME 1200 mg/kg showing distribution red pulp and white pulp.
			
Micrograph 85: Lung of male control mouse administered with DMSO showing normal alveolar wall of the lung.	Micrograph 86: Lung of the male mouse administered with LME at 300 mg/kg showing alveolar wall with scattered RBC.	Micrograph 87: Lung of male mouse administered with LME at 600mg/kg showing normal alveolar wall of the lung.	Micrograph 88: Lung of the male mouse administered with LME at 1200 mg/kg showing normal alveolar wall with scattered RBC.

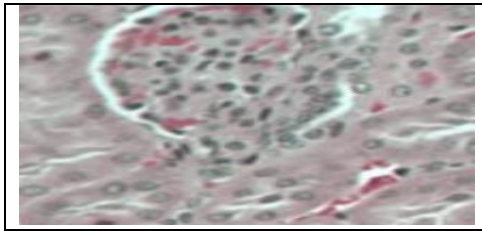
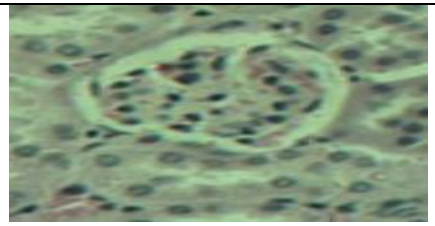
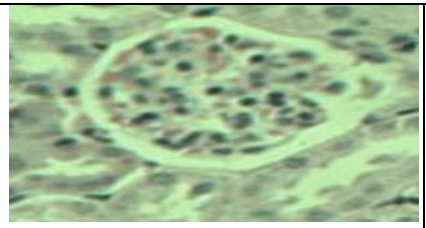
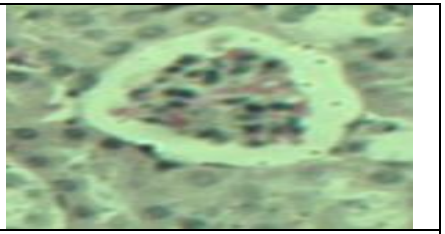
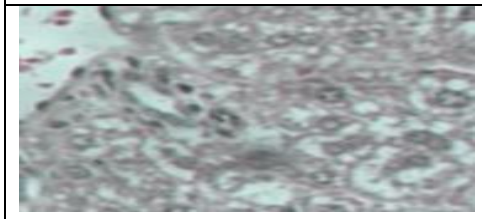
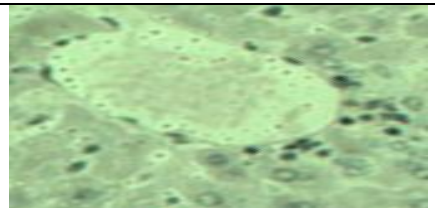
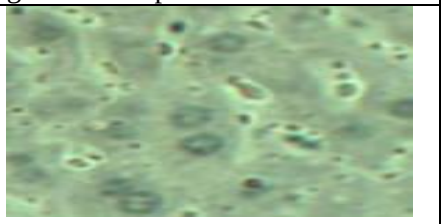
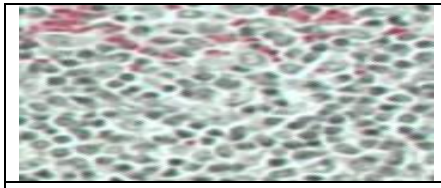
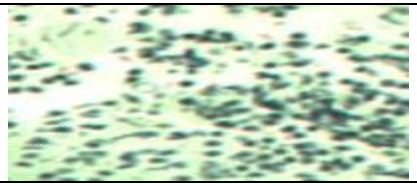
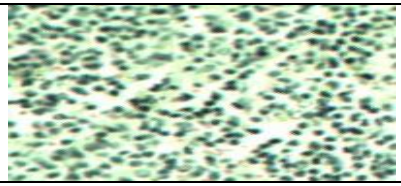
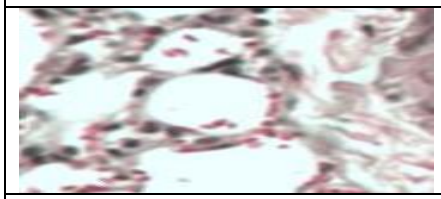
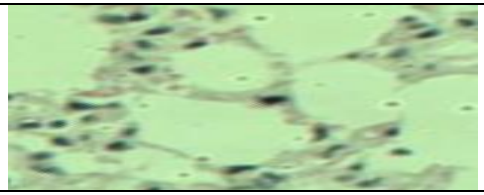
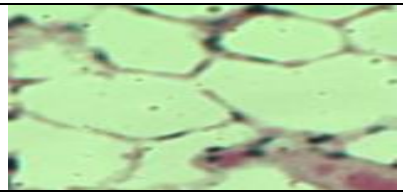
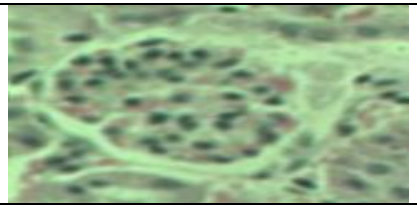
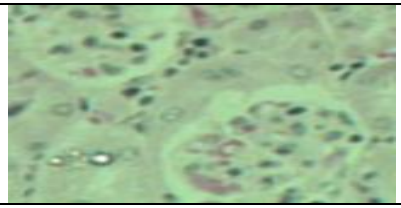
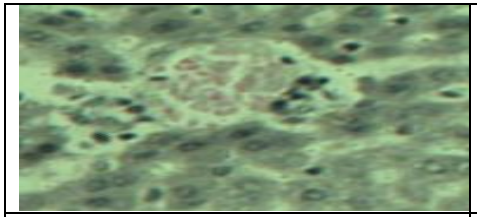
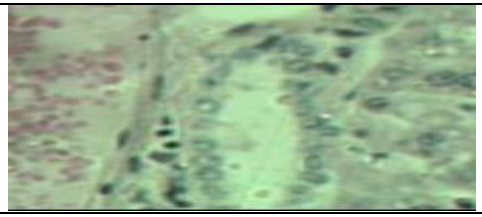
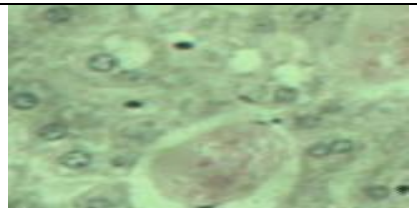
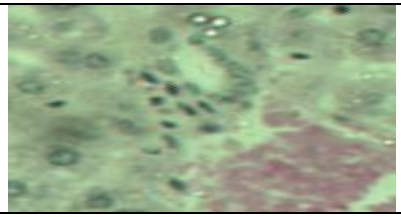
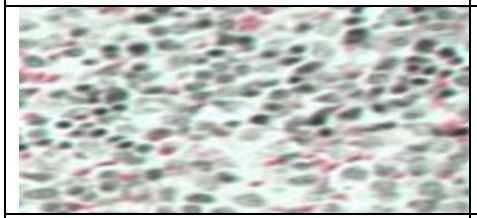
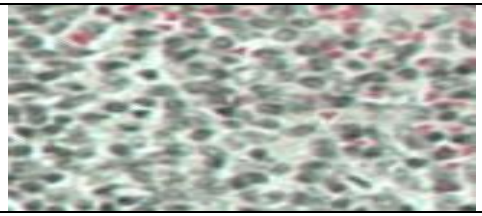
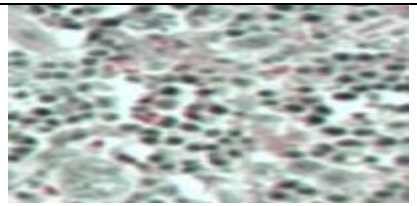
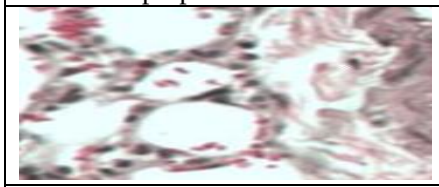
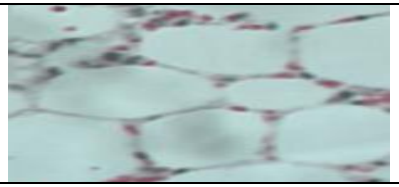
			
Micrograph 89: kidney of control male mouse administered with DMSO showing normal glomeruli and proximal convoluted tubules	Micrograph 90: Male mouse kidney administered with LME at 300 mg/kg showing increased glomerula space	Micrograph 91: Male mouse kidney administered with LME at 600 mg/kg showing widened glomerular	Micrograph 92: Kidney of male mouse administered with LME 1200 mg/kg showing widened glomerular space
			
Micrograph 93: Liver of control male mouse administered with DMSO showing normal bile duct and hepatocytes.	Micrograph 94: Liver of male mouse administered with LME extract at 300 mg/kg showing normal central vein.	Micrograph 95: liver of male mouse administered with LME at 600 mg/kg showing distended bile duct and central vein.	Micrograph 96: Liver of male mouse administered with LME at 1200 mg/kg normal hepatocytes

Figure 3: Mice vital organs micrographs after acute exposure to leaf methanolic extract (LME) and DMSO as control (H&E stain and magnification 40x).

			
Micrograph 97: Female control spleen administered with DMSO showing normal distribution of red and white pulp	Micrograph 98: Female mouse spleen administered with 300 mg/kg SCE showing normal red pulp and white pulp	Micrograph 99: Spleen of female mouse administered with 600 mg/kg SCE showing normal red and white pulp distribution	Micrograph 100: Spleen of a female mouse administered with 1200 mg/kg SCE showing disrtibution of red pulp and white pulp
			
Micrograph 101: Lung of the female mouse control mouse after administration of DMSO	Micrograph 102: Lung of the female mouse given a dose of 300 mg/kg SCE showing thickened alveolar walls with scattered RBC	Micrograph 103: Lung of female mouse given SCE at 600 mg/kg showing thickened alveolar walls with scattered RBC	Micrograph 104: lung of female mouse given SCE at a dose of 1200 mg/kg showing thickened alveolar wall with scattered RBC
			
Micrograph 105: Kidney of control female mouse administered with DMSO showing normal proximal convoluted tubules and glomeruli.	Micrograph 106: Kidney of a female mouse administered with SCE at 300 mg/kg showing disorganised glomeruli space.	Micrograph 107: Kidney of a female mouse administered with SCE at 600 mg/kg showing disorganised glomeruli.	Micrograph 108: Kidney of female mouse administered with SCE at 1200 mg/kg showing widened glomerular space and disorganised glomeruli

			
Micrograph 109: Liver of control female mouse showing bile ducts and central vein.	Micrograph 110: Liver of female mouse administered with SCE at 300 mg/kg showing normal bile duct and central vein.	Micrograph 111: Liver of the female mouse administered with SCE at 600 mg/kg showing central vein and normal bile duct.	Micrograph 112: Liver of a female mouse administered with SCE at 1200 mg/kg central vein and normal bile duct
			
Micrograph 113: Spleen of male control mouse administered with DMSO showing normal spleen red and white pulp.	Micrograph 114: Spleen of male mouse administered with SCE 300 mg/kg showing red pulp and white pulp.	Micrograph 115: Spleen of male mouse administered with SCE 600 mg/kg showing red and white pulp	
			
Micrograph 116: Lung of male control mouse administered with DMSO showing normal alveolar wall of the lung.	Micrograph 117: Lung of the male mouse administered with SCE at 300 mg/kg showing alveolar wall and scattered RBC.	Micrograph 118: Lung of male mouse administered with SCE at 600 mg/kg showing normal alveolar wall with scattered RBC.	

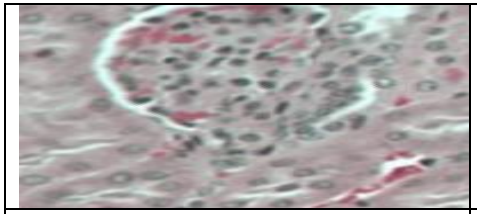
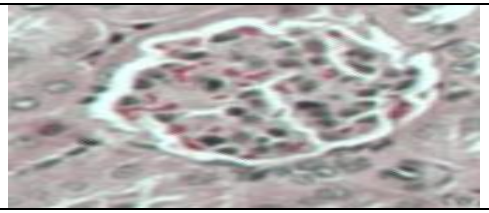
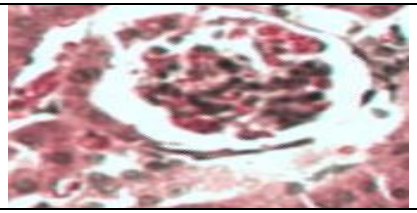
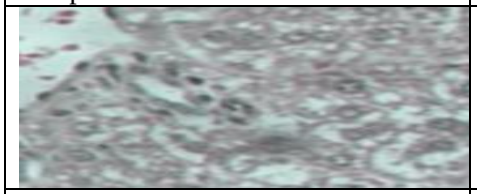
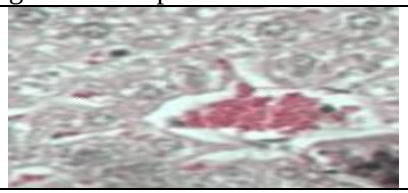
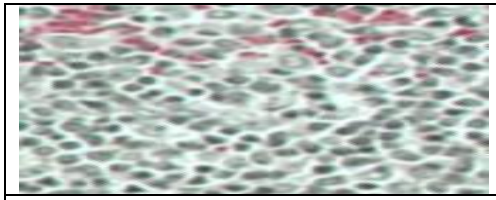
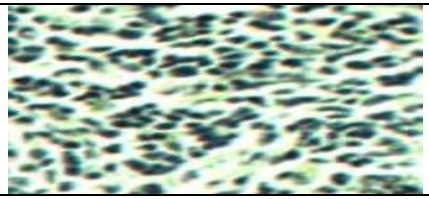
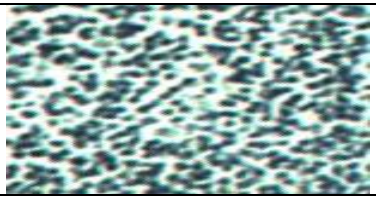
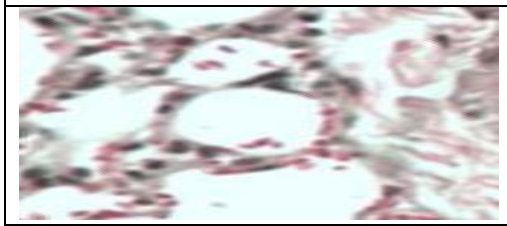
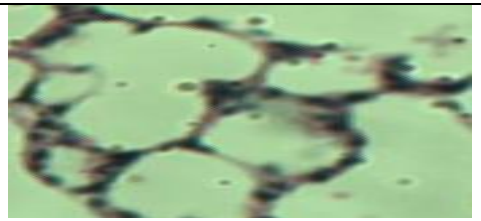
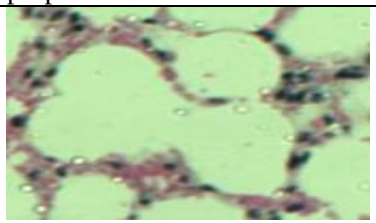
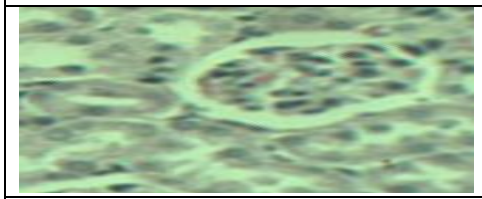
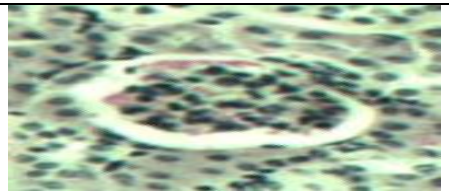
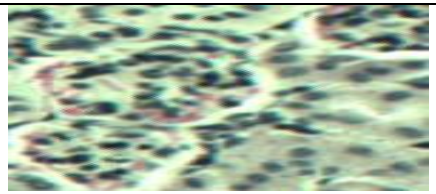
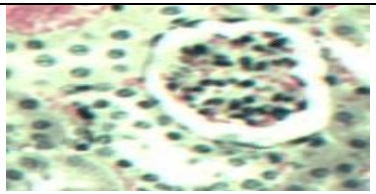
		
Micrograph 119: kidney of control male mouse administered with DMSO showing normal glomeruli and proximal convoluted tubules	Micrograph 120: Male mouse kidney administered with SCE at 300 mg/kg showing increased glomerula space	Micrograph 121: Male mouse kidney administered with SCE at 600 mg/kg showing widened glomerular space.
		
Micrograph 122: Liver of control male mouse administered with DMSO showing normal bile duct and normal hepatocytes	Micrograph 123: Liver of male mouse administered with SCE at 300 mg/kg showing normal central vein	Micrograph 124: liver of male mouse administered with SCE at 600 mg/kg showing normal central vein

Figure 4: Mice vital organs micrographs after acute exposure to stem chloroform extract (SCE) and DMSO as control (H&E stain and magnification 40x).

			
Micrograph 125: Female control spleen administered with DMSO showing normal distribution red and white pulp	Micrograph 126: Female mouse spleen administered with 300 mg/kg SEAE showing red and white pulp	Micrograph 127: Spleen of female mouse administered with 600 mg/kg SEAE showing reduced red and white pulp	Micrograph 128: Spleen of a female mouse administered with 1200 mg/kg SEAE showing more red and white pulp
			
Micrograph 129: Lung of the female mouse control mouse after administration of DMSO	Micrograph 130: Lung of the female mouse given a dose of 300 mg/kg SEAE showing thickened alveolar walls with scattered RBC	Micrograph 131: Lung of female mouse given SEAE at 600 mg/kg showing thickened alveolar walls with scattered RBC.	Micrograph 132: lung of female mouse given SEAE at a dose of 1200 mg/kg showing thickened alveolar wall with scattered RBC.
			
Micrograph 133: Kidney of control female mouse administered with DMSO showing normal proximal convoluted tubules and glomeruli.	Micrograph 134: Kidney of a female mouse administered with SEAE at 300 mg/kg showing widened glomeruli space.	Micrograph 135: Kidney of a female mouse administered with SEAE at 600 mg/kg with widened Bowman's space	Micrograph 136: Kidney of female mouse administered with SEAE at 1200 mg/kg showing widened glomerular space.

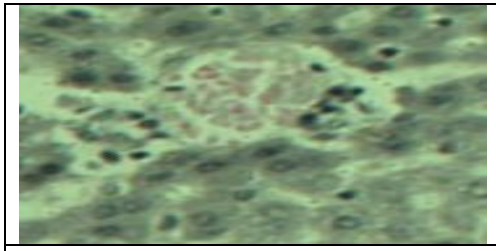
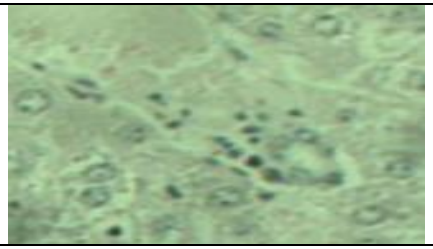
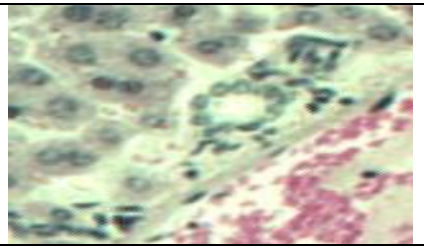
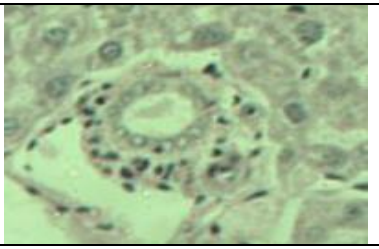
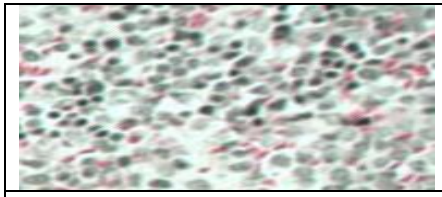
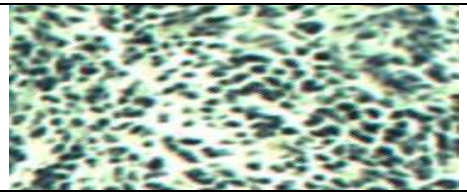
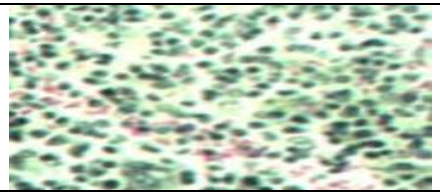
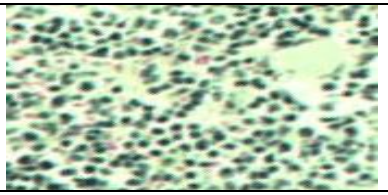
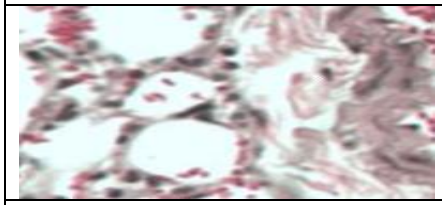
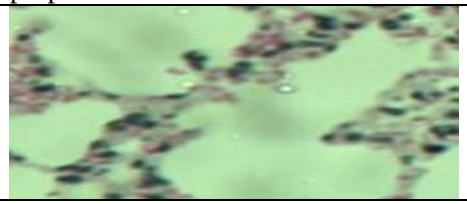
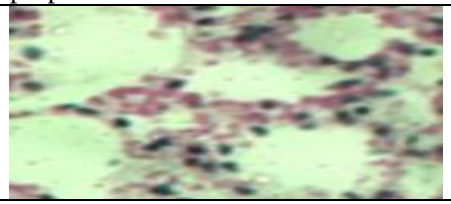
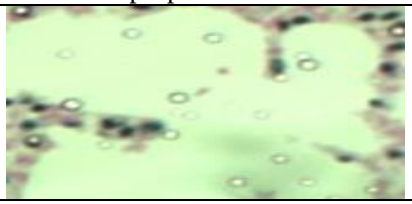
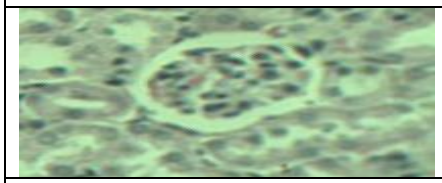
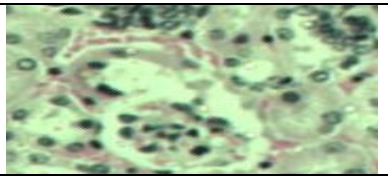
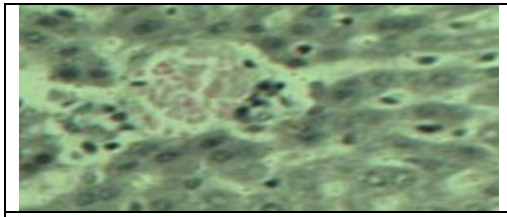
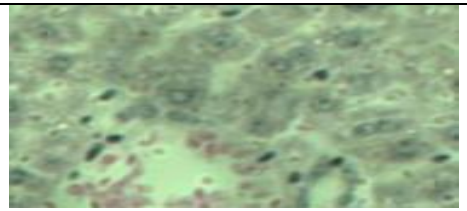
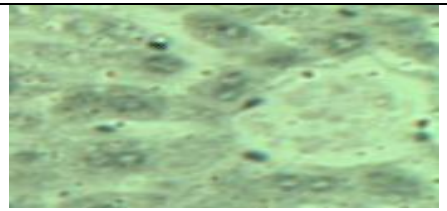
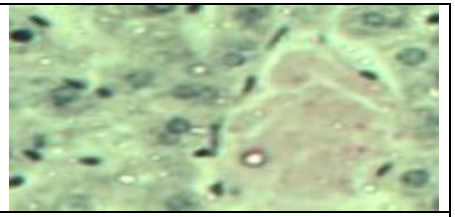
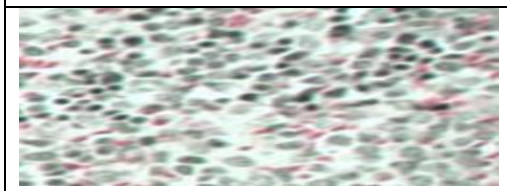
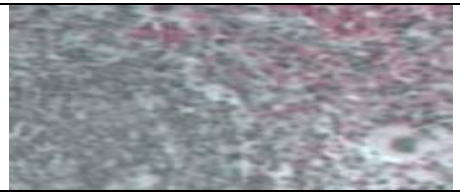
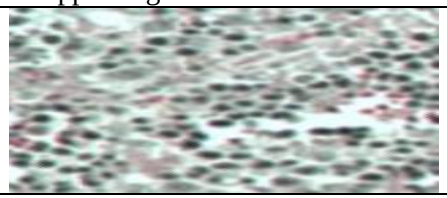
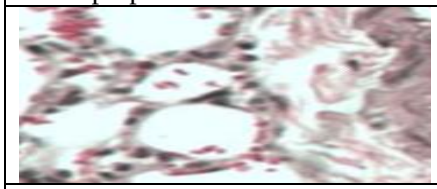
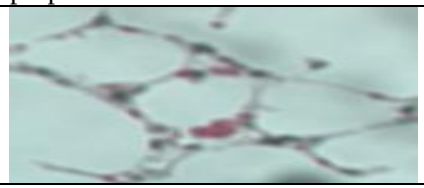
			
Micrograph 137: Liver of control female mouse showing normal bile duct and normal hepatocytes.	Micrograph 138: Liver of female mouse administered with SEAE at 300 mg/kg showing normal bile duct.	Micrograph 139: Liver of the female mouse administered with SEAE at 600 mg/kg showing normal bile duct.	Micrograph 140: Liver of a female mouse administered with SEAE at 1200 mg/kg normal central canal and normal bile duct.

Figure 5: Mice vital organs micrographs after acute exposure of stem ethyl acetate extract (SEAE) and DMSO as control (H&E stain and magnification 40x).

			
Micrograph 141: Female control spleen administered with DMSO showing normal distribution red and white pulp.	Micrograph 142: Female mouse spleen administered with 300 mg/kg SME showing red pulp and white pulp	Micrograph 143: Spleen of female mouse administered with 600 mg/kg SME showing red pulp and white pulp.	Micrograph 144**:** Spleen of a female mouse administered with 1200 mg/kg SME showing red pulp and white pulp.
			
Micrograph 145 Lung of the female control mouse after administration of DMSO	Micrograph 146 Lung of the female mouse given a dose of 300 mg/kg SME showing thickened alveolar walls	Micrograph 147 Lung of female mouse given SME at 600 mg/kg showing thickened alveolar walls	Micrograph 148: lung of female mouse given SME at a dose of 1200 mg/kg showing thickened alveolar wall
			
Micrograph 149: Kidney of control female mouse administered with DMSO showing normal proximal convoluted tubules and glomeruli.	Micrograph 150: Kidney of a female mouse administered with SME at 300 mg/kg showing widened glomeruli space.	Micrograph 151: Kidney of a female mouse administered with SME at 600 mg/kg showing disintegrated glomeruli, widened Bowman's space	Micrograph 152: Kidney of female mouse administered with SME at 1200 mg/kg showing widened glomerular space disintegration of glomeruli

			
Micrograph 153: Liver of control female mouse showing normal bile duct	Micrograph 154: Liver of female mouse administered with SME at 300 mg/kg showing bile duct and hepatocytes with disappearing nucleus.	Micrograph 155: Liver of the female mouse administered with SME at 600 mg/kg showing central canal and hepatocytes with disappearing nucleus.	Micrograph 156: Liver of a female mouse administered with SME at 1200 mg/kg normal central canal, hepatocytes and disappearing nucleus.
			
Micrograph 157: Spleen of male control mouse administered with DMSO showing normal spleen red pulp and white pulp.	Micrograph 158: Spleen of male mouse administered with SME 300 mg/kg showing red pulp and white pulp	Micrograph 159: Spleen of male mouse administered with SME 600 mg/kg showing red pulp and white pulp.	
			
Micrograph 160: Lung of male control mouse administered with DMSO showing normal alveolar wall of the lung.	Micrograph 161: Lung of the male mouse administered with SME at 300 mg/kg showing normal alveolar sac and alveolar wall.	Micrograph 162: Lung of male mouse administered with stem methanol at 600 mg/kg showing normal alveolar wall.	

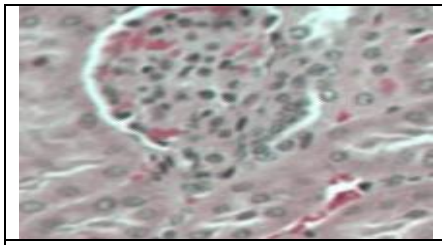
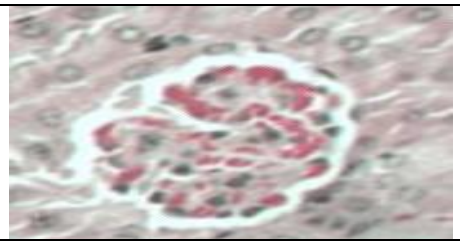
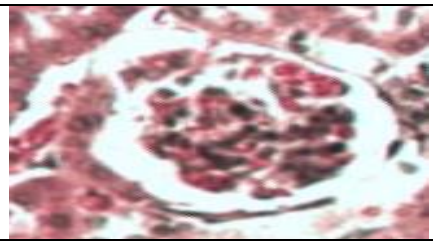
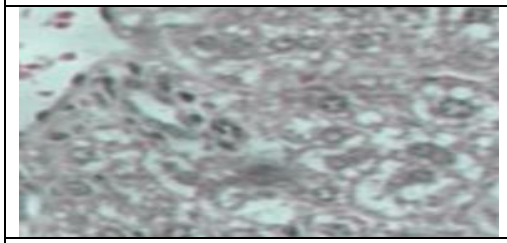
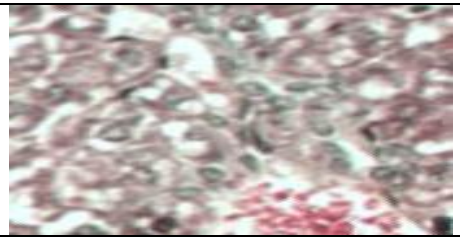
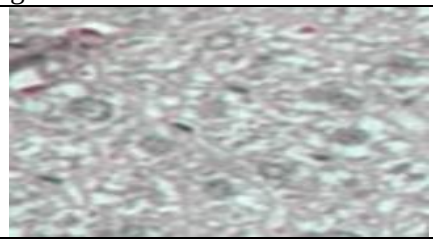
		
Micrograph 163: kidney of control male mouse administered with DMSO showing normal glomeruli and proximal convoluted tubules.	Micrograph 164: Male mouse kidney administered with SME at 300 mg/kg showing increased glomerula space and disorganized glomerula.	Micrograph 165: Male mouse kidney administered with SME at 600 mg/kg showing widened glomerular space, disorganized glomerula.
		
Micrograph 166: Liver of control male mouse administered with DMSO showing normal bile duct	Micrograph 167: Liver of male mouse administered with SME at 300 mg/kg showing multiple bile duct.	Micrograph 168: Liver of male mouse administered with SME at 600 mg/kg showing multiple bile duct.

Figure 6: Mice vital organs micrographs after acute exposure to stem bark methanolic extract (SME) and DMSO as control (H&E stain and magnification 40x).

2.4 Discussion

Medicinal herbs are used in increasing rates in rural and towns areas due to diseases they treat and its easy accessibility and affordability (WHO, 2002). *Commiphora campestris* has been used by the Pare Community for management of respiratory, skin, wounds and digestive tract disorders for many years but its toxicity to body tissues status had never been validated for safety. This acute toxicity study was conducted for 14 days to evaluate safety status of chloroform, ethyl acetate and methanolic extracts of *C. campestris*. All mice after been given the extracts showed behavioral changes that disappeared after short period of time. The animals appeared dull, closing eyes, huddling, rough hair coat and resumed to normal bright status within first four hours. All animals continued to feed and drink.

Mortality is one of the indicators of the toxicity of a particular compound. In this study no mortalities were recorded in female mice that were given leaf chloroform and leaf ethyl acetate extract, no mortalities were recorded in females that were given leaf methanolic, stem chloroform and stem ethyl acetate extract. One death was recorded to male mice given 600 mg/kg and 1200 mg/kg dose of leaf methanolic extract respectively. Higher mortalities were recorded in stem methanol extract, where at a 600 mg/kg two females died, at 1200 mg/kg three females died and at 1200 mg/kg all five males died. At 1200 mg/kg stem chloroform extract caused death of all five mice, this means that the extracts are toxic at certain doses above the dertemined lethal dose 50 (LD_{50}) which is 424 mg/kg.

Body weight and relative organ weight change are parameters that are essential in evaluation of preliminary indications of toxicity of the test substance (Sireeratawong *et al.,* 2008). Mice which were given oral dose of extracts at (300 mg/kg, 600 mg/kg and 1200 mg/kg) showed increase in body weights. Male and female mice that received leaf chloroform, leaf ethyl acetate, leaf methanolic, stem chloroform, stem ethyl acetate and stem bark methanolic extracts showed increase in body weight to controls, this increase in body weight means the extract do not interfere with body growth metabolism. In acute study relative organ weights of the tested mice showed changes in various vital organs. Kidney is an organ for excretion of different compounds, for male and female mice that received leaf chloroform extract had increased kidney relative organ weight which is might have been caused by compounds present in the extract which is associated with increased glomerural space (Burtis *et al*., 2008). Liver decreased relative organ weight for both male and females might have been caused by detoxification activity of the extract compounds. Males and females heart had no

significant change, the lung and spleen decrease in relative organ weight indicate that the extract had interference with the organ.

Hematological parameters are important in analyzing interference of the blood cell producing sites (Mukinda *et al.,* 2010). Increased WBC counts is a response of the cardiovascular system to the extracts this colloborates with other studies that revealed use of chemotherapy drugs do increase the percentage of white blood cells in patients who are receiving the medication (Lisson *et al.,* 1999) also other *Commiphora Spp* have shown to elevate WBC counts that colloborate to *C. campestris* (Haffor, 2010), this signifies that the plant has the effect on WBC count. Studies with other species of *Commiphora* plant reveled increase in RBC numbers (Rao *et al.,* 2001).

In Acute Studies male mice that received leaf chloroform extract, their hematological parameters showed that there is no significant changes in tested and control group, this means in males the extract is safe up to 1200 mg/kg dose. Females showed significant changes in blood parameters as compared to control. Increased females WBC means the extract stimulates hematopoietic system to produce more WBC, this is likely to be attributed by stress due to extract, decrease in RBC with dose level caused [HB] decreased except at 600 mg/kg where RBC increased, increased RDW (red blood cell distribution width) is a factor for determination of the level of destruction of the shape of RBC that might have also contributed to low [HB] levels, this signifies that at higher doses the extracts can cause anemia this collaborate with other studies that shows there is association of RDW and anaemia (Solak *et al.,* 2014).

Male mice that received leaf ethyl acetate extract had increased liver and decreased kidney relative organ weight respectively and these organs are essential for detoxification and excretion of different compounds so this shows the extract caused enlargement of the organs. Female mice had no significant statistical decreased relative organ weight of kidney and liver, this shows that the extract caused this due to detoxification and excretion activities of these organs. Male mice that received leaf ethyl acetate extract, had no significant change in WBC and RBC counts and its indices meaning that the extract had no effect hematopoietic processes, but data shows decrease in numbers of RBC and its indices like MCHC that is reflected in hemoglobin reduction, this can be explained by increase in spleen weight as trying to cover the amount of reduced blood in circulation, this means that the extract in higher doses it can cause anemia in mice. Females that received leaf ethyl acetate extract the WBC count, RBC, MCV, HCT and [HB] decreased significantly and MCH, MCHC, RDW

increased significantly. This decrease in total WBC, RBC counts and its indices is also supported by increase in RDW that changed shape of RBC leading to [HB] decrease which is related to decreased relative organ weight of liver, spleen and kidney which are key organs for blood production. Increased RDW means there are changes in red blood cells shapes this leads to anemia (Tekce *et al.,* 2014) but also low levels of RBC and [HB] signify liver disease (Burtis *et al.,* 2008), that might have been caused by extracts.

Male mice that received leaf methanolic extract showed significant decrease in WBC and RBC counts and its indices and [HB], RDW ($p<0.05$). This decrease in hematological values indicates there is production dysfunction in hematopoietic sites. The organ essential for this function is kidney that produces erythropoietin hormone for RBC synthesis (Burtis *et al.,* 2008), defects to kidneys results to low blood levels. Hemoglobin has decreased which is important protein component for RBC, this decrease is attributed by liver enlargement and dysfunction that might have been caused by extract detoxification. In females there was significant decrease in WBC count, RBC, MCV, HCT and [HB] ($P<0.05$) with significant increase in MCH, MCHC and RDW ($P<0.05$). Increased RDW means the RBC changed shapes from normal to abnormal that could be a cause of reduced [HB]. Studies shows that there is association of kidney diseases with RDW increment, meaning that the extract also caused kidney dysfunction (Solak *et al.,* 2014), this is also supported by decrease in relative organ weight of the spleen, kidney and liver resulting to reduced number of WBC and RBC synthesized as a result of reduced synthesis of essential proteins by liver and kidney. Decrease in RBC, HCT, [HB] and increase in RDW parameters could be due to liver and kidney dysfunction (Burtis *et al.,* 2008).

Male mice given leaf methanolic extract showed no significant change in relative organ weight of the heart ($P>0.05$), this means extract has no effect in the organ. Decreased relative organ weight of liver and increased kidneys relative organ weight was caused by extract due to detoxification and excretion of the compounds. Lung and spleen increased relative organ weight might be due to overwork of the lung and spleen to produce more blood to cover the lost blood and lung to capture more oxygen to far organs. In females that received leaf methanolic extract had decreased relative organ weight of the liver, kidneys this decrease is also linked to detoxification and excretion of the extract compounds. The extract had no impact to the heart. Spleen and lung decreased relative organ weight this might be caused so as to overcome shortage of blood in circulation.

The results showed that there was no significant change in WBC count, RBC count, MCH and RDW, ($P>0.05$) and decrease in [HB] for males that received stem bark chloroform extract. Reduced [HB] is likely to have been caused by change in RBC shape as the RDW was very high (Solak *et al.,* 2014). The extract did not cause death to mice up to a dose of 600 mg/kg, but at 1200 mg/kg all five mice died this means the extract was toxic at this dose. In females that were given stem chloroform extract there was no significant change in WBC counts but decrease in RBC counts, RBC indices and [HB] suggesting that the extract interferes with hematopoietic organs, this is linked with decreased relative organ weight of kidney and increased liver relative organ weight resulting to reduced hematopoietic proteins for synthesis of blood cells as summarized in Table 7.

Male mice that were administered with stem bark chloroform extract showed significant increase in weight of liver ($p<0.05$) and kidney (decrease at 300 mg/kg), this increase might be due to detoxification and excretion of extract. No significant increase in relative organ weight of spleen, heart and lung means the extract had no impact on the organ. In females liver and kidney decreased in relative organ weight this indicates that the extract caused atrophy of these organs as result of detoxification and excretion of the extract compounds. Heart had no significant change in relative organ weight meaning that extract had no interference with the organ. Lung and spleen however slightly increased in relative organ weight at 1200 mg/kg this might be caused by extract and spleen tried to overcome the blood shortage in circulation.

Male mice that received stem ethyl acetate extract, the results showed there is decrease in WBC count, RBC, RBC indices [HB] and significant increase RDW This signify that the extract has influence on blood production system and is supported by decreased relative organ weight of the liver and increased relative organ weight of the kidney as these two organs are important in production of RBC. In females there is significant decrease in WBC, RBC and [HB] and significant increase in RDW (Kim *et al.,* 2013), this suggest that the extract interfered the blood production sites.

Male mice that were administered with stem ethyl acetate extract had significant decrease in weight of the liver at 300 mg/kg and 1200 mg/kg and increase at 600 mg/kg this is not normal trend might be due to individual weakness and kidneys increased in size, increase or decrease to these organs means the extract causes changes due to detoxification and excretion activity hence the extract compounds causes tissue damage. Heart, lung and spleen also increased in relative organ weight this might be linked to compensation of blood in the circulation. In

female liver and kidney decrease and increase in relative organ weight respectively resulted from detoxification and excretion of compounds from the extract. Heart, lung and spleen also increased in relative organ weight this might be linked to compensation of blood in the circulation.

Male mice that received stem bark methanolic extract showed that there is significant decrease in WBC counts, RBC counts, RBC indices, HCT and [HB] ($P<0.05$) and significant increase in RDW ($P<0.05$). This means that the extract had influence on the production sites of blood cells resulting to low hemoglobin this is linked to decreased weights of the liver and kidney that caused reduced red blood cell production and increased relative organ weight of the spleen that could be a factor to compensate reduced blood supply, further more increased RDW resulted to decreased [HB] which is anaemia (Kim *et al.,* 2013). All male mice that were given a dose of 1200 mg/kg stem bark extract died, this shows that extract was toxic at this dose. Female mice that received stem bark extract showed decrease in WBC counts, RBC counts, [HB] ($P<0.05$) and significant increase seen in MCH and RDW ($P<0.05$). This means that the extract had influence on the production sites of blood cells resulting low WBC and RBC counts this is linked to decreased weights of the hemopoietic organs, like kidney and liver. The organs are important in ferritin production that is useful for blood production (Burtis *et al.,* 2008).

Male mice that were administered with stem bark metabolic extract showed decrease in weight of the liver, kidney, heart ($p<0.05$) and significant increase in weight of lungs and spleen ($p<0.05$). Females also showed significant decrease in weight of the liver, kidney, heart, lung ($p<0.05$) and significant increase of the spleen at 1200 mg/kg. Impaired functions of the vital organs may results to changes in relative organ weight as they will be compensating for their functions (Burtis *et al.,* 2008).

Extracts caused changes in histological arrangement of the vital organs, liver and kidney were changed their structure as compared to control mice, this is linked to excretory and detoxification functions of these organs. Changes include increased glomerural space of the kidney and increased sinusoid space of the liver. These organs are crucial in blood production so changes in blood components are related to the organ changes.

2.5 Conclusion

Different extracts have shown to be toxicity at different dose levels, the extracts caused changes in behavior of mice in the first four hours but later they resumed to normal behavior,

some extracts caused mortality in mice this indicates that extracts are toxic to body tissues. Hematological changes in different parameters were noted. These changes cannot be used to conclude toxicity as the time to run the acute test was very short to induce the recognizable tissues changes. This attracted sub-acute toxicity study to evaluate toxicity of the plant extracts for 28 days using rats so as to get enough blood for hematology, biochemical and histopathology analysis.

CHAPTER THREE

Sub-acute Oral Toxicity Evaluation of Commiphora campestris extracts in Rats.

Abstract

This study evaluated the in vivo sub acute toxicity of *Camiphora campestris* leaf and stem bark extract. Rats were given *C. campestris* extract by gavage at doses 150 mg/kg, 200 mg/kg and 250 mg/kg for 28 days. Body clinical changes were monitored. Rats were bright, no mortality and increased in body weights. Leaf chloroform and stem chloroform extract showed increase in relative organ weights. Leaf ethyl acetate, leaf methanolic, stem ethyl acetate extract and stem bark methanolic extract caused decrease in relative organ weight. Leaf chloroform extract caused increase in glucose, cholesterol, total protein, albumin, triglycerides. Leaf ethyl acetate and leaf methanolic extract caused significant decrease cholesterol and total protein. Stem chloroform extract caused significant decreased albumin, cholesterol and ALP. Stem ethyl acetate and stem bark methanolic extract caused decreased albumin, cholesterol, creatinine and AST. In hematology, leaf chloroform, leaf ethyl acetate, leaf methanol, stem chloroform and stem ethyl acetate extracts caused significantly decreased WBC, RBC, [HB] and stem bark methanolic extract caused increase in RDW, WBC and decrease in RBC and [HB]. This study concludes that in sub acute doses *C. campestris* extracts are toxic at higher doses. The extracts reduced cholesterol levels indicating antilipidemic effect. More research to validate safety doses in humans is still needed.

Key words: *Commiphora campestris*, acute toxicity, sub acute toxicity, Dimethyl Sulphoxide (DSMO), Mortality, Hematology, Histopathology and Serum biochemistry

3.0 Introduction

3.1 Background

Commiphora campestris stem barks are used by the Pare tribe for treatment human infectious diseases, therefore this study evaluated *in vivo* toxicity profile of *C. campestris* using sub-acute study in a rat model. Medicinal plants prepared using African knowledge have played a significant role in provision of health care services for many years even before the introduction of orthodox conventional medicines in African countries. The growing demand for herbal products necessitates the provision of evidence on the efficacy, safety and quality of herbal medicines (WHO, 2002).

It is in this vein that medicinal plants used for management of microbial infections and non infectious diseases in Tanzania are being evaluated for their antimicrobial and efficacy properties by Natural Products Research (NPR) in Tanzania. One of such plants is *C. campestris* also called "Msighe" in the Pare language, has been used for many years by Pare communities for the management of coughs, wounds, diarrhoea, gastrointestinal infections and has recently found its way to the markets. The latex from the chopped stem bark inhibits the growth of *Klebsiella oxytoca* (clinical isolate), *Klebsiella pneumoniae* (ATCC700603), *Salmonella kisarawe* (clinical isolate), *Proteus mirabilis* (NCTC 1075), *Salmonella typhi* (NCTC 8385), *Pseudomonas aeruginosa* (ATCC 29953), *Escherichia coli* (ATCC 25922), *Cryptococcus neuformans* (clinical isolate) and *Candida albicans* (ATCC90028). It is therefore likely *C. campestris* is a potential source of drug templates for the management of *Gram* negative bacteria and fungi. This discovery necessitated toxicological studies on the *C. campestris* (Godfrey *et al.*, 2016).

3.2 Material and Methods

3.2.1 Plant materials

Commiphora campestris leaf and stem barks were collected from Pare Mountains at Kisiwani Village in Same District, Kilimanjaro, Tanzania in April, 2016. Identification was performed by Mr. Emmanuel Mboya, a botanist from Tropical Pesticides Research Institute (TPRI), Arusha, Tanzania. Voucher specimens were stored at NM-AIST.

3.2.2Preparation of the extracts

Fresh collected *C. campestris* leaf and stem barks were washed and dried under the shade, then pulverized into powder using Swinging Traditional Chinese Medicine Pulveriser,

Diaxiang Electronic Equipment (DXF-20D). Leaves and stem bark powder weighing 500 g each was consecutively extracted in three solvents namely chloroform, ethyl acetate and methanol, by soaking in 1 litre of a solvent for 72 hours then sieved and filtered using Whatman filter size 40. After extraction crude extracts were stored at -20°C until further use.

3.2.3 Animals and treatment

In this sub acute toxicity study, forty (40) Winstar albino rats (20 females and 20 males) weighing 62 – 96 g for male sex and 45 - 77 g for female sex for each extract testing, bought from the Sokoine University of Agriculture (SUA) were used. Same sex and litter were kept in a meshed cage with dimensions of 10 cm x 10 cm x10 cm (1000 cm^3) covered with saw dust beddings to avoid mating and fighting. Light was set at 12 hour light supply and 12 hour darkness. Each cage was identified for extract and dose level. Animals were left to acclimatize for 1 week before administration extracts. The ethical clearance number NIMR/HQ/R.8a/Vol.IX/2396 was sought from NIMR Tanzania.

3.2.4 Sub-acute toxicity evaluation

Sub acute toxicity was carried out according to OECD (OECD, 2008). Forty (40) rats were divided into two groups per sex (20 male rats and 20 female rats) for each extract. 20 rats per each sex were grouped into four groups of five rats per group. Males and females were separated to avoid mating and fighting. The male rats were grouped such that, the first group was a control group and received 10% DMSO, the second group received a dose of 150 mg/kg, third group received 200 mg/kg and the fourth group received 250 mg/kg of extract. The same grouping was done to female sex rats. Three dose categories of (150 mg/kg, 200 mg/kg and 250 mg/kg) were determined following determination of the Lethal Dose 50 in the acute toxicity study. The rats were marked such that first group (control) was unmarked, the second group (150 mg/kg) was marked by one tail mark, the third group (200 mg/kg) by two tail marks and the third group (250 mg/kg) by three tail marks. Body weights were measured and recorded at every time of *C. campestris* extract administration.

3.3 Collection of tissue samples

3.3.1 Blood sample collection

Blood samples were collected on the 28^{th} day (the last day of the experiment) from retro orbital plexus of the eye using VITREX NRIS soda lime glass 80IU/ml heparinised microhematocrit tubes into EDTA and plain vacutainer tubes for whole blood and serum

samples respectively thereafter rats were sacrificed and vital organs (liver, lung, kidney and spleen) collected, weighed and fixed in 10% buffered formalin.

3.3.2 Relative organ weights (row) assay

The relative vital organ weights were taken by using Meltzer sensitive weighing balance.

3.3.3 Hematology

In sub acute tests, blood samples were drawn from the test rats on day 28^{th} and were analyzed for different hematological blood parameters using *NS4s* automated analyzer (Germany), The hematological parameters analyzed included: packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count (lymphocytes and monocytes), and hemoglobin concentration [Hb] concentration. Others were; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT), red cell distribution width (RDW) and recorded as Mean ± Standard Error.

3.3.4 Biochemical parameters examination

Biochemical parameters were analysed using procedures outlined in the Biosystems Laboratory Kit (S.A. Costa Brava, 30. 08030, Barcelona, Spain). The serum samples were assayed for lipid profile (cholesterol and triglycerides), liver enzymes bio marker (ALT, AST and ALP), kidney function tests (serum urea and creatinine), blood glucose, albumin, total protein and bilirubin.

3.3.5 Histopathology

At the end of the experiments all rats were humanely sacrificed, vital organs were taken and fixed in buffered formalin 10%, for one week, then washed in ascending grades of ethanol, cleared with xylene, embedded in paraffin wax, sectioned by microtome and stained with Haematoxylin and Eosin (H & E) then mounted on Canada balsam. All sections were examined under a light microscope at 4x, 10x, 20x 40x and 100x magnifications and photomicrographs taken at 40 x magnification using Olympus photomicroscope for observation and documentation of histopathological lesions.

3.3.6 Statistical data analysis

Results are presented as mean ± SEM and were analyzed by One Way – ANOVA using Statistica Version 8, (StatSoft, 2007). The values of $P< 0.05$ were considered statistically significant.

3.4 Results

3.4.1 Clinical observation in sub-acute toxicity

Throughout 28 days of the sub acute toxicity study, no sign of toxicity or mortality were observable. All test rats survived throughout the testing time.

3.4.2 Body weight in sub-acute toxicity study

Body weights of rats that received *C. campestris* extract increased from day 0 to day 28. The ($P<0.05$) suggests significant weight increase in each group. At Day 0, for males ($P<0.05$) and for females at Day 14 ($P<0.05$) suggests there was no significant weight change. Female rats that received leaf and stem ethyl acetate extracts showed statistically non significant change in body weight, however relative organ weight values shows increase in weight as indicated in Table 10.

Table 10: Body weight of male and female rats after sub acute exposure to leaf chloroform extract expressed as mean ± sem

			Dose (mg/kg Bwt)				
Extract	**Sex**	**Bwt (g)**	**0**	**150**	**200**	**250**	**p-value**
LCE	Males	Day 0	101.1±0.33	115.37±6.37	110.17±8.91	113.77±6.04	0.012
		Day 7	117.12±0.38	143.96±8.78	128.56±8.7	148.36±5.12	0.006
		Day 14	131.13±0.43	136.16±8.93	136.16±8.93	148.36±4.27	0.003
		Day 21	154.15±0.5	166.96±8.92	145.76±6.01	163.56±3.23	0.002
		Day 28	161.16±0.52	205.74±13.61	151.97±11.78	195.95±2.2	0.002
	Females	Day 0	122.12±0.4	70.16±2.8	62.96±0.17	108.53±1.42	0.002
		Day 7	135.14±0.44	94.94±4.99	90.35±0.48	141.31±1.63	0.001
		Day 14	139.14±0.45	91.15±0.33	91.15±0.33	131.92±1.08	0.002
		Day 21	162.16±0.53	115.33±5.59	109.33±0.66	145.51±2.41	0.000
		Day 28	163.16±0.53	121.72±4.2	156.5±9.39	142.71±3.2	0.0000012
LEA	Male	Day 0	101.1±0.33	114.37±0.92	109.97±10.76	116.57±12.48	0.0022
		Day 7	117.12±0.38	143.56±4.22	114.97±11.67	134.77±10.37	0.0001
		Day 14	133.36±3.63	133.36±3.63	145.36±12.05	135.77±8.34	0.0003
		Day 21	154.15±0.5	156.56±4.48	158.16±10.88	145.16±5.41	0.0069
		Day 28	161.16±0.52	197.55±5.25	188.15±11.47	193.35±7.13	0.0129
	Female	Day 0	122.12±0.4	52.77±2.81	42.78±2.81	93.34±12.59	0.0108
		Day 7	135.14±0.44	85.15±4.79	66.36±1.6	118.52±13.39	0.0445
		Day 14	85.95±4.99	85.95±4.99	68.36±0.62	110.33±11.59	0.0508
		Day 21	162.16±0.53	107.13±5.79	84.55±0.45	88.35±12.41	0.0726
		Day 28	163.16±0.53	114.93±4.99	93.14±0.33	95.55±9.62	0.0340
SME	Males	Day 0	101.1±0.33	123.97±14.41	84.78±5.22	77.58±8.02	0.0183
		Day 7	117.12±0.38	142.97±14.46	118.57±8.21	93.17±9.25	0.0040
		Day 14	131.13±0.43	116.37±6.64	116.37±6.64	90.38±6.88	0.0110
		Day 21	154.15±0.5	129.37±20.09	124.77±3.58	105.17±4.65	0.0078
		Day 28	161.16±0.53	130.97±21.07	154.36±3.04	132.16±9.06	0.0528
	Females	Day 0	122.12±0.4	117.13±0.85	66.36±0.62	79.56±5.61	0.0005
		Day 7	135.14±0.44	140.71±3.2	90.14±1.8	91.75±4.82	0.0010
		Day 14	139.14±0.45	87.35±1.61	87.35±1.61	101.94±4.99	0.0007
		Day 21	162.16±0.53	138.12±1.82	98.54±0.67	99.14±3.23	0.0008
		Day 28	163.16±0.53	152.91±1.06	123.73±1.23	120.13±2.24	0.0000

SCE	Males	Day 0	101.1±0.33	109.97±2.56	136.96±9.57	109.57±7.03	0.0001
		Day 7	117.12±0.38	134.77±2.17	140.37±9.13	120.97±7.97	0.0069
		Day 14	131.13±0.43	144.16±7.76	144.16±7.76	126.17±5.32	0.0000
		Day 21	154.15±0.5	157.56±7.37	168.15±9.59	138.56±3.79	0.00005
		Day 28	161.16±0.52	163.16±7.61	171.96±4.33	172.75±4.2	0.0082
	Females	Day 0	122.12±0.4	54.97±3	50.57±3.4	48.57±1.4	0.0309
		Day 7	135.14±0.44	60.36±3.59	46.17±2.21	52.17±0.25	0.0031
		Day 14	139.14±0.45	54.97±3.01	55.17±1.8	60.36±1.6	0.0046
		Day 21	162.16±0.53	72.36±1.6	72.36±1.6	78.55±1.41	0.0034
		Day 28	163.16±0.53	88.54±3.4	87.75±0.31	81.75±2.82	0.0007
SEA	Males	Day 0	101.1±0.33	99.57±3.92	88.78±8.84	120.57±8.97	0.0022
		Day 7	117.12±0.38	117.77±3.21	114.77±5.59	125.37±6.82	0.0001
		Day 14	127.76±6.05	127.76±6.05	122.97±5.51	117.97±6.53	0.0003
		Day 21	154.15±0.5	139.36±4.61	143.36±7.13	153.16±7.39	0.0069
		Day 28	161.16±0.52	155.76±7.86	155.36±6.06	170.56±9.76	0.0129
	Females	Day 0	122.12±0.4	64.56±1.4	93.75±3.82	69.56±0.64	0.0108
		Day 7	135.14±0.44	86.95±2	127.32±6.59	64.36±2.42	0.0445
		Day 14	78.35±2.6	78.35±2.6	118.73±5.19	74.15±3.79	0.0508
		Day 21	162.16±0.53	87.35±4.42	126.32±1.62	94.94±5.99	0.0726
		Day 28	163.16±0.53	118.33±1.62	144.51±1.44	102.14±6.22	0.034
SME	Males	Day 0	68.12±1.53	68.12±0.09	70.38±3.21	70.98±3.42	0.1743
		Day 7	76.88±1.73	81.8±0.48	85.8±2.03	89.98±2.67	0.0419
		Day 14	87.58±1.97	92.1±0.6	93.8±2.71	96.58±2.5	0.1743
		Day 21	98.29±2.21	97.8±0.58	112.57±7.14	117.8±6.69	0.0450
		Day 28	118.72±2.67	121.26±1.11	130.96±1.16	133.16±2.49	0.0101
	Females	Day 0	59.36±1.33	66±0.09	64.96±4.91	69.76±0.4	0.9510
		Day 7	69.58±1.56	76.8±3.23	81.95±4.41	84.18±6.28	0.0001
		Day 14	77.85±1.75	85.34±8.59	86.35±3.6	90.63±4.56	0.0139
		Day 21	86.61±1.94	98.13±11.79	101.16±4.56	123.72±9.79	0.0282
		Day 28	100.23±2.25	113.12±12.59	115.33±2.6	128.72±5.56	0.0001

Values are expressed as Mean ± Sem, Bwt = Body Weight, Row = Relative organ weight, LCE = leaf chloroform extract, LEA = leaf ethyl acetate extract, LME = leaf methanolic extract, SCE = stem bark chloroform extract, SEA = stem ethyl acetate extract, SME = stem bark methanolic extract.

3.4.3 Relative organ weights (ROW) in sub acute toxicity study

Relative organ weight of vital organs of tested rats significantly decreased ($P<0.05$) however the lungs of male rats significantly increased ($P<0.05$) compared to control. Male rats that received leaf chloroform extract showed increase in Row ($p<0.05$) while in females the ROW significantly decreased in ROW ($P<0.05$). Male rats that received leaf ethyl acetate extract shows decrease in ROW except the lung which shows increase in row ($p<0.05$) while in females the ROW significantly decreased in weight ($P<0.05$). Male rats that received leaf methanolic extract showed decrease in ROW except the lung and spleen which shows increase in row ($p<0.05$) while in females the ROW significantly decreased in weight ($P<0.05$). Male rats that received stem chloroform extract shows increase in row of all vital and decrease in spleen ($p<0.05$) while in females the ROW significantly decreased in weight ($P<0.05$). Male rats that received stem ethyl acetate extract shows decrease in ROW except the lung which shows increase in row ($p<0.05$) while in females the ROW significantly decreased in weight ($P<0.05$). Male rats that received stem bark methanolic extract shows decrease in relative organ weight (ROW) except the lung which shows increase in row ($p<0.05$) while in females the ROW significantly decreased in weight ($P<0.05$). The results are summarized in Table 11.

Table 11: Relative organ weight of rats after sub acute exposure to leaf chloroform extract expressed as Mean ± Sem

			Dose (mg/kgBwt)				
Extract	**Sex**	**ROW(g)**	**0**	**150**	**200**	**250**	**p-value**
LCE	Males	Lungs	0.82±0.01	0.92±0.01	1.17±0.01	1.56±0.01	0.0005
		Kidney	0.99±0.01	1.13±0.01	1.24±0.01	1.31±0.01	0.0005
		Liver	3.74±0.02	6.22±0.03	6.22±0.03	7.44±0.01	0.0006
		Heart	0.66±0.01	0.56±0.01	0.69±0.01	0.84±0.02	0.0007
		Spleen	0.35±0.01	0.36±0.02	0.34±0.01	0.28±0.01	0.0006
	Females	Lung	1.5±0.01	1.28±0.03	0.94±0.03	0.58±0.03	0.0005
		Kidney	1.25±0.00	1.11±0.02	0.98±0.02	0.83±0.03	0.00001
		Liver	6.04±0.02	3.32±0.10	3.32±0.10	3.16±0.08	0.0007
		Heart	0.63±0.00	0.6±0.01	0.59±0.01	0.44±0.03	0.0003
		Spleen	0.5±0.00	0.39±0.01	0.3±0.01	0.27±0.03	0.0000
LEAE	Males	Lungs	0.82±0.01	1.36±0.02	1.3±0.01	1.36±0.04	0.0026
		Kidney	0.99±0.01	1.09±0.03	1.03±0.01	0.97±0.01	0.0026
		Liver	5.39±0.24	5.39±0.24	4.86±0.01	4.96±0.04	0.0007
		Heart	0.66±0.01	0.72±0.01	0.57±0.02	0.69±0.01	0.0009
		Spleen	0.35±0.01	0.39±0.01	0.33±0.02	0.3±0.01	0.000018
	Females	Lung	1.5±0.01	0.77±0.03	0.65±0.02	0.87±0.02	0.0005
		Kidney	1.25±0.01	0.82±0.03	0.78±0.02	0.93±0.02	0.0009
		Liver	6.04±0.03	4.15±0.12	3.76±0.11	4.42±0.11	0.0013
		Heart	0.63±0.00	0.47±0.03	0.32±0.03	0.48±0.02	0.00002
		Spleen	0.5±0.00	0.25±0.02	0.17±0.01	0.28±0.01	0.0008
LME	Males	Lungs	0.82±0.01	0.97±0.01	1.27±0.04	1.4±0.01	0.0007
		Kidney	0.99±0.00	0.77±0.01	0.95±0.02	0.86±0.01	0.0000
		Liver	3.74±0.02	4.6±0.01	4.6±0.01	4.54±0.01	0.0005
		Heart	0.66±0.01	0.58±0.01	0.98±0.01	0.62±0.01	0.0012
		Spleen	0.35±0.01	0.41±0.01	0.35±0.01	0.42±0.01	0.0022
	Female	Lung	1.5±0.01	0.76±0.02	0.72±0.01	0.55±0.01	0.0007
		Kidney	1.25±0.00	0.98±0.02	0.84±0.01	0.8±0.02	0.0008
		Liver	6.04±0.02	3.57±0.07	3.57±0.07	3.27±0.07	0.0007
		Heart	0.63±0.00	0.48±0.01	0.51±0.01	0.51±0.01	0.0126
		Spleen	0.5±0.00	0.31±0.02	0.3±0.01	0.26±0.01	0.0024

Extract	Sex	ROW(g)	Dose (mg/kgBwt)				p-value
			0	150	200	250	
SCE	Males	Lungs	0.82±0.01	1.31±0.01	1.16±0.01	1.44±0.01	0.0005
		Kidney	0.99±0.01	1.2±0.01	1.38±0.02	1.12±0.02	0.0000
		Liver	3.74±0.02	4.78±0.01	4.78±0.01	4.82±0.01	0.0006
		Heart	0.66±0.01	0.75±0.01	0.69±0.01	0.58±0.02	0.0000
		Spleen	0.35±0.01	0.36±0.01	0.34±0.01	0.27±0.01	0.0045
	Females	Lung	1.5±0.01	0.87±0.02	0.95±0.02	1.17±0.03	0.0006
		Kidney	1.25±0.01	0.87±0.03	0.94±0.03	1.09±0.03	0.0000
		Liver	6.04±0.03	4.6±0.15	4.6±0.15	5.8±0.14	0.0011
		Heart	0.63±0	0.47±0.02	0.49±0.06	0.56±0.02	0.0337
		Spleen	0.5±0	0.35±0.01	0.29±0.01	0.24±0.02	0.0009
SEAE	Males	Lungs	0.82±0.01	1.2±0.01	2.03±0.01	1.06±0.01	0.0004
		Kidney	0.98±0.02	0.98±0.02	1.19±0.02	1.28±0.04	0.00001
		Liver	3.74±0.02	4.33±0.1	5.27±0.36	4.94±0.03	0.0003
		Heart	0.66±0.01	0.71±0.02	0.58±0.02	0.75±0.03	0.0044
		Spleen	0.35±0.01	0.38±0.01	0.36±0.02	0.39±0.02	0.0007
	Females	Lung	1.5±0.01	0.85±0.04	0.94±0.03	1.18±0.04	0.0007
		Kidney	1.25±0.01	0.92±0.04	1.07±0.04	0.86±0.05	0.0008
		Liver	4.5±0.13	4.5±0.13	5.73±0.25	4.49±0.13	0.0037
		Heart	0.63±0	0.41±0.03	0.54±0.03	0.49±0.03	0.0082
		Spleen	0.5±0	0.27±0.03	0.34±0.02	0.24±0.02	0.0027
SME	Males	Lungs	0.82±0.01	0.92±0.09	0.83±0.01	0.85±0.01	0.0037
		Kidney	0.99±0.02	0.97±0.01	0.8±0.02	0.72±0.03	0.0000
		Liver	3.74±0.02	3.68±0.01	3.66±0.01	3.22±0.11	0.0009
		Heart	0.66±0.01	0.59±0.01	0.53±0.01	0.46±0.02	0.0000
		Spleen	0.35±0.01	0.31±0.01	0.29±0.01	0.25±0.01	0.00003
	Females	Lung	1.5±0.01	1.28±0.05	0.92±0.02	0.59±0.07	0.0005
		Kidney	1.25±0.02	1.07±0.04	0.99±0.03	0.84±0.05	0.0000
		Liver	6.04±0.02	4.83±0.27	3.35±0.06	3.07±0.06	0.0007
		Heart	0.6±0.04	0.59±0.03	0.57±0.02	0.49±0.03	0.0034
		Spleen	0.53±0.02	0.4±0.03	0.29±0.01	0.3±0.02	0.0000

Values are expressed as Mean ± Sem, Bwt = Body Weight, ROW = Relative organ weight, LCE = leaf chloroform extract, LEA = leaf ethyl acetate extract, LME = leaf methanolic extract, SCE = stem bark chloroform extract, SEA = stem ethyl acetate extract, SME = stem bark methanolic extract.

3.4.4 Hematology in sub acute toxicity study

Male rats that received leaf chloroform extract shows decrease in WBC Counts, RBC and [HB] with increase in RDW ($p<0.05$) while in females the hematological parameters significantly increased and decrease in RDW ($P<0.05$) as indicated in Table 12.

Table 12: Effects of leaf chloroform extract on white blood cell counts, RBC counts and its indices expressed as mean ± sem

		Dose in mg/kgBwt				
Sex	**Parameter**	**0**	**150**	**200**	**250**	**p- value**
Males	WBC (m/mm^3)	127.75±0.42	69.41±9.76	80.25±9.76	67.26±2.78	0.095
	Lymp (#)	60.96±0.2	96.00±10.68	71.94±10.68	85.27±3.69	0.0026
	Mon (#)	17.72±0.06	4.00±2.88	12.95±2.88	11.13±2.08	0.0026
	RBC(m/mm^3)	16.41±0.05	8.92±0.64	7.74±0.64	7.46±0.21	0.0052
	MCV(fl)	55.96±0.18	64.36±0.25	61.58±0.25	61.48±0.13	0.0011
	HCT (%)	91.69±0.3	57.26±4.22	47.6±4.22	45.71±1.19	0.0052
	MCH(pg)	10.31±0.03	17.92±1.4	21.34±1.4	21.55±0.11	0.0031
	MCHC(g/dl)	18.42±0.06	27.93±2.46	34.89±2.46	35.21±0.24	0.0010
	RDW	19.82±0.06	24.92±3.68	17.37±3.68	11.09±0.15	0.018
	[Hb] (g/dl)	16.92±0.06	16.02±0.26	16.2±0.26	16.11±0.48	0.098
Females	WBC (m/mm^3)	20.03±0.09	120.19±0.38	115.97±0.38	101.83±0.33	0.0002
	Lymp (#)	8.01±0.03	52.36±0.17	51.75±0.17	53.85±0.18	0.0022
	Mon (#)	4.00±0.01	15.85±0.05	16.32±0.05	25.33±0.08	0.001
	RBC(m/mm^3)	5.27±0.02	14.40±0.05	16.66±0.05	16.99±0.06	0.0005
	MCV(fl)	59.26±0.19	57.68±0.19	58.16±0.19	58.06±0.19	0.00002
	HCT (%)	31.13±0.1	82.86±0.31	96.7±0.31	98.5±0.32	0.0005
	MCH(pg)	23.02±0.07	10.54±0.03	8.71±0.03	8.91±0.03	0.0005
	MCHC(g/dl)	38.94±0.13	18.27±0.05	15.12±0.05	15.42±0.05	0.0005
	RDW	11.71±0.04	21.81±0.05	14.11±0.05	12.61±0.04	0.0005
	[Hb] (g/dl)	12.11±0.04	15.1±0.05	14.61±0.05	15.22±0.05	0.01

Values are expressed as Mean ± Sem, Bwt = Body Weight, WBC = White blood cells, Lymph = Lymphocytes, Mon = Monocytes, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, HCT = Hematocrit, MCH = Mean Cell Hemoglobin, MCHC = Mean Cell Hemoglobin Concetration, RDW = Red Cell Distribution Width, [HB] = Hemoglobin concentration, # = Number of cells.

Male rats that received leaf ethyl acetate showed decrease in WBC Counts, RBC and [HB] with increase in RDW ($p<0.05$) while in females the hematological parameters significantly increased and decrease in RDW ($P<0.05$) as indicated in Table 13.

Table 13: Effects of leaf ethyl acetate extract on white blood cell counts, RBC counts and its indices expressed as mean ± sem

		Dose in mg/kgBwt				
Sex	**Parameter**	**0**	**150**	**200**	**250**	**p-value**
Males	WBC (m/mm^3)	127.75±0.42	103.66±0.34	101.95±0.46	99.1±0.34	0.0005
	Lymp (#)	60.96±0.2	48.35±0.16	48.71±0.14	46.21±0.1	0.0007
	Mon (#)	17.72±0.06	21.52±0.07	28.54±0.08	29.13±0.06	0.0005
	RBC(m/mm^3)	16.41±0.05	13.07±0.04	8.79±0.03	8.52±0.02	0.0005
	MCV(fl)	55.96±0.18	57.46±0.19	64.65±0.18	62.68±0.13	0.0005
	HCT (%)	91.69±0.3	74.97±0.24	56.28±0.16	53.14±0.11	0.0005
	MCH(pg)	10.31±0.03	11.31±0.04	18.66±0.05	18.58±0.04	0.0008
	MCHC(g/dl)	18.42±0.06	19.72±0.06	29.25±0.08	29.73±0.06	0.0005
	RDW	19.82±0.06	22.12±0.07	13.92±0.04	26.12±0.06	0.0005
	[Hb] (g/dl)	16.92±0.06	14.81±0.05	16.34±0.05	15.77±0.03	0.0000
Females	WBC (m/mm^3)	20.03±0.09	97.33±0.32	101.55±0.33	120.11±0.39	0.0005
	Lymp (#)	8.01±0.03	53.75±0.18	48.35±0.16	50.05±0.16	0.0005
	Mon (#)	4±0.01	13.71±0.05	28.33±0.09	15.42±0.05	0.0005
	RBC(m/mm^3)	5.27±0.02	9.68±0.03	8.73±0.03	13.9±0.05	0.0005
	MCV(fl)	59.26±0.19	59.66±0.19	64.16±0.21	57.76±0.19	0.0005
	HCT (%)	31.13±0.1	57.66±0.19	55.86±0.18	80.18±0.26	0.0005
	MCH(pg)	23.02±0.07	13.91±0.05	18.52±0.06	10.91±0.04	0.0005
	MCHC(g/dl)	38.94±0.13	23.42±0.08	29.03±0.1	18.92±0.06	0.0005
	RDW	11.71±0.04	23.42±0.08	13.81±0.05	18.72±0.06	0.0005
	[Hb] (g/dl)	12.11±0.04	13.51±0.04	16.22±0.05	15.22±0.05	0.0000

Values are expressed as Mean ± Sem, Bwt = Body Weight, WBC = White blood cells, Lymph = Lymphocytes, Mon = Monocytes, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, HCT = Hematocrit, MCH = Mean Cell Hemoglobin, MCHC = Mean Cell Hemoglobin Concetration, RDW = Red Cell Distribution Width, [HB] = Hemoglobin concentration, # = Number of cells.

Male rats that received leaf methanolic extract showed decrease in WBC Counts, RBC and [HB] with increase in RDW ($p<0.05$) while in females the hematological parameters significantly increased ($P<0.05$) as indicated in Table 14.

Table 14: Effects of leaf methanolic extract on white blood cell counts, RBC counts and its indices expressed as mean ± sem

		Dose in mg/kgBwt				
Sex	**Parameter**	**0**	**150**	**200**	**250**	**p-value**
Males	WBC (m/mm^3)	127.75±0.42	97.75±107.6	107.6±0.23	79.51±4.42	0.0000
	Lymp (#)	60.96±0.2	47.45±0.15	47.08±0.58	76.36±10.13	0.0023
	Mon (#)	17.72±0.06	30.83±0.1	23.17±1.88	17.44±6.37	0.0552
	RBC(m/mm^3)	16.41±0.05	10.72±0.03	9.15±0.17	9.5±0.07	0.0009
	MCV(fl)	55.96±0.18	60.46±0.2	64.81±0.25	64.17±0.73	0.011
	HCT (%)	91.69±0.3	64.66±0.21	59.19±1.04	60.82±0.18	0.001
	MCH(pg)	10.31±0.03	14.41±0.05	18.61±0.12	17.72±0.17	0.0005
	MCHC(g/dl)	18.42±0.06	23.92±0.08	28.84±0.2	27.68±0.07	0.0005
	RDW	19.82±0.06	23.12±0.08	13.84±0.05	18.24±2.67	0.018
	[Hb] (g/dl)	16.92±0.06	15.52±0.05	17.06±0.23	16.86±0.04	0.0115
Female	WBC (m/mm^3)	20.03±0.09	115.12±0.37	110.11±0.36	113.7±0.37	0.0006
	Lymp (#)	8.01±0.03	48.35±0.16	51.75±0.17	51.15±0.17	0.0006
	Mon (#)	4±0.01	16.92±0.06	19.92±0.06	17.32±0.06	0.0005
	RBC(m/mm^3)	5.27±0.02	16.58±0.05	12.21±0.04	15.68±0.05	0.0005
	MCV(fl)	59.26±0.19	57.06±0.19	58.86±0.19	56.86±0.19	0.0005
	HCT (%)	31.13±0.1	94.39±0.31	71.77±0.23	88.99±0.29	0.0005
	MCH(pg)	23.02±0.07	9.21±0.03	12.01±0.04	9.91±0.03	0.0005
	MCHC(g/dl)	38.94±0.13	16.32±0.05	20.52±0.07	17.52±0.06	0.0005
	RDW	11.71±0.04	21.42±0.07	8.11±0.03	17.62±0.06	0.0005
	[Hb] (g/dl)	12.11±0.04	15.42±0.05	14.71±0.05	15.62±0.05	0.0006

Values are expressed as Mean ± Sem, Bwt = Body Weight, WBC = White blood cells, Lymph = Lymphocytes, Mon = Monocytes, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, HCT = Hematocrit, MCH = Mean Cell Hemoglobin, MCHC = Mean Cell Hemoglobin Concetration, RDW = Red Cell Distribution Width, [HB] = Hemoglobin concentration, # = number of cells, # = Number of cells.

Male rats that received stem chloroform extract showed decrease in WBC Counts, RBC and [HB] with increase in RDW ($p<0.05$) while in females the hematological parameters significantly increased ($P<0.05$) as indicated in Table 15.

Table 15: Effects of stem chloroform extract on white blood cell counts, RBC counts and its indices expressed as mean ± sem

		Dose in mg/kgBwt				
Sex	**Parameter**	**0**	**150**	**200**	**250**	**p - value**
Males	WBC (m/mm^3)	127.75±0.42	92.96±3.78	81.44±1.85	73.75±4.07	0.0032
	Lymp (#)	60.96±0.2	54.93±2.73	69.85±4.72	79.54±7.32	0.009
	Mon (#)	17.72±0.06	29.04±0.08	24.86±2.79	16.25±5.27	0.0288
	RBC(m/mm^3)	16.41±0.05	8.56±0.04	8.31±0.15	7.57±0.37	0.0037
	MCV(fl)	55.96±0.18	63.93±0.29	63.11±0.47	62.9±0.21	0.0053
	HCT (%)	91.69±0.3	54.59±0.32	52.34±1.22	47.54±2.58	0.0032
	MCH(pg)	10.31±0.03	18.17±0.27	18.41±0.45	21.18±1.5	0.0107
	MCHC(g/dl)	18.42±0.06	28.52±0.39	29.27±0.89	33.83±2.56	0.0107
	RDW	19.82±0.06	26.38±0.17	26.19±0.05	17.15±3.61	0.0073
	[Hb] (g/dl)	16.92±0.06	15.56±0.26	15.29±0.14	15.8±0.36	0.0107
Females	WBC (m/mm^3)	20.03±0.09	113.48±0.37	63.36±0.21	37.51±0.12	0.0005
	Lymp (#)	8.01±0.03	49.45±0.16	90.29±0.29	99±0.32	0.0005
	Mon (#)	4.0±0.01	17.12±0.06	7.91±0.03	1.1±0	0.0005
	RBC(m/mm^3)	5.27±0.02	13.24±0.04	3.83±0.01	0.56±0	0.0005
	MCV(fl)	59.26±0.19	58.16±0.19	63.16±0.21	61.86±0.2	0.0005
	HCT (%)	31.13±0.1	76.88±0.25	24.12±0.08	3.4±0.01	0.0005
	MCH(pg)	23.02±0.07	11.11±0.04	19.52±0.06	1.7±0.01	0.0005
	MCHC(g/dl)	38.94±0.13	19.22±0.06	31.13±0.1	2.9±0.01	0.0005
	RDW	11.71±0.04	24.42±0.08	12.01±0.04	11.81±0.04	0.0011
	[Hb] (g/dl)	12.11±0.04	14.81±0.05	7.51±0.02	0.1±0	0.0005

Values are expressed as Mean ± Sem, Bwt = Body Weight, WBC = White blood cells, Lymph = Lymphocytes, Mon = Monocytes, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, HCT = Hematocrit, MCH = Mean Cell Hemoglobin, MCHC = Mean Cell Hemoglobin Concetration, RDW = Red Cell Distribution Width, [HB] = Hemoglobin concentration, # = number of cells.

Male rats that received stem ethyl acetate extract shows decrease in WBC Counts, RBC and [HB] with increase in RDW ($p<0.05$) while in females the hematological parameters significantly increased ($P<0.05$) and decrease in RDW at a dose of 250 mg/kg as indicated in Table 16.

Table 16: Effects of stem ethyl acetate extract on white blood cell counts, RBC counts and its indices expressed as mean ± sem

		Dose in mg/kgBwt				
Sex	**Parameter**	**0**	**150**	**200**	**250**	**p- value**
Males	WBC (m/mm^3)	127.75±0.42	59.18±1.05	38.19±15.53	106.03±1.45	0.001
	Lymp (#)	60.96±0.2	93.29±0.82	99.79±0.25	49.13±1.16	0.0005
	Mon (#)	17.72±0.06	5.53±0.67	0.24±0.10	18.5±1.42	0.001
	RBC(m/mm^3)	16.41±0.05	7.83±0.12	7.65±0.06	8.3±0.54	0.0102
	MCV(fl)	55.96±0.18	62.89±0.78	61.24±0.44	61.6±0.43	0.0063
	HCT (%)	91.69±0.3	49.12±1.2	46.71±0.59	51.13±3.79	0.0095
	MCH(pg)	10.31±0.03	21.69±0.09	20.68±0.54	18.31±0.12	0.001
	MCHC(g/dl)	18.42±0.06	34.56±0.24	33.91±1.07	29.8±0.47	0.001
	RDW	19.82±0.06	11.04±0.2	12.57±0.99	21.7±3.21	0.0005
	[Hb] (g/dl)	16.92±0.06	16.96±0.26	15.82±0.33	15.17±0.92	0.1909
Females	WBC (m/mm^3)	20.03±0.09	107.9±0.35	112.2±0.37	113.41±0.37	0.0006
	Lymp (#)	8.01±0.03	47.05±0.15	49.95±0.16	52.45±0.17	0.0005
	Mon (#)	4±0.01	20.72±0.07	18.02±0.06	17.72±0.06	0.0006
	RBC(m/mm^3)	5.27±0.02	7.39±0.02	12.87±0.04	14.96±0.05	0.0005
	MCV(fl)	59.26±0.19	60.66±0.2	55.36±0.18	57.26±0.19	0.0000
	HCT (%)	31.13±0.1	44.74±0.15	71.17±0.23	85.59±0.28	0.0005
	MCH(pg)	23.02±0.07	18.42±0.06	11.11±0.04	9.71±0.03	0.0005
	MCHC(g/dl)	38.94±0.13	30.43±0.1	20.22±0.07	17.02±0.06	0.0005
	RDW	11.71±0.04	26.83±0.09	19.82±0.06	10.51±0.03	0.0005
	[Hb] (g/dl)	12.11±0.04	13.61±0.04	14.41±0.05	14.61±0.05	0.0006

Values are expressed as Mean ± Sem, Bwt = Body Weight, WBC = White blood cells, Lymph = Lymphocytes, Mon = Monocytes, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, HCT = Hematocrit, MCH = Mean Cell Hemoglobin, MCHC = Mean Cell Hemoglobin Concetration, RDW = Red Cell Distribution Width, [HB] = Hemoglobin concentration, # = number of cells.

Male rats that received stem bark methanolic extract showed significant increase in WBC count ($P < 0.05$) at dose 250 mg/kg, which is reflected with increase of lymphocytes and monocytes with significant decrease in neutrophils. At a dose of 150 mg/kg and 200 mg/kg, WBC count decreased significantly compared to control. In females WBC count increased significantly ($P<0.05$) and was reflected in lymphocytes, monocytes and neutrophils in all doses to control groups. Red blood cells, hematocrit, hemoglobin concentration in males significantly decreased at all doses ($P<0.05$) compared to control. Hematological indices MCV, MCH and [MCHC] were significantly increased ($P<0.05$) compared to control. Red Cell Distribution Width significantly decreased at 150 mg/kg and significantly increased at 200 mg/kg and 250 mg/kg in both sexes compared to control group as seen in Table 17.

Table 17: Effects of stem bark methanolic extract on white blood cell counts, RBC counts and its indices expressed as mean ± sem

		Dose in mg/kgBwt				
Sex	**Parameter**	**0**	**150**	**200**	**250**	**p-value**
Males	WBC (m/mm^3)	127.75±0.42	103±6.01	111.23±0.36	161.25±12.16	0.0005
	Lymp (#)	60.96±0.2	45.84±3.7	55.26±0.18	99±10.77	0.0005
	Mon (#)	17.72±0.06	23.42±1.41	25.86±0.24	27.93±0.43	0.0005
	RBC(m/mm^3)	16.41±0.05	11.95±1.08	8.29±0.35	7.34±0.3	0.0005
	MCV(fl)	55.96±0.18	56.62±0.24	60.92±0.81	58.34±0.89	0.0004
	HCT (%)	91.69±0.3	67.67±5.84	50.38±1.47	42.84±1.97	0.0005
	MCH(pg)	10.31±0.03	11.01±0.18	19.58±0.79	20.12±0.52	0.0010
	MCHC(g/dl)	18.42±0.06	19.45±0.27	32.09±0.85	34.46±0.74	0.0005
	RDW	19.82±0.06	11.64±2.14	19.91±0.54	25.88±1.63	0.0011
	[Hb] (g/dl)	16.92±0.06	16.16±0.19	14.81±0.05	13.21±0.39	0.0005
Females	WBC (m/mm^3)	20.03±0.09	30.43±13.61	91.09±0.3	105.73±3.67	0.0005
	Lymp (#)	8.01±0.03	22.74±10.17	58.66±0.19	58.86±0.21	0.001
	Mon (#)	4.00±0.01	6.03±2.7	20.02±0.07	29.63±2.37	0.0005
	RBC(m/mm^3)	8.02±0.05	1.66±0.74	12.41±0.04	17.24±1.2	0.0005
	MCV(fl)	5.27±0.02	5.19±0.02	10.86±0.04	16.53±2.32	0.0006
	HCT (%)	59.26±0.19	64.48±1.31	59.65±0.03	58.15±1.28	0.0008
	MCH(pg)	31.13±0.1	33.43±0.59	64.76±0.21	96.1±12.6	0.0005
	MCHC(g/dl)	23.02±0.07	31.47±2.14	11.82±0.04	7.99±2.52	0.0005
	RDW	38.94±0.13	38.34±0.17	19.81±0.07	13.41±5.62	0.0005
	[Hb] (g/dl)	11.71±0.04	10.81±0.22	12.16±0.04	13.51±0.31	0.0005
	WBC (m/mm^3)	12.11±0.04	12.8±0.17	12.83±0.03	12.86±0.04	0.0094

Values are expressed as Mean ± Sem, Bwt = Body Weight, WBC = White blood cells, Lymph = Lymphocytes, Mon = Monocytes, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, HCT = Hematocrit, MCH = Mean Cell Hemoglobin, MCHC = Mean Cell Hemoglobin Concetration, RDW = Red Cell Distribution Width, [HB] = Hemoglobin concentration, # = number of cells.

3.4.5 Biochemical Assays in Sub acute Toxicity

The 28 day repeated dose administration produced changes in serum parameters in dose related fashion. The exposure of male rats to leaf chloroform extract caused significant decrease in glucose, cholesterol, total protein, albumin and triglycerides ($P<0.05$) and increase in urea, bilirubin, ALT, ALP, creatinine and AST ($p<0.05$) compared to control groups while female rates showed decrease in cholesterol, total protein, albumin, triglycerides, urea and ALP ($P<0.05$) and increase in glucose, bilirubin, ALT, creatinine and AST ($P<0.05$) as indicated in Table 18.

Table 18: Effects of sub acute exposure of leaf chloroform extract on rats biochemical parameters expressed as mean ± sem

		Dose in mg/kgBwt				
Sex	**Parameter**	**0**	**150**	**200**	**250**	**p-value**
Males	Glucose (mg/dl)	33.9±0.03	28.58±0.03	27.21±0.03	22.94±0.02	0.0005
	Cholesterol (mg/dl)	129.96±0.12	68.3±0.06	69.46±0.15	71.34±0.08	0.0005
	Total Protein (g/dl)	8.44±0.01	8.13±0.01	8.13±0.01	7.96±0.01	0.0000
	Albumin (g/dl)	3.36±0.00	3.42±0.00	3.27±0.00	2.91±0.00	0.0005
	Triglycerides (mg/d)	39.63±0.04	47.36±0.04	87.96±0.08	90.88±0.10	0.0005
	Urea (mg/dl)	29.01±0.03	32.84±0.03	35.75±0.04	42.24±0.04	0.0005
	Bilirubin (mg/dl)	0.67±0.01	0.64±0.01	0.71±0.01	2±0.03	0.0016
	Creatinine (mg/dl)	27.26±0.56	29.93±0.5	39.91±0.67	43.75±0.59	0.0006
	ALT (U/L)	54.37±0.70	57.92±0.97	57.92±0.97	60.56±0.81	0.0011
	AST (U/L)	0.74±0.01	0.67±0.01	0.89±0.01	0.97±0.01	0.0000
	ALP (U/L)	58.19±1.06	69.84±1.17	59.86±1.01	74.04±1.00	0.0000
Female	Glucose (mg/dl)	15.62±0.01	18.49±0.02	21.27±0.02	30.25±0.03	0.0005
	Cholesterol (mg/dl)	103.97±0.1	59.25±0.06	45.94±0.04	44.13±0.04	0.0005
	Total Protein (g/dl)	8.11±0.01	8.11±0.01	7.69±0.01	6.68±0.01	0.0005
	Albumin (g/dl)	4.04±0.00	3.23±0.00	3.04±0.00	2.96±0.00	0.0005
	Triglycerides (mg/dl)	144.02±0.14	149.82±0.14	133.38±0.13	130.51±0.14	0.0005
	Urea (mg/dl)	48.51±0.05	28.78±0.03	20.66±0.02	19.39±0.02	0.0005
	Bilirubin (mg/dl)	0.35±0.05	0.44±0.01	0.57±0.01	1.23±0.02	0.0008
	Creatinine (mg/dl)	16.67±0.06	40.24±0.68	41.9±0.70	43.41±0.58	0.0031
	ALT (U/L)	85.73±0.33	66.74±1.12	66.74±1.12	77.59±1.04	0.0005
	AST (U/L)	0.82±0.01	0.74±0.01	0.89±0.01	0.95±0.01	0.0000
	ALP (U/L)	49.84±1.17	63.19±1.06	69.84±1.17	74.49±1.00	0.0000

Values are expressed as Mean ± Sem, Bwt = Body Weight, ALT = Alanine Amino Transferase, AST = Aspartate Amino Transferase, ALP = Alkaline Phosphatase

The exposure of male rats to leaf ethyl acetate extract caused significant increase in glucose, albumin, triglyceride, urea, bilirubin, ALT, ALP and creatinine ($P<0.05$) and decrease in cholesterol, total protein and AST ($p<0.05$) compared to control groups while female rat shows increase in glucose, total protein, triglycerides, bilirubin, ALT, AST and ALP

(P<0.05) and decrease in cholesterol albumin urea and creatinine (P<0.05) as seen in Table 19.

Table 19: Effects of sub acute exposure of leaf ethyl acetate extract on rats biochemical parameters expressed as mean ± sem

		Dose in mg/kgBwt				
Sex	**Parameter**	**0**	**150**	**200**	**250**	**p-value**
Males	Glucose (mg/dl)	33.9±0.03	44.73±0.37	47.94±0.49	67.81±0.07	0.001
	Cholesterol (mg/dl)	129.96±0.12	74.95±0.07	82.21±0.08	101.57±0.11	0.001
	Total Protein (g/dl)	8.44±0.01	7.61±0.01	7.52±0.01	7.68±0.01	0.001
	Albumin (g/dl)	3.06±0.00	3.06±0.00	2.66±0.00	3.19±0.00	0.001
	Triglycerides (mg/d)	39.63±0.04	81.19±0.08	81.19±0.08	139.21±0.15	0.001
	Urea (mg/dl)	29.01±0.03	39.23±0.04	44.1±0.04	52.93±0.06	0.001
	Bilirubin (mg/dl)	0.67±0.01	0.23±0.00	1.01±0.02	0.99±0.16	0.001
	Creatinine (mg/dl)	0.74±0.01	49.88±0.84	39.91±0.67	59.86±1.5	0.000
	ALT (U/L)	27.26±0.56	71.15±1.2	71.15±1.2	88.8±4.45	0.000
	AST (U/L)	58.19±1.06	0.89±0.01	1.04±0.02	1.11±0.04	0.000
	ALP (U/L)	54.37±0.69	79.81±1.34	69.84±1.17	56.54±1.34	0.001
Female	Glucose (mg/dl)	15.62±0.01	54.51±0.05	38.89±0.04	64.82±0.07	0.001
	Cholesterol (mg/dl)	103.97±0.10	51.98±0.05	51.98±0.05	51.99±0.06	0.012
	Total Protein (g/dl)	6.42±0.01	6.42±0.01	5.86±0.01	7.2±0.01	0.001
	Albumin (g/dl)	4.04±0.00	2.64±0.00	2.68±0.00	2.95±0.00	0.002
	Triglycerides(mg/dl)	144.02±0.14	167.21±0.16	129.52±0.12	179.82±0.19	0.001
	Urea (mg/dl)	48.51±0.05	28.55±0.03	31.1±0.03	23.37±0.02	0.001
	Bilirubin (mg/dl)	0.35±0.05	0.99±0.02	1.03±0.02	0.16±0.02	0.001
	Creatinine (mg/dl)	16.67±0.06	23.28±0.39	26.6±0.45	36.58±10	0.001
	ALT (U/L)	85.73±0.33	47.71±0.80	59.02±0.99	87.15±2.94	0.001
	AST (U/L)	0.82±0.01	0.52±0.01	0.59±0.01	0.81±0.02	0.001
	ALP (U/L)	49.84±1.17	53.21±0.89	56.54±0.95	66.51±1.47	0.000

Values are expressed as Mean ± Sem, Bwt = Body Weight, ALT = Alanine Amino Transferase, AST = Aspartate Amino Transferase, ALP = Alkaline Phosphatase

The exposure of male rats to leaf methanolic extract caused significant increase in glucose, albumin, triglyceride, urea, bilirubin, ALT, ALP, AST and cretinine (P<0.05) and decrease in cholesterol and total protein (p<0.05) compared to control groups while female rat shows increase in glucose, albumin, bilirubin ALT and AST, and ALP (P<0.05) and decrease in, cholesterol urea total protein, triglycerides and creatinine (P<0.05) as seen in Table 20.

Table 20: Effects of sub-acute exposure of leaf methanolic extract on rats biochemical parameters expressed as mean ± sem

		Dose in mg/kgBwt				
Sex	**Parameter**	**0**	**150**	**200**	**250**	**p - value**
Males	Glucose(mg/dl)	33.9±0.03	51.9±0.03	41.21±0.04	55.31±3.53	0.0005
	Cholesterol(mg/dl)	129.96±0.12	84.62±0.08	73.86±0.07	63.13±2.67	0.0005
	Total Protein (g/dl)	8.44±0.01	7.03±0.01	7.37±0.01	6.94±0.11	0.0005
	Albumin (g/dl)	3.36±0.00	3.15±0.00	156.58±0.15	38.07±0.04	0.0005
	Triglycerides (mg/d)	39.63±0.04	156.58±0.15	132.42±0.12	125.46±1.71	0.0005
	Urea (mg/dl)	29.01±0.03	38.07±0.04	33.31±0.03	35.63±0.59	0.0005
	Bilirubin (mg/dl)	0.67±0.01	0.96±0.02	0.62±0.67	1.12±0.89	0.0005
	Creatinine (mg/dl)	26.61±0.38	66.51±1.12	39.91±0.67	53.21±0.89	0.0000
	ALT (U/L)	53.56±0.47	63.71±1.07	1.48±0.02	96.44±1.62	0.0000
	AST (U/L)	0.72±0.01	1.48±0.02	1.08±0.02	0.89±1.4	0.0005
	ALP (U/L)	56.95±0.73	96.44±1.62	69.84±1.17	83.14±1.4	0.0000
Female	Glucose (mg/dl)	15.62±0.01	93.06±0.09	65.81±0.06	60.5±0.06	0.0005
	Cholesterol (mg/dl)	103.97±0.1	38.69±0.04	42.92±0.04	42.93±0.05	0.0011
	Total Protein (g/dl)	8.7±0.01	8.41±0.01	8.43±0.01	8.35±0.01	0.0005
	Albumin (g/dl)	4.04±0.00	3.1±0.00	148.85±0.14	21.59±0.02	0.0005
	Triglycerides (mg/dl)	144.02±0.14	148.85±0.14	53.16±0.05	62.84±0.07	0.0005
	Urea (mg/dl)	48.51±0.05	21.59±0.02	25.07±0.02	17.99±0.02	0.0005
	Bilirubin (mg/dl)	0.35±0.05	0.5±0.01	0.8±0.01	1.61±0.03	0.0006
	Creatinine (mg/dl)	16.67±0.06	19.95±0.34	33.26±0.56	36.58±0.61	0.0005
	ALT (U/L)	85.73±0.33	66.74±1.12	6.4.4±0.01	49.88±0.84	0.0016
	AST (U/L)	0.82±0.01	0.44±0.01	0.74±0.01	0.81±0.01	0.0012
	ALP (U/L)	49.84±1.17	49.88±0.84	63.19±1.06	66.51±1.12	0.0019

Values are expressed as Mean ± Sem, Bwt = Body Weight, ALT = Alanine Amino Transferase, AST = Aspartate Amino Transferase, ALP = Alkaline Phosphatase

The exposure of male rats to stem chloroform extract caused significant increase in glucose, total protein, triglycerides, urea, bilirubin, ALT, AST and cretinine ($P<0.05$) and decrease in albumin cholesterol and ALP ($p<0.05$) compared to control groups while female rat shows increase in glucose, bilirubin, ALT, ALP, creatinine and AST ($P<0.05$) and decrease in cholesterol, urea, total protein, triglycerides and albumin ($P<0.05$) as seen in Table 21

Table 21: Effects of sub-acute exposure of stem chloroform extract on rats biochemical parameters expressed as mean ± sem

		Dose in mg/kgBwt				
Sex	**Parameter**	**0**	**150**	**200**	**250**	**p-value**
Males	Glucose(mg/dl)	33.9±0.03	29.92±0.03	36.56±0.03	43.87±0.04	0.0005
	Cholesterol (mg/dl)	129.96±0.12	67.09±0.06	69.51±0.07	69.53±0.07	0.0011
	Total Protein (g/dl)	8.15±0.01	8.15±0.01	7.55±0.01	7.48±0.01	0.0005
	Albumin (g/dl)	3.36±0.00	3.4±0.00	3.02±0.00	2.62±0.00	0.0005
	Triglycerides (mg/d)	39.63±0.04	66.69±0.06	80.24±0.08	92.79±0.09	0.0005
	Urea (mg/dl)	29.01±0.03	29.59±0.03	36.25±0.69	38.07±0.04	0.0005
	Bilirubin (mg/dl)	0.67±0.01	1.95±0.03	0.57±0.01	0.53±0.01	0.0050
	Creatinine (mg/dl)	27.26±0.56	29.93±0.5	39.91±0.67	43.23±0.73	0.0060
	ALT (U/L)	54.37±0.69	43.57±0.73	25.65±0.43	32.82±0.55	0.0050
	AST (U/L)	0.74±0.01	0.89±0.01	0.67±0.01	0.96±0.02	0.0000
	ALP (U/L)	58.19±1.06	59.86±1.01	69.84±1.17	69.84±1.17	0.0001
Female	Glucose (mg/dl)	15.62±0.01	43.87±0.04	48.86±0.05	68.15±0.07	0.0005
	Cholesterol (mg/dl)	103.97±0.1	134.19±0.13	99.13±0.09	67.71±0.07	0.0005
	Total Protein (g/dl)	9±0.01	9±0.01	8.65±0.01	7.48±0.01	0.0005
	Albumin (g/dl)	4.04±0.00	3.42±0.00	2.91±0.00	2.76±0.00	0.0005
	Triglycerides (mg/dl)	144.02±0.14	119.85±0.11	101.49±0.1	93.93±0.56	0.0000
	Urea (mg/dl)	48.51±0.05	27.74±0.03	24.72±0.02	23.91±0.02	0.0005
	Bilirubin (mg/dl)	0.35±0.05	0.9±0.02	0.78±0.01	0.23±0.13	0.0008
	Creatinine (mg/dl)	16.67±0.06	59.86±1.01	33.26±0.56	33.24±0.64	0.0011
	ALT (U/L)	85.73±0.33	98.18±1.65	98.31±1.65	100.03±2.7	0.000004
	AST (U/L)	0.82±0.01	1.33±0.02	0.74±0.01	0.74±0.01	0.0011
	ALP (U/L)	49.84±1.17	89.79±1.51	63.19±1.06	63.15±1.22	0.0011

Values are expressed as Mean ± Sem, Bwt = Body Weight, ALT = Alanine Amino Transferase, AST = Aspartate Amino Transferase, ALP = Alkaline Phosphatase

The exposure of male rats to stem etyhl acetate extract caused significant increase in glucose, total protein, triglycerides, urea, bilirubin, ALT and ALP ($P<0.05$) and decrease in albumin, cholesterol, cretinine and AST ($p<0.05$) compared to control groups while female rat shows increase in glucose, bilirubin, ALT, ceatinine and AST ($P<0.05$) and decrease in cholesterol, urea, total protein, triglycerides and albumin and ALP ($P<0.05$) as indicated in Table 22.

Table 22: Effects of sub-acute exposure of stem ethyl acetate extract on rats biochemical parameters expressed as mean ± sem.

		Dose in mg/kgBwt				
Sex	**Parameter**	**0**	**0**	**150**	**250**	**p-value**
Males	Glucose (mg/dl)	33.9±0.03	35.23±0.03	37.89±0.04	65.16±0.07	0.0005
	Cholesterol (mg/dl)	129.96±0.12	87.04±0.08	59.24±0.06	78.6±0.08	0.0005
	Total Protein (g/dl)	8.44±0.01	8.48±0.01	8.26±0.01	9.37±0.01	0.0005
	Albumin (g/dl)	3.36±0.00	3.02±0.00	2.4±0.00	2.38±0	0.0005
	Triglycerides (mg/d)	39.63±0.04	362.46±0.34	116.95±0.11	48.34±0.05	0.0005
	Urea (mg/dl)	29.01±0.03	38.53±0.04	46.42±0.04	43.41±0.05	0.0005
	Bilirubin (mg/dl)	0.67±0.01	1.29±0.16	1.06±0.04	0.87±0.03	0.0000
	Creatinine (mg/dl)	0.74±0.01	23.28±3.82	39.91±2.71	53.21±5.43	0.0000
	ALT (U/L)	27.26±0.56	47.16±2.69	52.4±2.2	60.67±5.38	0.0005
	AST (U/L)	58.19±1.06	0.52±0.08	0.89±0.06	1.18±0.12	0.0005
	ALP (U/L)	54.37±0.69	46.56±8.14	53.21±2.56	83.14±5.84	0.0000
Females	Glucose (mg/dl)	15.62±0.01	66.33±0.06	65.81±0.06	54.19±0.06	0.0005
	Cholesterol (mg/dl)	103.97±0.1	65.8±0.06	65.28±0.06	50.79±0.05	0.0005
	Total Protein (g/dl)	8.7±0.01	7.96±0.01	7.89±0.01	6.03±0.01	0.0005
	Albumin (g/dl)	4.04±0.00	3.08±0.00	3.06±0.00	2.95±0.00	0.0005
	Triglycerides (mg/dl)	144.02±0.14	88.66±0.08	87.96±0.08	79.82±0.19	0.0008
	Urea (mg/dl)	48.51±0.05	27.49±0.03	27.27±0.03	13.81±0.01	0.0005
	Bilirubin (mg/dl)	0.35±0.35	0.9±0.16	1.33±0.06	0.163±0.10	0.0000
	Creatinine (mg/dl)	0.82±0.01	0.74±0.01	0.84±0.04	0.96±0.05	0.0000
	ALT (U/L)	16.67±0.06	29.93±3.43	33.26±1.26	33.26±0.64	0.0011
	AST (U/L)	49.84±1.17	63.43±5.07	91.84±7.94	119.14±8.77	0.0000
	ALP (U/L)	85.73±0.33	57.2±2.69	59.86±1.53	63.19±1.95	0.0000

Values are expressed as Mean ± Sem, Bwt = Body Weight, ALT = Alanine Amino Transferase, AST = Aspartate Amino Transferase, ALP = Alkaline Phosphatase

The exposure of male rats to stem bark methanolic extract caused significant increase in glucose, total protein, triglyeride, urea, bilirubin, ALT and ALP ($P<0.05$) and decrease in albumin cholesterol cretinine and AST ($p<0.05$) compared to control groups while female rat shows increase in glucose, bilirubin, ALT, creatinine, AST ($P<0.05$) and decrease in cholesterol, urea, total protein, triglycerides, albumin and ALP ($P<0.05$) as indicated in Table 23.

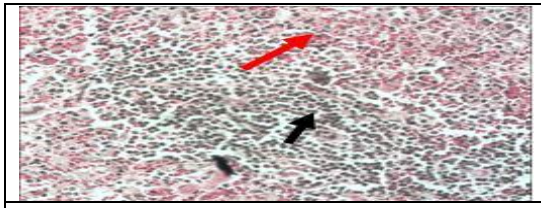
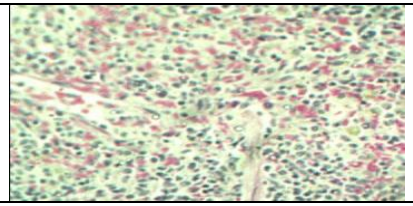
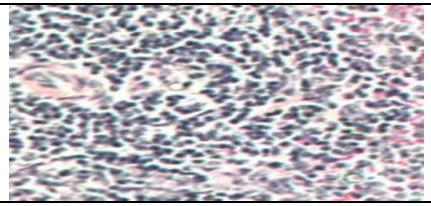
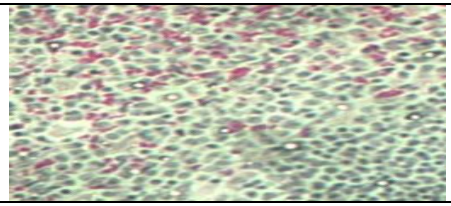
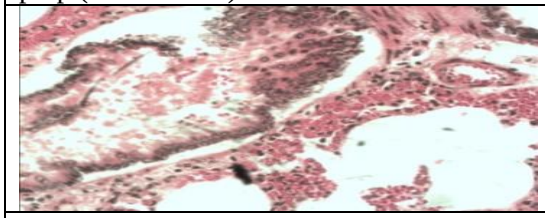
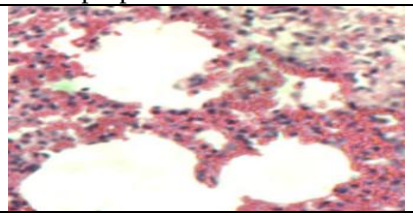
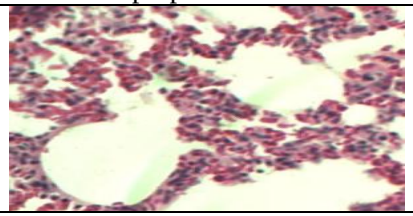
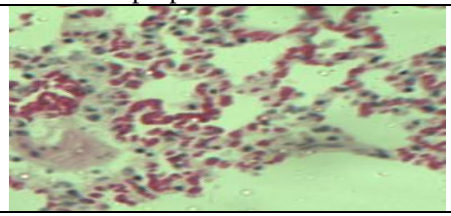
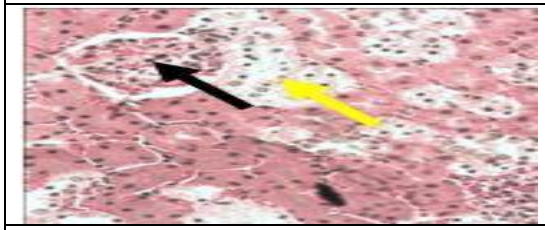
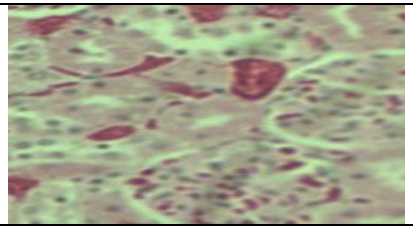
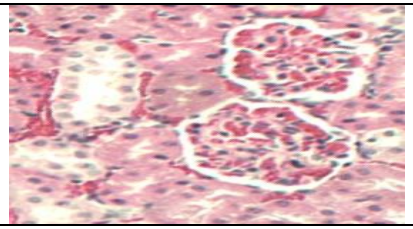
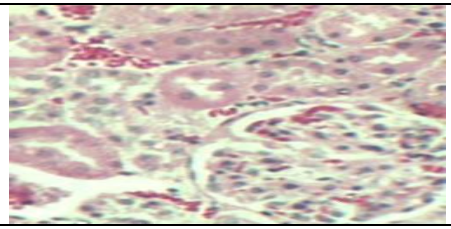
Table 23: Effects of sub-acute exposure of stem bark methanolic extract on rats biochemical parameters expressed as mean ± sem.

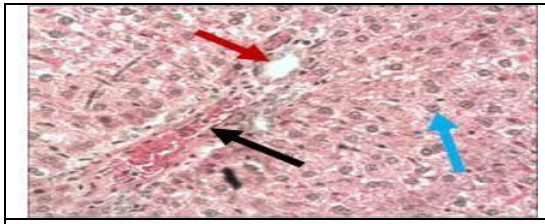
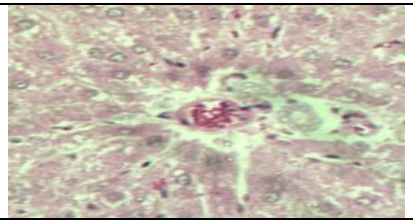
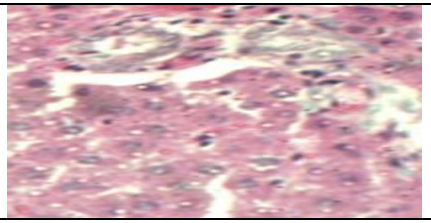
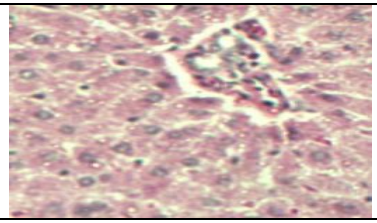
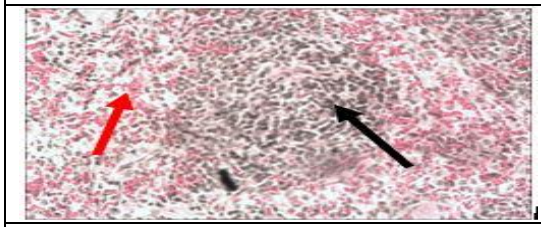
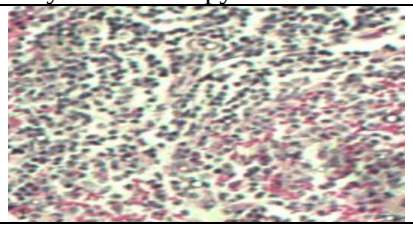
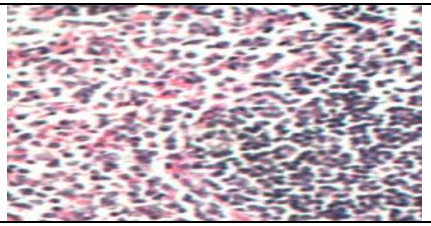
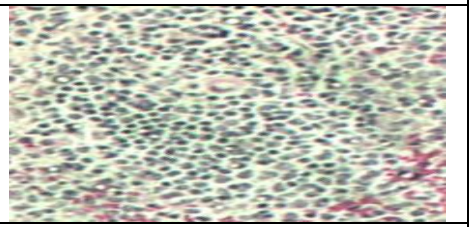
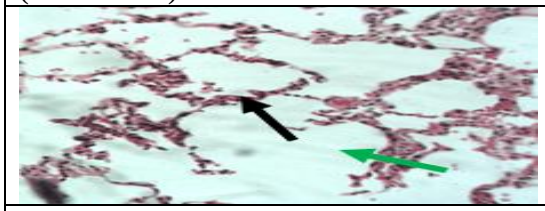
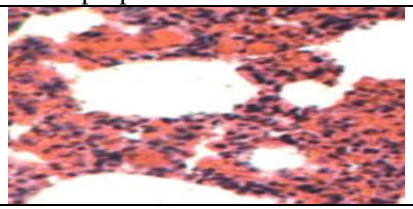
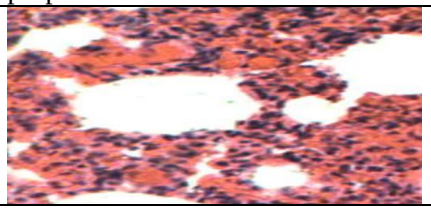
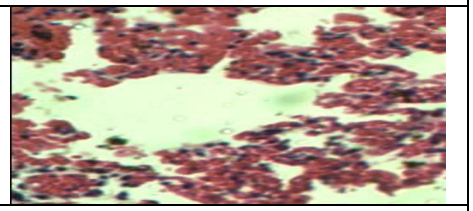
		Dose in mg/kgBwt				
Sex	**Parameter**	**0**	**150**	**200**	**250**	**p-values**
Males	Glucose (mg/dl)	33.9±0.03	68.46±8.49	72.45±0.07	81.76±2.32	0.0005
	Cholesterol (mg/dl)	129.96±0.12	70.17±14.6	62.86±0.06	48.96±3.37	0.0005
	Total Protein (g/dl)	8.44±0.01	8.28±0.03	7.57±0.01	6.31±0.30	0.0005
	Albumin (g/dl)	3.36±0.00	3.38±0.01	2.64±0.24	2.49±0.16	0.0011
	Triglycerides (mg/d)	39.63±0.04	115.99±18.74	114.82±0.1	69.59±11.07	0.0005
	Urea (mg/dl)	29.01±0.06	24.34±1.05	16.36±0.02	13.58±0.69	0.0005
	Bilirubin (mg/dl)	0.67±0.01	0.5±0.03	0.85±0.01	1.06±0.06	0.0000
	Creatinine (mg/dl)	0.74±0.01	0.67±0.03	0.89±0.01	1.03±0.05	0.0000
	ALT (U/L)	27.26±0.56	29.93±1.11	39.91±0.67	46.56±2.22	0.0005
	AST (U/L)	58.19±1.06	59.89±1.36	69.84±1.17	76.49±2.67	0.0000
	ALP (U/L)	54.37±0.69	53.92±0.78	42.47±0.71	35.02±1.4	0.0011
Female	Glucose (mg/dl)	64.48±0.25	77.44±3.28	97.71±0.09	98.04±0.16	0.0007
	Cholesterol (mg/dl)	129.1±0.5	59.24±16.92	56.21±0.05	41.1±3.67	0.0005
	Total Protein (g/dl)	6.5±0.02	7.85±0.34	7.09±0.01	8.15±0.26	0.0005
	Albumin (g/dl)	2.55±0.04	3.13±0.16	3.03±0.05	2.76±0.06	0.0008
	Triglycerides (mg/dl)	81.91±2.31	132.42±13.07	70.56±0.07	39.63±7.54	0.0005
	Urea (mg/dl)	48.51±0.05	27.97±5.01	24.84±0.02	18.22±1.61	0.0005
	Bilirubin (mg/dl)	0.35±0.35	0.5±0.41	0.8±0.01	1.61±0.21	0.0006
	Creatinine (mg/dl)	0.82±0.01	0.44±0.19	0.74±0.03	0.81±0.03	0.0012
	ALT (U/L)	16.67±0.06	19.95±0.95	33.26±0.56	36.58±1.31	0.0005
	AST (U/L)	49.84±1.17	49.88±1.00	63.19±1.06	68.51±1.78	0.0013
	ALP (U/L)	85.73±0.33	74.7±2.32	69.22±2.60	66.74±0.80	0.0017

Values are expressed as Mean ± Sem, Bwt = Body Weight, ALT = Alanine Amino Transferase, AST = Aspartate Amino Transferase, ALP = Alkaline Phosphatase.

3.4.6 Histopathology

Oral administration of the extract at different doses of extracts showed different pathological changes in vital organs on micrographs. The spleen showed normal red and white pulp in females and male spleen. Female lungs alveolar walls are thickened with narrowed respiratory ducts, where males and control groups appears normal. Kidneys in both sexes shows increased glomerular space with lost structure. Liver in both sexes showed increased sinusoid lumen as shown in Fig. 7 - 12.

			
Micrograph 169**:** Female control spleen administered with DMSO showing normal distribution red pulp (red arrow) and white pulp (black arrow).	Micrograph 170: Female rat spleen administered with 150 mg/kg LCE showing red pulp and white pulp.	Micrograph 171: Spleen of female rat administered with 200 mg/kg LCE showing reduced red pulp and white pulp.	Micrograph 172: Spleen of a female rat administered with 250 mg/kg LCE showing more reduced red pulp and white pulp.
			
Micrograph 173: Lung of the female control rat after administration of DMSO	Micrograph 174: Lung of the female rat given a dose of 150 mg/kg LCE showing thickened alveolar walls	Micrograph 175: Lung of female rat given LCE at 200 mg/kg showing thickened alveolar walls.	Micrograph 176: lung of female rats given LCE at a dose of 250 mg/kg showing thickened alveolar wall.
			
Micrograph 177**:** Kidney of control female rat administered with DMSO showing normal proximal convoluted tubules (yellow arrow) and glomeruli (black arrow).	Micrograph 178: Kidney of a female rat administered with LCE at 150 mg/kg showing widened glomeruli space	Micrograph 179: Kidney of a female rat administered with LCE at 200 mg/kg showing disintegrated glomeruli	Micrograph 180: Kidney of female rate administered with LCE at 250mg/kg showing disintegration of glomeruli

			
Micrograph 181: Liver of control female rat showing normal bile duct (brown arrow), central vein (black arrow) and normal hepatocytes (blue arrow).	Micrograph 182: Liver of female rat administered with LCE at 150 mg/kg showing normal bile duct, slightly distendended sinusoids, karyorhexis and pyknosis.	Micrograph 183: Liver of the female rat administered with LCE at 200 mg/kg showing distended sinusoids and Pyknosis.	Micrograph 184: Liver of a female rat administered with LCE at 250 mg/kg distended sinusoids lumen, pyknosis.
			
Micrograph 185: Spleen of male control rat administered with DMSO showing normal spleen red pulp (red arrow) and white pulp (black arrow).	Micrograph 186: Spleen of male rat administered with LCE 150 mg/kg showing red pulp and white pulp.	Micrograph 187: Spleen of male rat administered with LCE 200 mg/kg showing red pulp and white pulp.	Micrograph 188: Spleen of female rat administered with LCE 250 mg/kg showing red pulp and white pulp.
			
Micrograph 189: Lung of male control rat administered with DMSO showing normal alveolar wall (black arrow) of the lung.	Micrograph 190: Lung of the male rat administered with LCE at 150 mg/kg showing scattered RBC and thickened alveolar wall.	Micrograph 192: Lung of male rat administered with LCE at 200 mg/kg showing thickened alveolar wall and scattered RBC on the lung.	Micrograph 191: Lung of the male rat administered with LCE at 250 mg/kg showing thickened alveolar wall with scattered RBC.

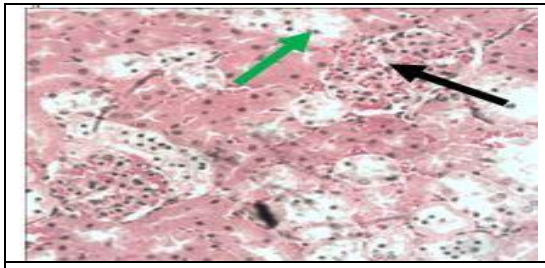
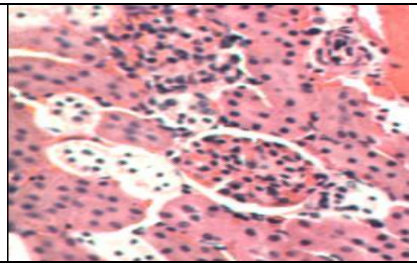
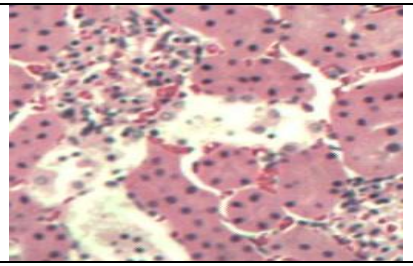
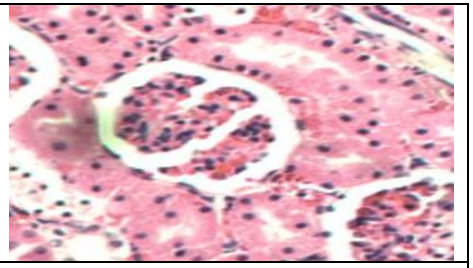
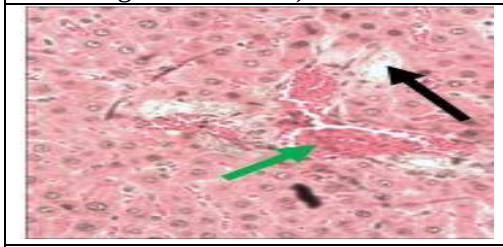
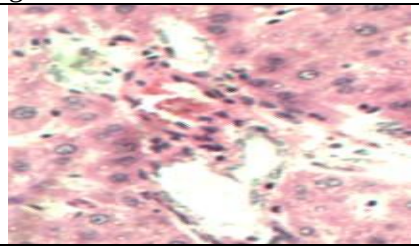
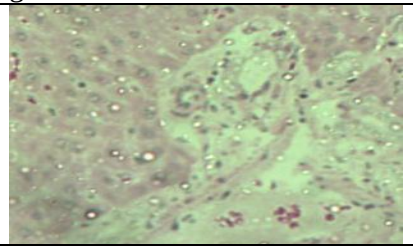
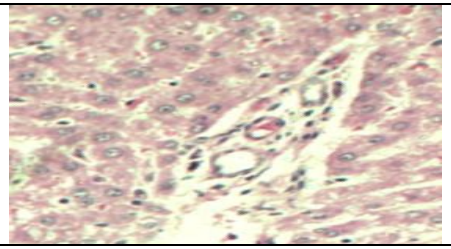
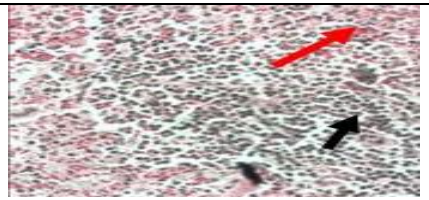
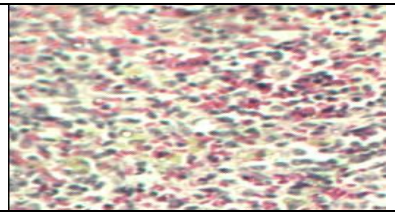
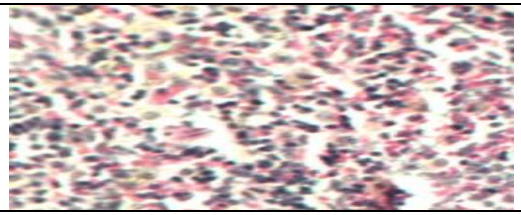
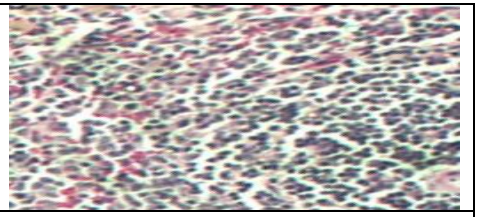
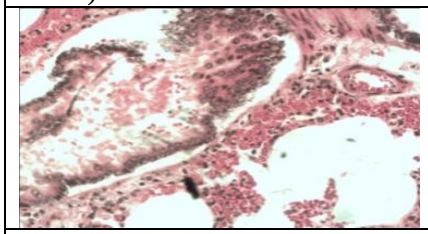
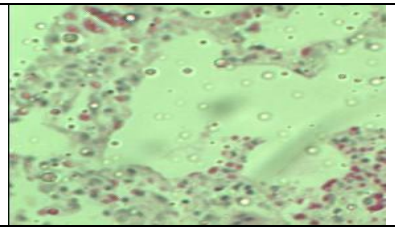
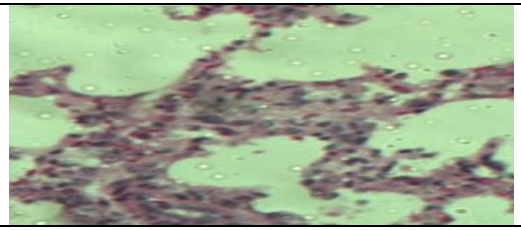
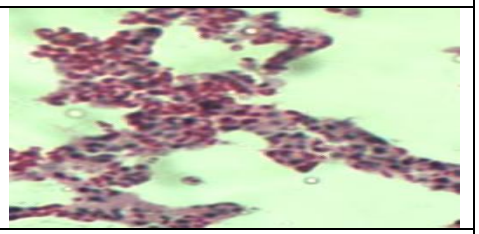
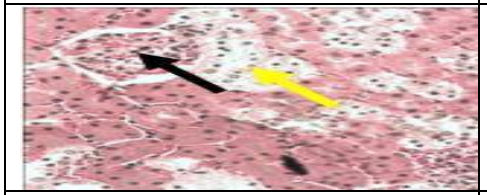
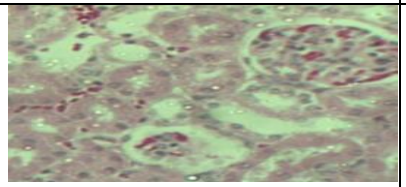
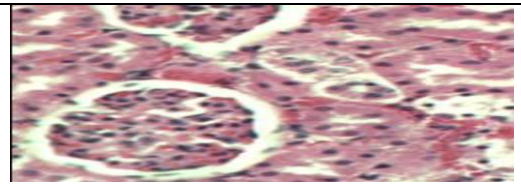
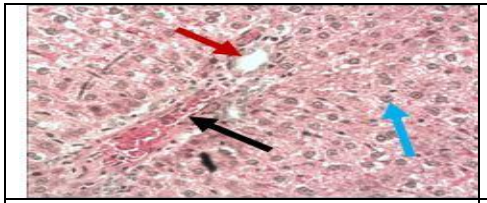
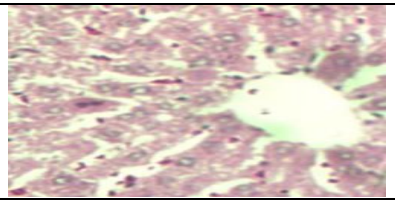
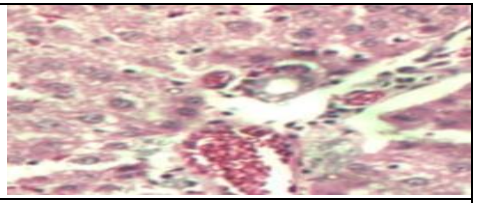
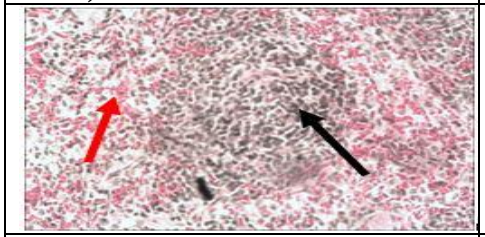
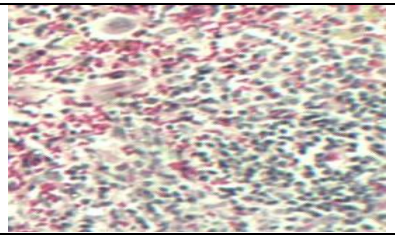
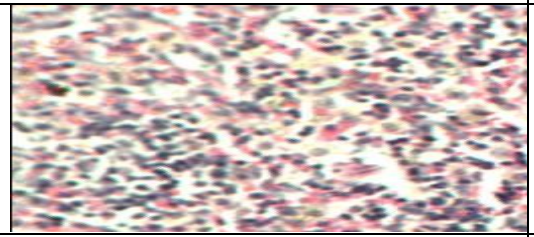
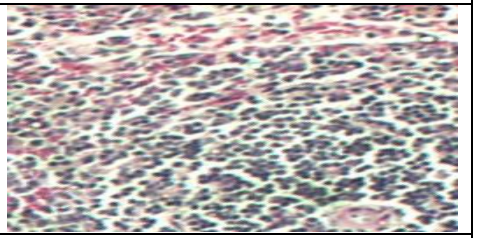
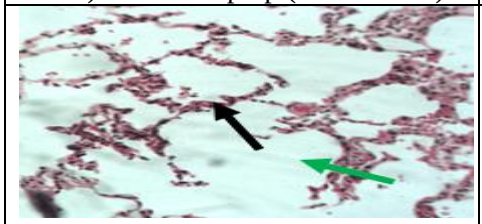
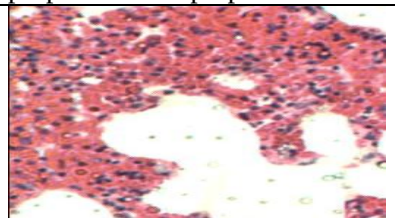
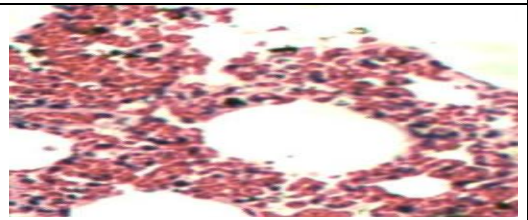
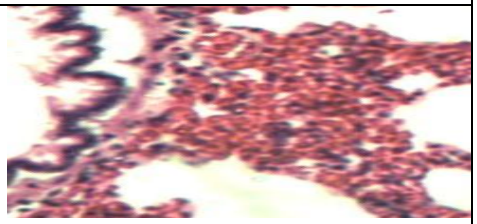
			
Micrograph 192: Kidney of control male rat administered with DMSO showing normal glomeruli (black arrow) and proximal convoluted tubules (green arrow). (H&E stain magnification 40x)	Micrograph 193: Kidney of male rat administered with LCE at 150 mg/kg showing increased glomerula space and disorganized glomeruli..	Micrograph 194: Male rat kidney administered with LCE at 200 mg/kg showing widened glomerular space and disorganized glomerula	Micrograph 195: Kidney of male rat administered with LCE 250 mg/kg showing widened glomerular space
			
Micrograph 196: Liver of control male rat administered with DMSO showing normal bile duct (black arrow), normal hepatocytes (yellow arrow) and normal central vein (green arrow).	Micrograph 197: Liver of male rat administered with LCE extract at 150 mg/kg showing distended sinusoids.	Micrograph 200: liver of male rat administered with LCE at 200 mg/kg showing distended sinusoids.	Micrograph 198: Liver of male rat administered with LCE at 250 mg/kg showing distended sinusoids and multiple bile ducts

Figure 7: Microscopic micrographs of vital organs after sub-acute exposure to leaf chloroform extract (LCE) and DMSO as control (H&E stain and magnification 40x)

			
Micrograph 199: Female control spleen administered with DMSO showing normal distribution red pulp (red arrow) and white pulp (black arrow).	Micrograph 200: Spleen of female rat administered with 200 mg/kg LEAE showing red pulp and white pulp	Micrograph 201: Spleen of female rat administered with 200 mg/kg LEAE showing red pulp and white pulp	Micrograph 202: Spleen of a female rat administered with 250 mg/kg LEAE showing red pulp and white pulp
			
Micrograph 203: Lung of the female control rat after administration of DMSO.	Micrograph 204: Lung of the female rat given a dose of 150 mg/kg LEAE showing thickened alveolar walls.	Micrograph 205: Lung of female rat given LEAE at 200 mg/kg showing thickened alveolar walls.	Micrograph 206: Lung of female rats given LEAE at a dose of 250 mg/kg showing thickened alveolar wall
			
Micrograph 207: Kidney of control female rat administered with DMSO showing normal proximal convoluted tubules (yellow arrow) and glomeruli (black arrow).	Micrograph 208: Kidney of a female rat administered with LEAE at 200 mg/kg showing widened Bowman's space	Micrograph 209: Kidney of female rate administered with LEAE at 250 mg/kg showing widened glomerular space.	Micrograph 210: Kidney of a female rat administered with LEAE at 150 mg/kg showing widened glomeruli space.

			
Micrograph 211: Liver of control female rat showing normal bile duct (brown arrow),central vein (black arrow) and normal hepatocytes (blue arrow)	Micrograph 212: Liver of female rat administered with LEAE at 150 mg/kg showing distended sinusoids.	Micrograph 213: Liver of the female rat administered with LEAE at 200 mg/kg showing central canal and multiple normal bile duct	Micrograph 214: Liver of a female rat administered with LEAE at 250 mg/kg distended sinusoids lumen.
			
Micrograph 215: Spleen of male control rat administered with DMSO showing normal spleen red pulp (red arrow) and white pulp (black arrow)	Micrograph 216: Spleen of male rat administered with LEAE 150 mg/kg showing red pulp and white pulp.	Micrograph 217: Spleen of male rat administered with LEAE 200 mg/kg showing red pulp and white pulp.	Micrograph 218: Spleen of female rat administered with LEAE 250 mg/kg showing red pulp and white pulp.
			
Micrograph 219: Lung of male control rat administered with DMSO showing normal alveolar wall (black arrow) of the lung.	Micrograph 220: Lung of the male rat administered with LEAE at 150 mg/kg showing scattered RBC and thickened alveolar sac	Micrograph 221: Lung of male rat administered with LEAE at 200 mg/kg showing thickened alveolar wall.	Micrograph 222: Lung of the male rat administered with LEAE at 250 mg/kg showing thickened alveolar wall.

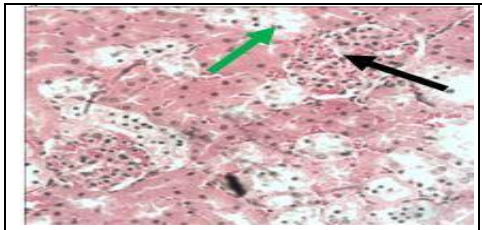
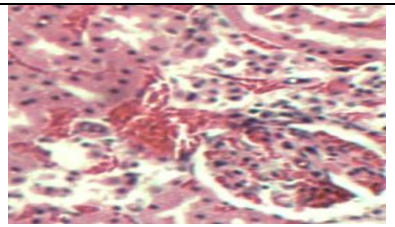
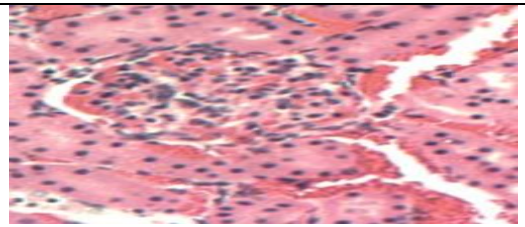
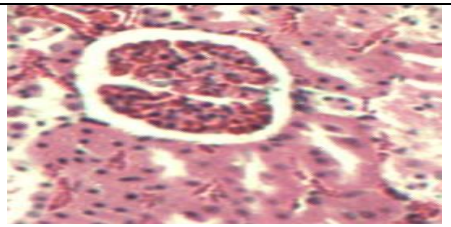
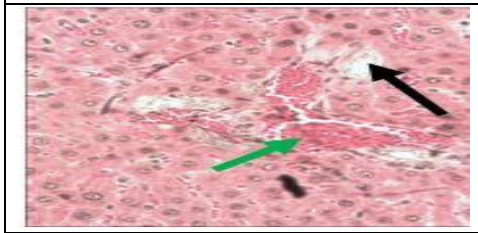
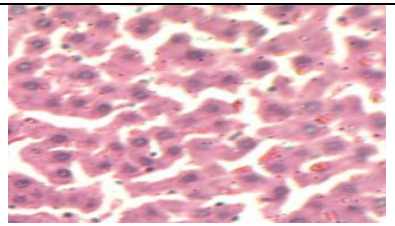
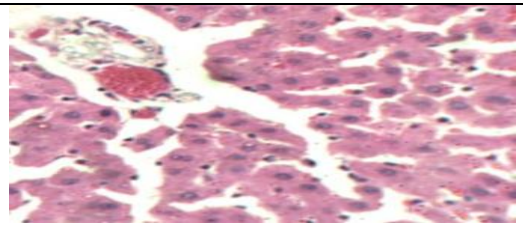
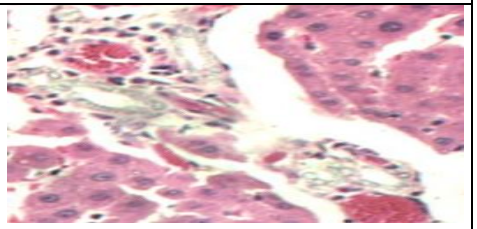
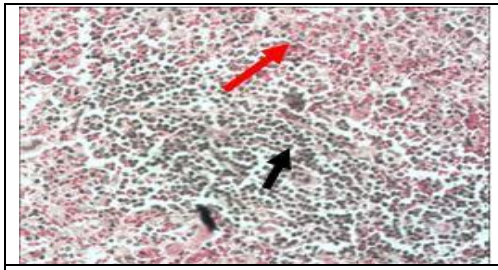
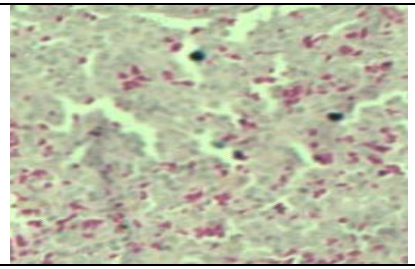
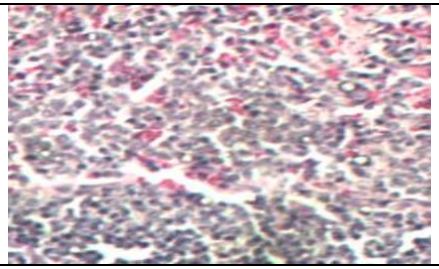
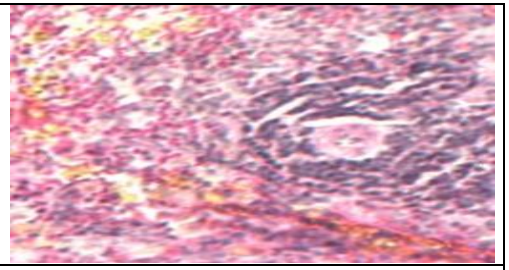
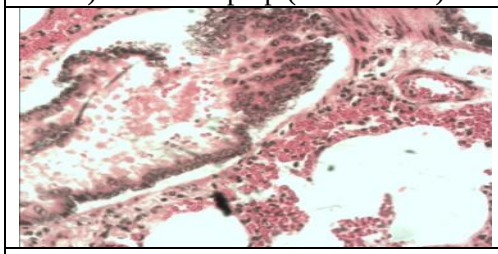
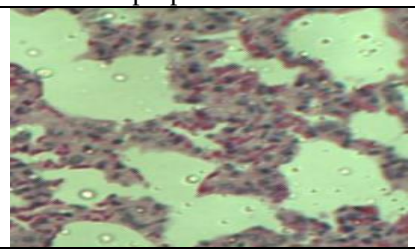
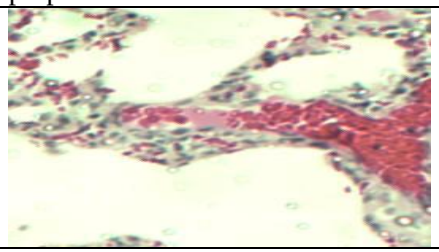
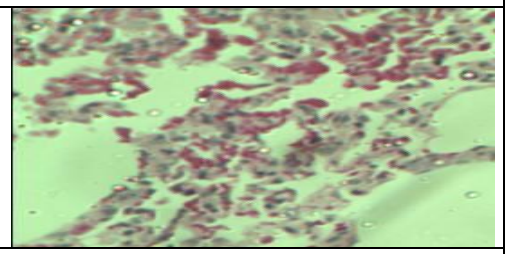
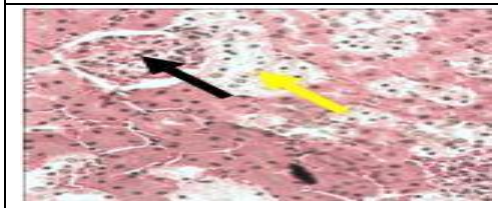
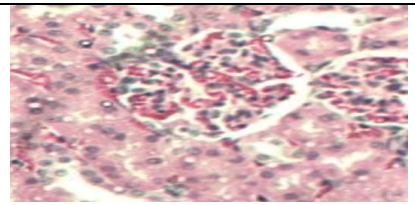
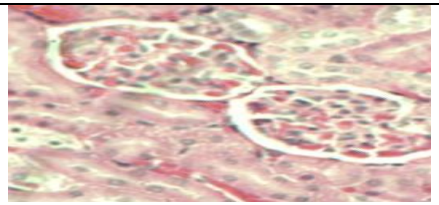
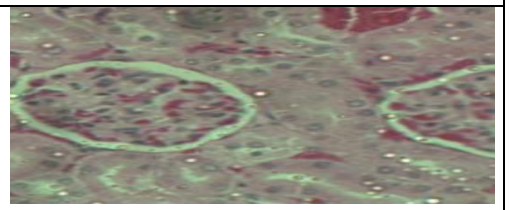
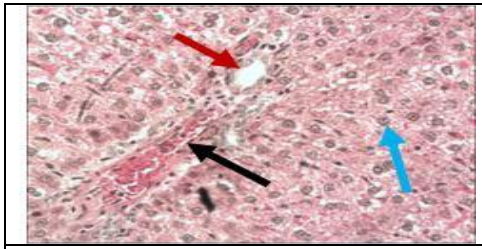
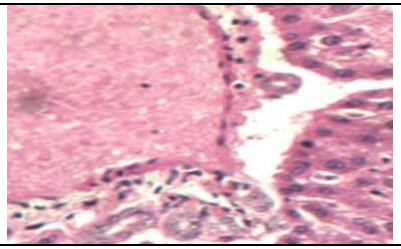
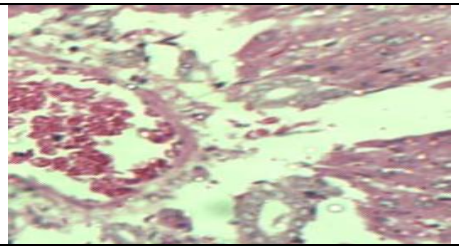
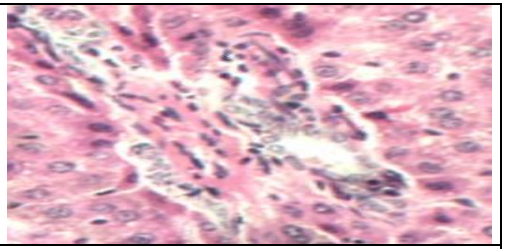
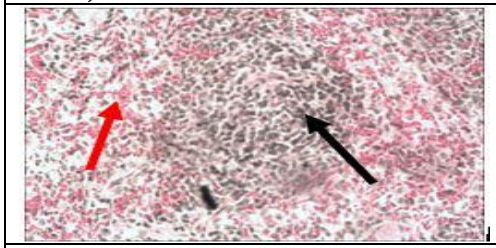
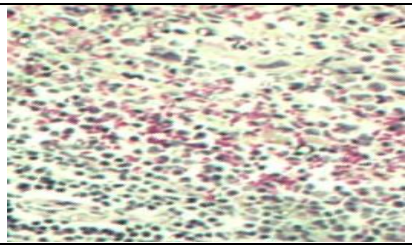
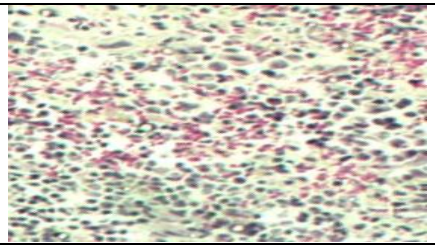
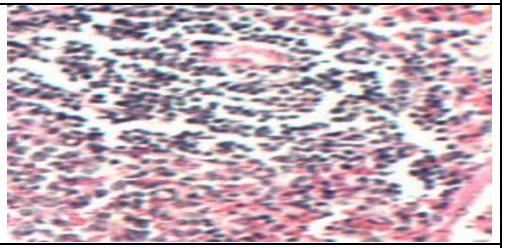
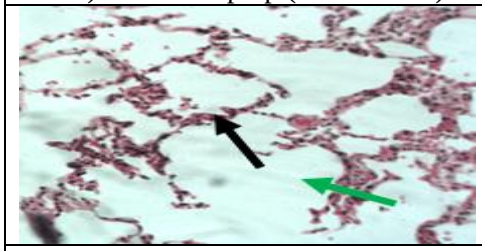
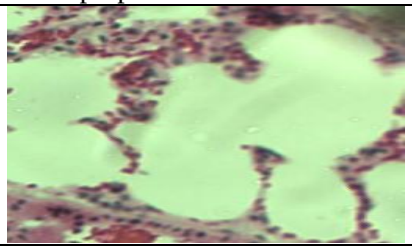
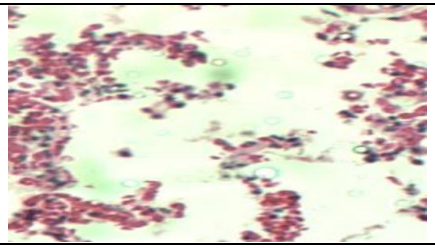
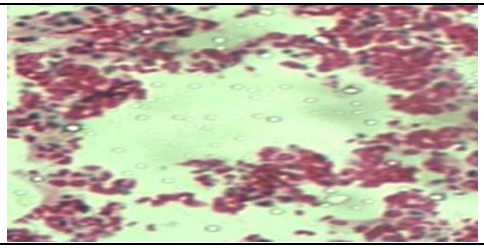
			
Micrograph 223 kidney of control male rat administered with DMSO showing normal glomeruli (black arrow) and proximal convoluted tubules (green arrow).	Micrograph 224: Male rat kidney administered with LEAE at 200 mg/kg showing widened glomerular space and disorganized glomerular.	Micrograph 225 Kidney of male rat administered with LEAE 250 mg/kg showing disorganized glomeruli	Micrograph 226 Male rat kidney administered with LEAE at 150 mg/kg showing increased glomerula space.
			
Micrograph 227: Liver of control male rat administered with DMSO showing normal bile duct(black arrow),normal hepatocytes (yellow arrow) and normal central vein (green arrow)	Micrograph 228: Liver of male rat administered with LEAE at 150 mg/kg showing distended sinusoids.	Micrograph 229: liver of male rat administered with LEAE at 200 mg/kg showing distended sinusoids.	Micrograph 230: Liver of male rat administered with LEAE at 250 mg/kg showing distended sinusoids**.**

Figure 8: Micrographs of vital organs after sub-acute exposure to leaf ethyl acetate extract (LEAE) and DMSO as control (H&E stain and magnification 40x)

			
Micrograph 231: Female control spleen administered with DMSO showing normal distribution red pulp (red arrow) and white pulp (black arrow)	Micrograph 232: Female rat spleen administered with 150 mg/kg LME showing red pulp and white pulp.	Micrograph 233: Spleen of female rat administered with 200 mg/kg LME showing red pulp and white pulp.	Micrograph 234: Spleen of a female rat administered with 250 mg/kg LME showing red pulp and white pulp.
			
Micrograph 235: Lung of the female control rat after administration of DMSO	Micrograph 236: Lung of the female rat given a dose of 150 mg/kg LME showing thickened alveolar walls.	Micrograph 237: Lung of female rat given LME at 200 mg/kg showing thickened alveolar	Micrograph 238: lung of female rats given LME at a dose of 250 mg/kg showing thickened alveolar wall.
			
Micrograph 239: Kidney of control female rat administered with DMSO showing normal proximal convoluted tubules (yellow arrow) and glomeruli (black arrow).	Micrograph 240: Kidney of a female rat administered with LME at 200 mg/kg showing disintegrated glomeruli and widened Bowman's space.	Micrograph 241 Kidney of female rate administered with LME at 250 mg/kg showing widened glomerular space and disintegration of glomeruli.	Micrograph 242: Kidney of a female rat administered with LME at 150 mg/kg showing widened glomeruli space.

			
Micrograph 243: Liver of control female rat showing normal bile duct (brown arrow),central vein (black arrow) and normal hepatocytes (blue arrow)	Micrograph 244: Liver of female rat administered with LME at 150 mg/kg showing distended sinusoids	Micrograph 245: Liver of the female rat administered with LME at 200 mg/kg showing distended.	Micrograph 246: Liver of a female rat administered with LME at 250 mg/kg distended sinusoids lumen
			
Micrograph 247: Spleen of male control rat administered with DMSO showing normal spleen red pulp (red arrow) and white pulp (black arrow).	Micrograph 248: Spleen of male rat administered with LME 150 mg/kg showing red pulp and white pulp.	Micrograph 249: Spleen of male rat administered with LME 200 mg/kg showing red pulp and white pulp.	Micrograph 250: Spleen of female rat administered with LME 250 mg/kg showing red pulp and white pulp.
			
Micrograph 251: Lung of male control rat administered with DMSO showing normal alveolar wall (black arrow) of the lung.	Micrograph 252: Lung of the male rat administered with LME at 150 mg/kg showing thickened alveolar wall.	Micrograph 253: Lung of male rat administered with LME at 200 mg/kg showing thickened alveolar wall sac.	Micrograph 254: Lung of the male rat administered with LME at 250 mg/kg showing thickened alveolar wall

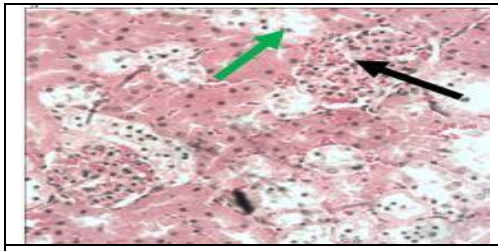
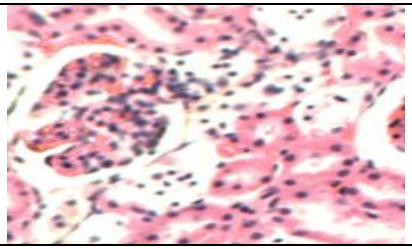
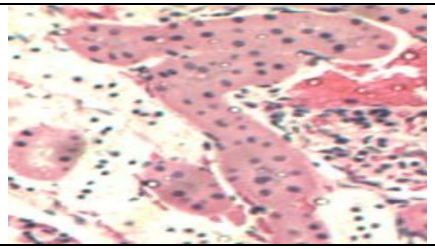
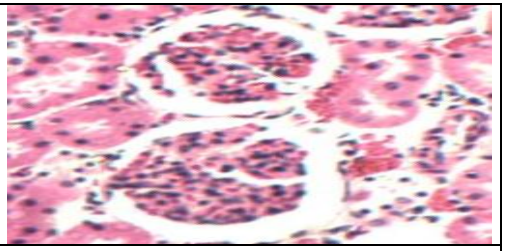
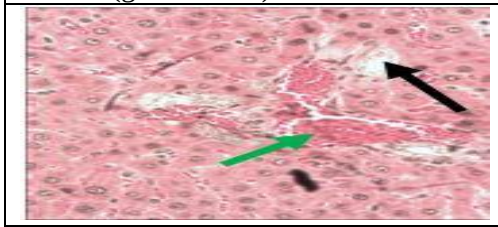
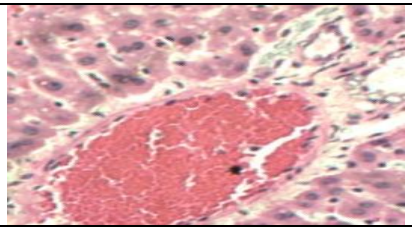
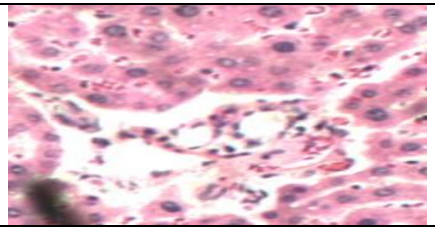
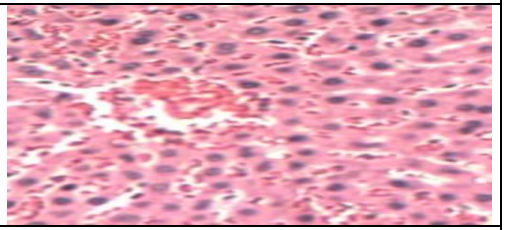
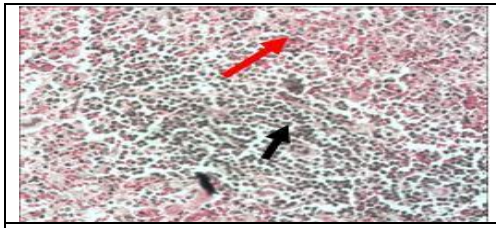
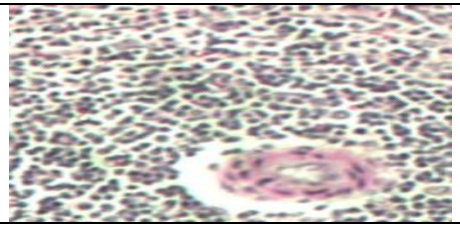
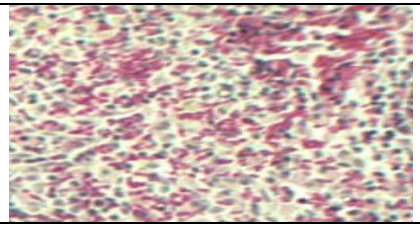
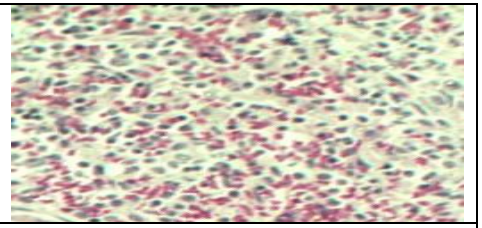
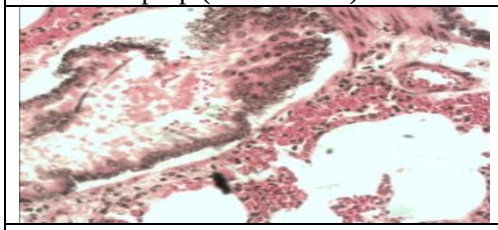
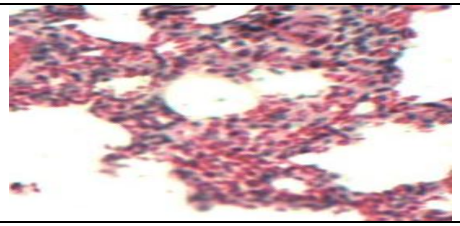
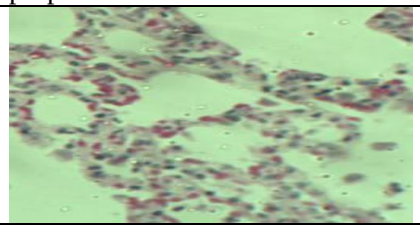
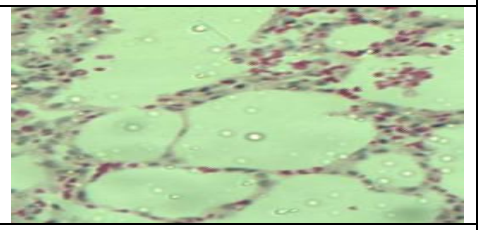
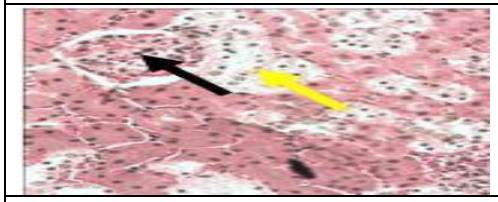
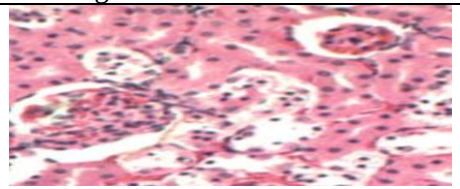
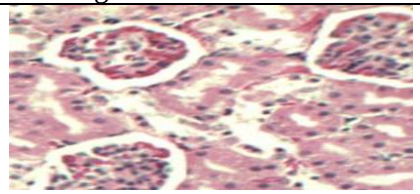
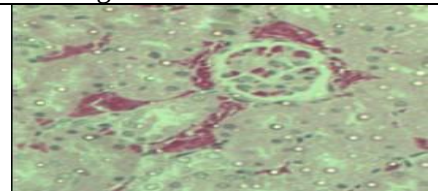
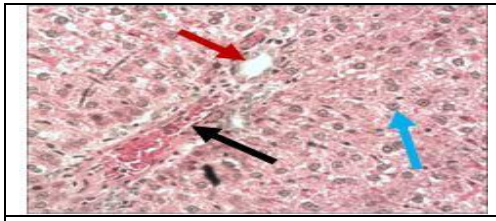
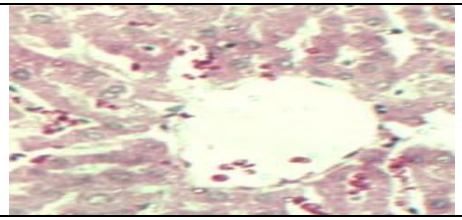
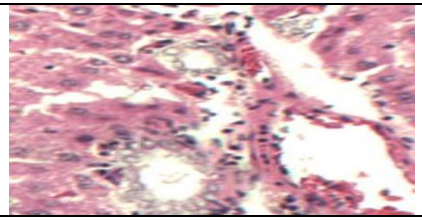
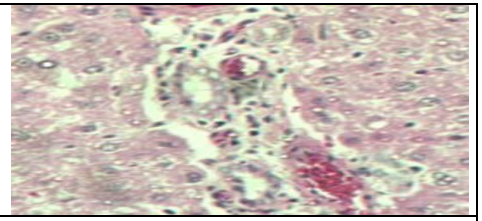
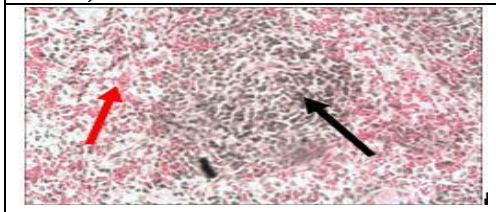
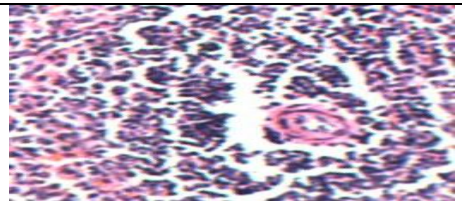
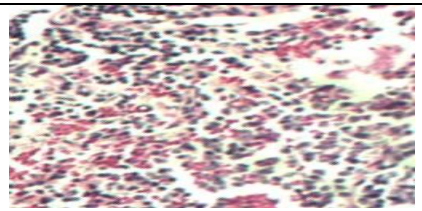
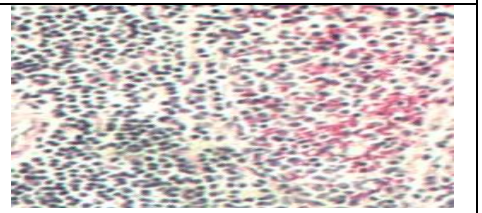
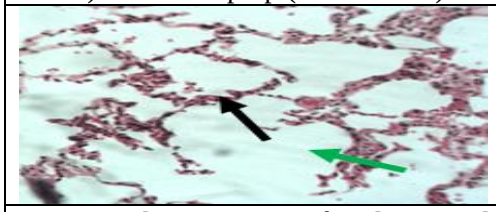
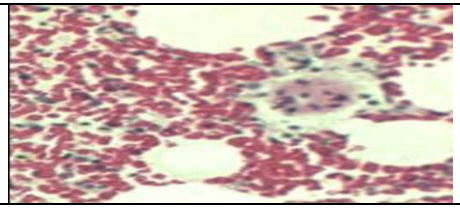
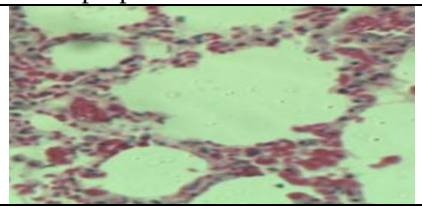
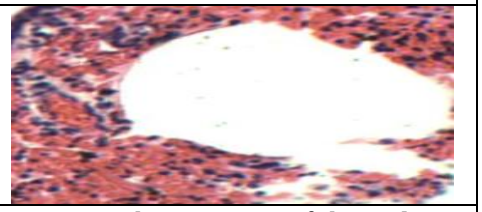
			
Micrograph 255: kidney of control male rat administered with DMSO showing normal glomeruli (black arrow) and proximal convoluted tubules (green arrow).	Micrograph 256: Male rat kidney administered with LME at 150 mg/kg showing increased glomerula space	Micrograph 257: Male rat kidney administered with LME at 200 mg/kg showing disorganized glomerula	Micrograph 258: Kidney of male rat administered with LME 250 mg/kg showing widened glomerular space and and disorganized glomeruli
			
Micrograph 259: Liver of control male rat administered with DMSO showing normal bile duct (black arrow),normal hepatocytes (yellow arrow) and normal central vein (green arrow)	Micrograph 260: Liver of male rat administered with LME extract at 150 mg/kg showing distended sinusoids.	Micrograph 261: Liver of male rat administered with LME at 200 mg/kg showing distended sinusoids.	Micrograph 262: Liver of male rat administered with LME at 250 mg/kg showing distended sinusoids

Figure 9: Micrographs of vital organs after sub acute exposure to leaf methanolic extact (LME) and DMSO as control (H&E stain and magnification 40x)

			
Micrograph 263: Female control spleen administered with DMSO showing normal distribution red pulp (red arrow) and white pulp (black arrow).	Micrograph 264: Female rat spleen administered with 150 mg/kg SCE showing red pulp and white pulp.	Micrograph 265: Spleen of female rat administered with 200 mg/kg SCE showing red pulp and white pulp	Micrograph 266: Spleen of a female rat administered with 250 mg/kg SCE showing red pulp and white pulp.
			
Micrograph 267: Lung of the female control rat after administration of DMSO	Micrograph 268: Lung of the female rat given a dose of 150 mg/kg SCE showing thickened alveolar walls.	Micrograph 269: Lung of female rat given SCE at 200 mg/kg showing thickened alveolar walls.	Micrograph 270: lung of female rats given SCE at a dose of 250 mg/kg showing thickened alveolar wall.
			
Micrograph 271: Kidney of control female rat administered with DMSO showing normal proximal convoluted tubules (yellow arrow) and glomeruli (black arrow).	Micrograph 272: Kidney of a female rat administered with SCE at 150 mg/kg showing widened glomeruli space and disorganized glomeruli.	Micrograph 273: Kidney of a female rat administered with SCE at 200 mg/kg showing disintegrated glomeruli and widened Bowman's space)	Micrograph 274: Kidney of female rate administered with SCE at 250 mg/kg showing widened glomerular space and disintegration of glomeruli

			
Micrograph 275: Liver of control female rat showing normal bile duct (brown arrow), central vein (black arrow) and normal hepatocytes (blue arrow).	Micrograph 276: Liver of female rat administered with SCE at 150 mg/kg showing distended sinusoids.	Micrograph 277: Liver of the female rat administered with SCE at 200 mg/kg showing distended sinusoids.	Micrograph 278: Liver of a female rat administered with SCE at 250 mg/kg slight distended sinusoids lumen.
			
Micrograph 279: Spleen of male control rat administered with DMSO showing normal spleen red pulp (red arrow) and white pulp (black arrow)	Micrograph 280: Spleen of male rat administered with SCE 150 mg/kg showing red pulp and white pulp	Micrograph 281: Spleen of male rat administered with SCE 200 mg/kg showing red pulp and white pulp.	Micrograph 282: Spleen of female rat administered with SCE 250 mg/kg showing red pulp and white pulp).
			
Micrograph 283: Lung of male control rat administered with DMSO showing normal alveolar wall (black arrow) of the lung.	Micrograph 284: Lung of the male rat administered with SCE at 150 mg/kg showing scattered RBC thickened alveolar wall.	Micrograph 285: Lung of male rat administered with SCE at 200 mg/kg showing thickened alveolar wall.	Micrograph 286: Lung of the male rat administered with SCE at 250 mg/kg showing thickened alveolar wall.

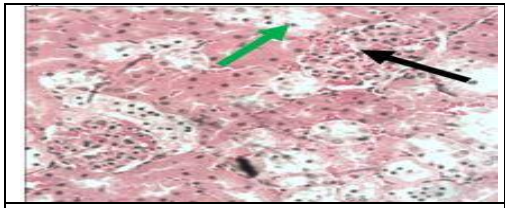
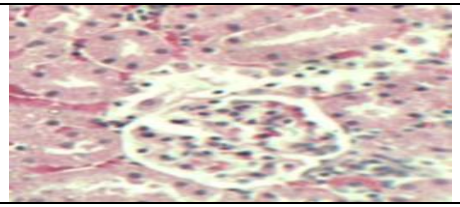
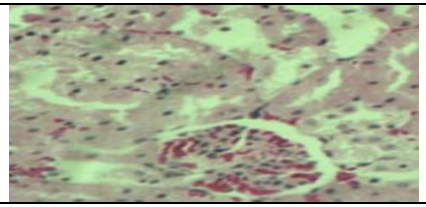
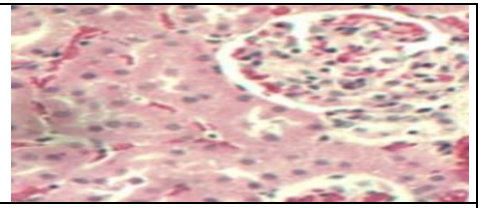
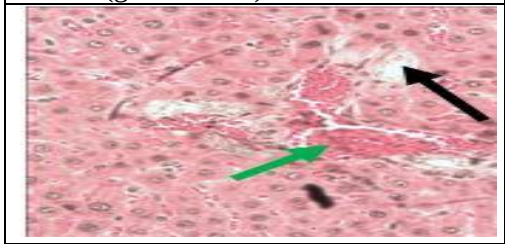
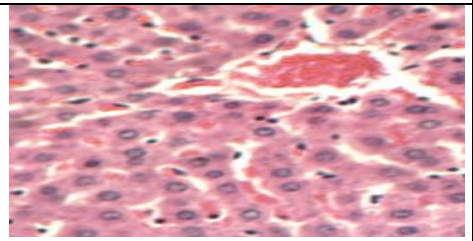
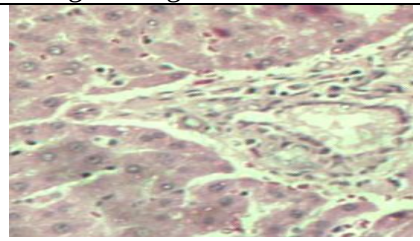
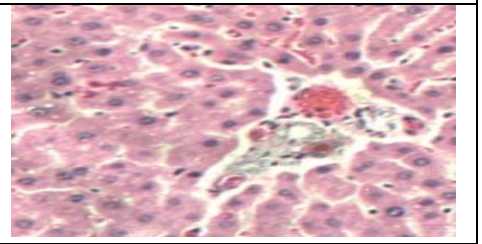
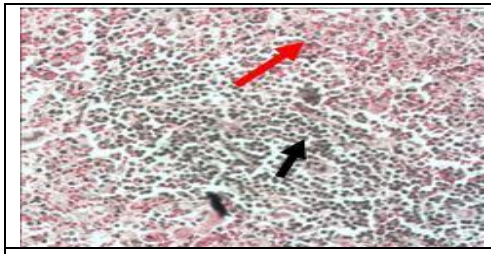
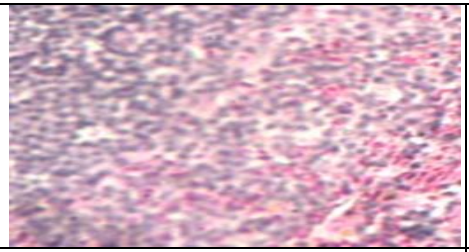
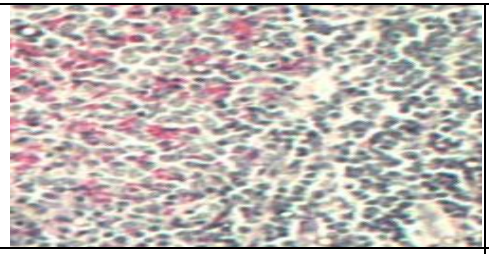
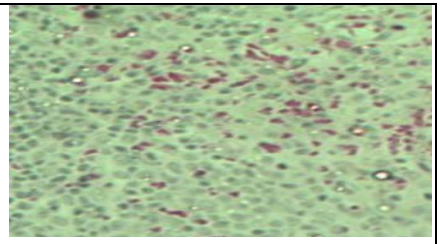
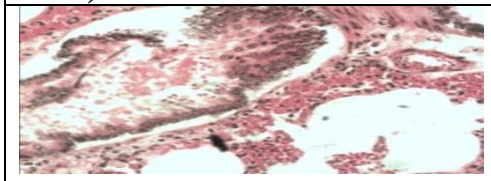
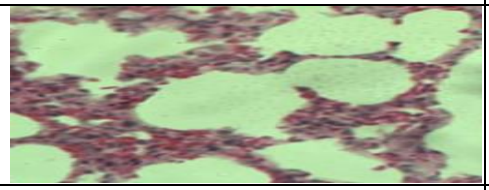
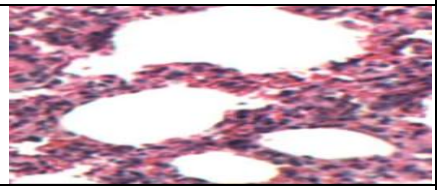
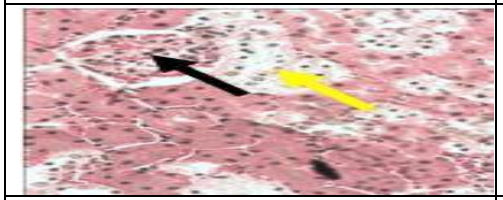
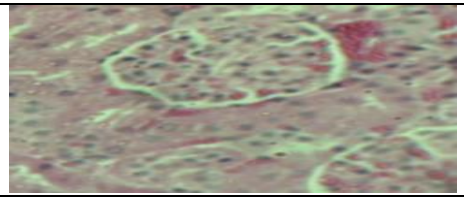
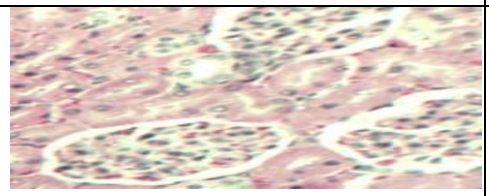
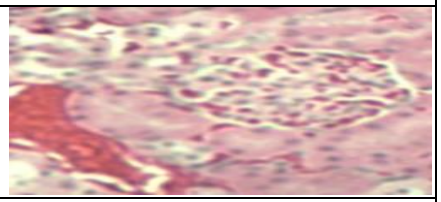
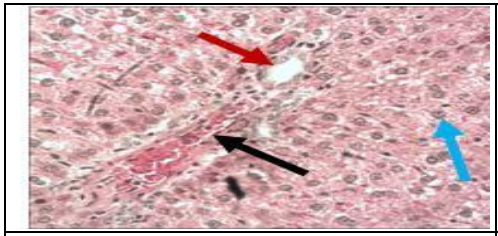
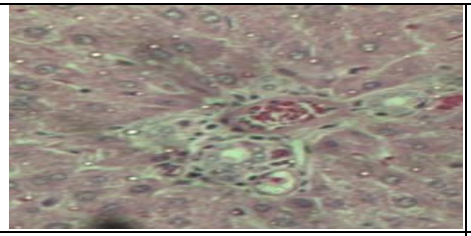
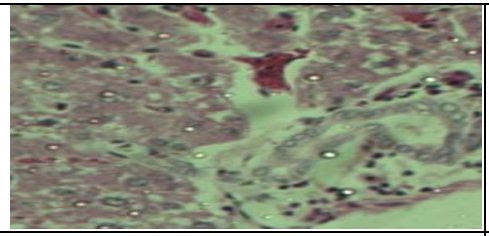
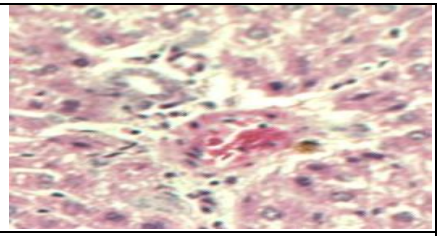
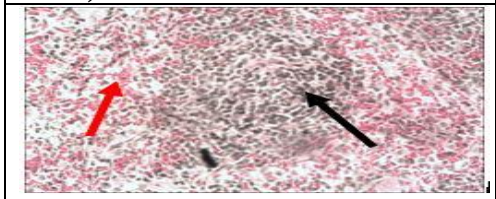
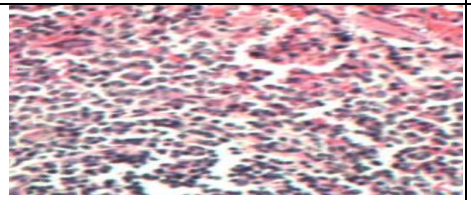
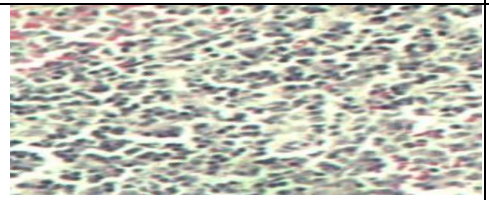
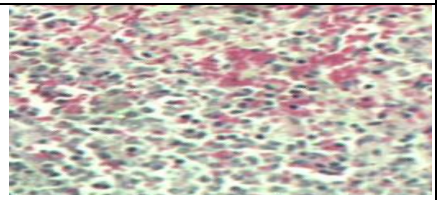
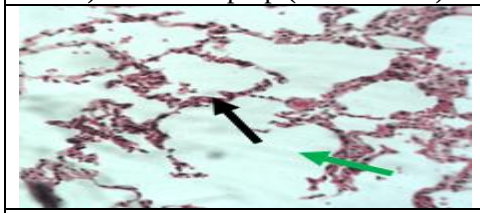
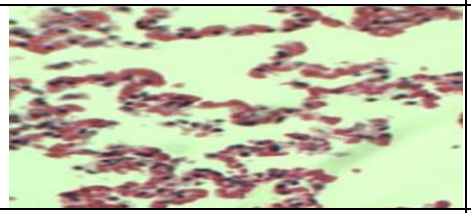
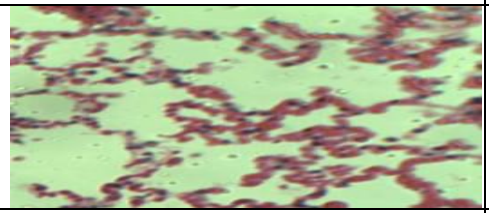
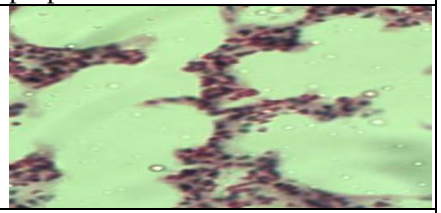
			
Micrograph 287: Kidney of control male rat administered with DMSO showing normal glomeruli (black arrow) and proximal convoluted tubules(green arrow).	Micrograph 288: Male rat kidney administered with SCE at 150 mg/kg showing increased glomerula space.	Micrograph 289: Male rat kidney administered with SCE at 200 mg/kg showing widened glomerular space and disorganized glomerula.	Micrograph 290: Kidney of male rat administered with SCE 250 mg/kg showing widened glomerular space and disorganized glomeruli.
			
Micrograph 291 Liver of control male rat administered with DMSO showing normal bile duct(black arrow),normal hepatocytes (yellow arrow) and normal central vein (green arrow)	Micrograph 292: Liver of male rat administered with SCE extract at 150 mg/kg showing distended sinusoids.	Micrograph 293: Liver of male rat administered with SCE at 200 mg/kg showing distended sinusoids.	Micrograph 294: Liver of male rat administered with SCE at 250 mg/kg showing distended sinusoids.

Figure 10: Micrographs of vital organs after sub-acute exposure of stem chloroform extract (SCE) and DMSO as control (H&E stain and magnification 40x)

			
Micrograph 295: Female control spleen administered with DMSO showing normal distribution red pulp (red arrow) and white pulp (black arrow).	Micrograph 296: Female rat spleen administered with 150 mg/kg STEA showing red pulp and white pulp.	Micrograph 300: Spleen of female rat administered with 200 mg/kg STEA showing red pulp and white pulp**.**	Micrograph 297: Spleen of a female rat administered with 250 mg/kg STEA showing red pulp and white pulp.
			
Micrograph 298: Lung of the female control rat after administration of DMSO	Micrograph 299: Lung of the female rat given a dose of 150 mg/kg STEA showing thickened alveolar walls.	Micrograph 300: Lung of female rat given STEA at 200 mg/kg showing thickened alveolar walls.	Micrograph 301: lung of female rats given STEA at a dose of 250 mg/kg showing thickened alveolar wall.
			
Micrograph 302 Kidney of control female rat administered with DMSO showing normal proximal convoluted tubules (yellow arrow) and glomeruli(black arrow)	Micrograph 303: Kidney of a female rat administered with STEA at 150 mg/kg showing normal glomeruli space.	Micrograph 304: Kidney of a female rat administered with STEA at 200 mg/kg showing disintegrated glomeruli and widened Bowman's space.	Micrograph 305: Kidney of female rate administered with STEA at 250 mg/kg showing widened glomerular space and disintegration of glomeruli.

			
Micrograph 306: Liver of control female rat showing normal bile duct (brown arrow), central vein (black arrow) and normal hepatocytes (blue arrow)	Micrograph 307: Liver of female rat administered with STEA at 150 mg/kg showing slightly distendended sinusoids	Micrograph 308: Liver of the female rat administered with STEA at 200 mg/kg showing central canal	Micrograph 309: Liver of a female rat administered with STEA at 250 mg/kg normal central canal.
			
Micrograph 310: Spleen of male control rat administered with DMSO showing normal spleen red pulp (red arrow) and white pulp (black arrow).	Micrograph 311: Spleen of male rat administered with STEA 150 mg/kg showing red pulp and white pulp.	Micrograph 312: Spleen of male rat administered with STEA 200 mg/kg showing red pulp and white pulp	Micrograph 313: Spleen of female rat administered with STEA 250 mg/kg showing red pulp and white pulp.
			
Micrograph 314: Lung of male control rat administered with DMSO showing normal alveolar wall (black arrow) of the lung.	Micrograph 315: Lung of the male rat administered with STEA at 150 mg/kg showing scattered RBC and thickened alveolar sac and alveolar wall	Micrograph 316: Lung of male rat administered with STEA at 200 mg/kg showing thickened alveolar wall.	Micrograph 317: Lung of the male rat administered with STEA at 250 mg/kg showing thickened alveolar wall.

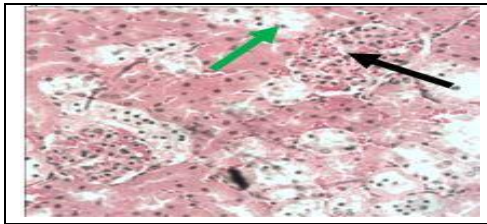
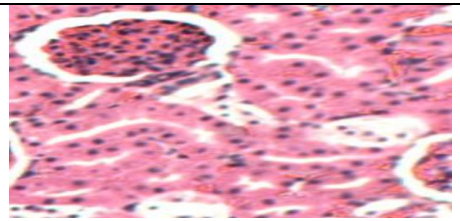
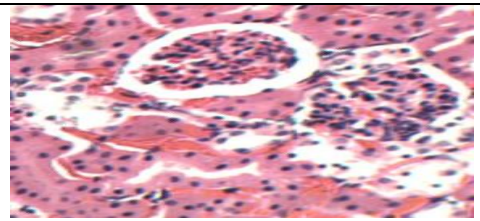
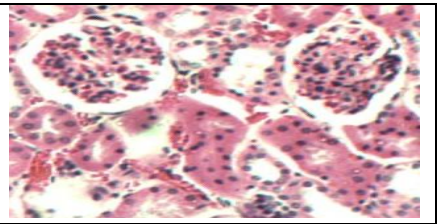
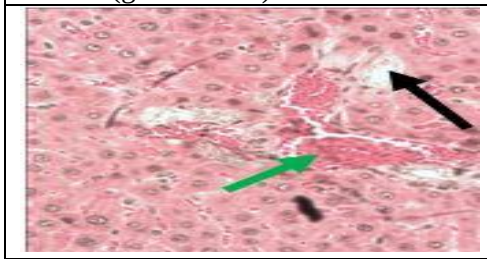
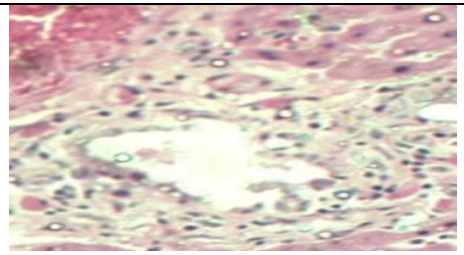
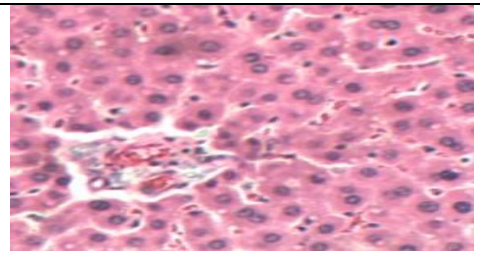
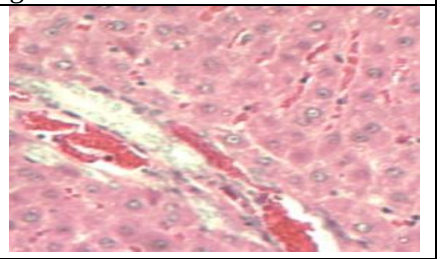
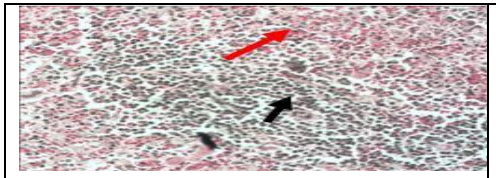
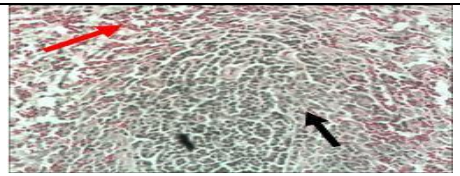
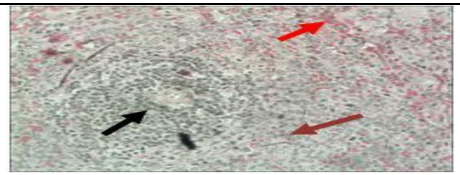
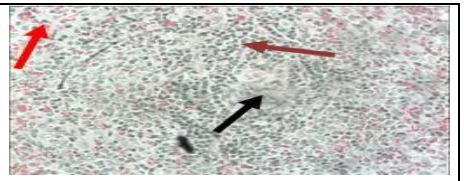
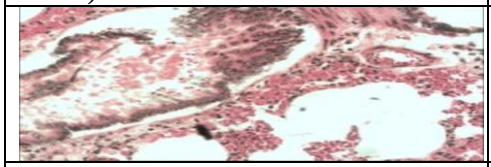
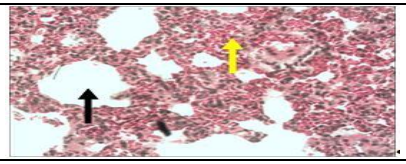
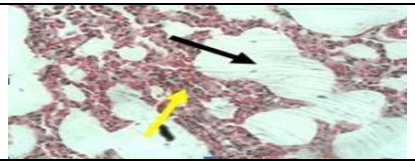
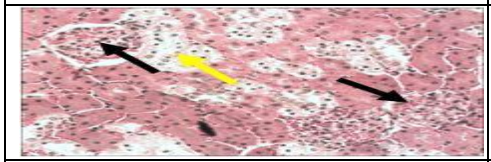
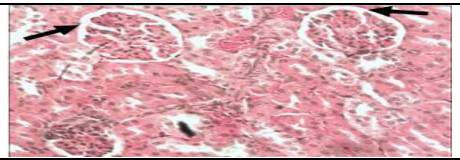
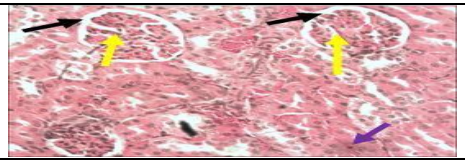
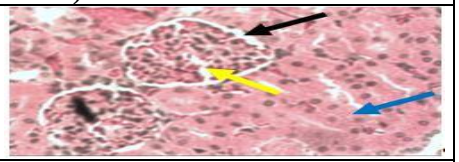
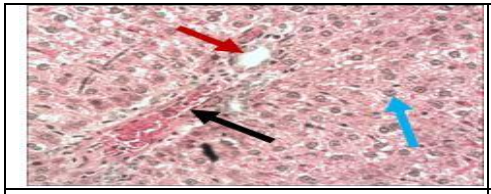
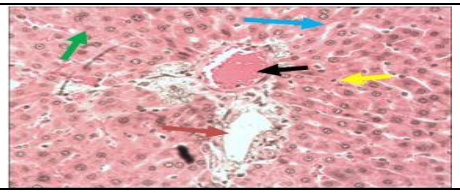
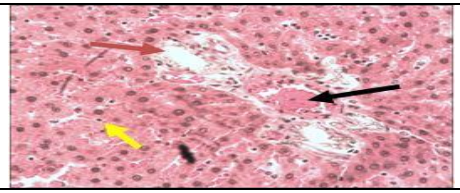
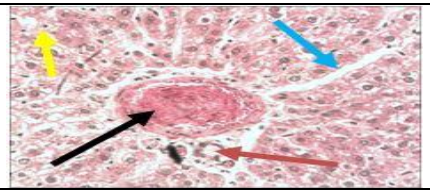
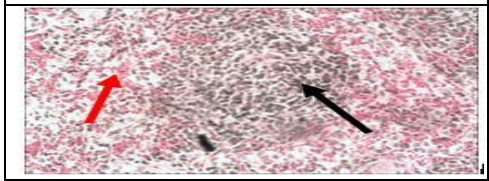
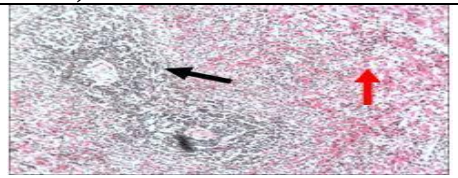
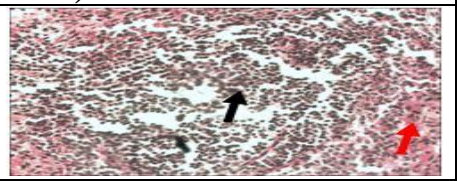
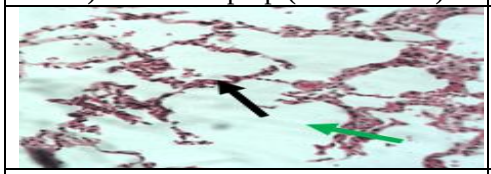
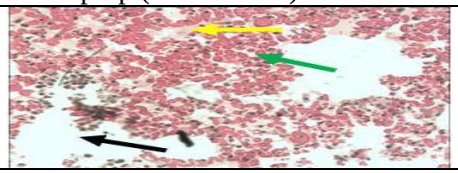
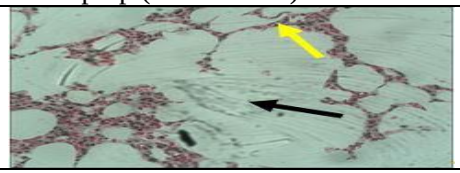
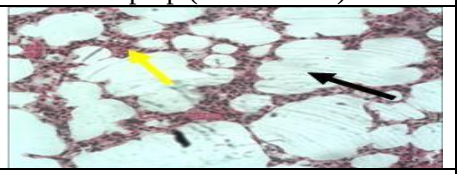
			
Micrograph 318: kidney of control male rat administered with DMSO showing normal glomeruli (black arrow) and proximal convoluted tubules (green arrow).	Micrograph 319: Male rat kidney administered with STEA at 150 mg/kg showing increased glomerula space.	Micrograph 320: Male rat kidney administered with STEA at 200 mg/kg showing widened glomerular space and disorganized glomeruli.	Micrograph 321: Kidney of male rat administered with STEA at 250 mg/kg showing widened glomerular space and disorganized glomeruli.
			
Micrograph 322: Liver of control male rat administered with DMSO showing normal bile duct(black arrow), normal hepatocytes (yellow arrow) and normal central vein (green arrow)	Micrograph 323: Liver of male rat administered with STEA extract at 150 mg/kg showing distended sinusoids.	Micrograph 324: Liver of male rat administered with STEA at 200 mg/kg showing distended sinusoids.	Micrograph 325: Liver of male rat administered with STEA at 250 mg/kg showing congested and distended sinusoids.

Figure 11: Micrographs of vital organs after sub-acute exposure to stem ethyl acetate extract (STEA) and DMSO as control (H&E stain and magnification 40x)

			
Micrograph 326: Female control spleen administered with DMSO showing normal distribution red pulp (red arrow) and white pulp (black arrow)	Micrograph 327: Female rat spleen administered with 150 mg/kg SME showing red pulp (red arrow) and white pulp (black arrow)	Micrograph 328: Spleen of female rat administered with 200 mg/kg SME showing reduced red pulp and white pulp (atrophy).	Micrograph 329: Spleen of a female rat administered with 250 mg/kg SME showing more reduced red pulp and white pulp (atrophy).
			
Micrograph 330: Lung of the female control rat after administration of DMSO	Micrograph 331: Lung of the female rat given a dose of 150 mg/kg SME showing thickened alveolar walls (yellow arrow) and surrounded alveolar sac (black arrow).	Micrograph 332: Lung of female rat given SME at 200 mg/kg showing thickened alveolar walls (yellow arrow) and respiratory tract (black arrow).	Micrograph 333: Lung of female rats given SME at a dose of 250 mg/kg showing thickened alveolar wall (yellow arrow) and circumscribed alveolar sac (black arrow)
			
Micrograph 334: Kidney of control female rat administered with DMSO showing normal proximal convoluted tubules (yellow arrow) and glomeruli (black arrow).	Micrograph 335: Kidney of a female rat administered with SME at 150 mg/kg showing widened glomeruli space (black arrow).	Micrograph 336: Kidney of a female rat administered with SME at 200 mg/kg showing disintegrated glomeruli (yellow arrow), widened Bowman's (black arrows), Pyknosis (purple arrow).	Micrograph 337: Kidney of female rate administered with SME at 250 mg/kg showing widened glomerular space (black arrow), disintegration of glomeruli (yellow arrow), Karyolysis (blue arrow)

			
Micrograph 338: Liver of control female rat showing normal bile duct (brown arrow), central vein (black arrow) and normal hepatocytes (blue arrow)	Micrograph 339: Liver of female rat administered with SME at 150 mg/kg showing normal bile duct (brown arrow) ,slightly distended sinusoids (blue arrow), karyorhexis (green arrow) and pyknosis (yellow arrow)	Micrograph 340: Liver of the female rat administered with SME at 200 mg/kg showing central canal (black arrow), normal bile duct (brown arrow), Pyknosis (yellow arrow).	Micrograph 341: Liver of a female rat administered with SME at 250 mg/kg normal central canal(black arrow), distended sinusoids lumen (blue arrow), pyknosis (yellow arow) and normal bile duct (brown arrow)
			
Micrograph 342: Spleen of male control rat administered with DMSO showing normal spleen red pulp (red arrow) and white pulp (black arrow)	Micrograph 343: Spleen of male rat administered with SME 150 mg/kg showing red pulp (red arrow) and white pulp (black arrow)	Micrograph 344: Spleen of male rat administered with SME 200 mg/kg showing red pulp (red arrow) and white pulp (black arrow)	Micrograph 345: Spleen of female rat administered with SME 250 mg/kg showing red pulp (red arrow) and white pulp (black arrow).
			
Micrograph 346: Lung of male control rat administered with DMSO showing normal alveolar wall (black arrow) of the lung.	Micrograph 347: Lung of the male rat administered with SME at 150 mg/kg showing scattered RBC (green arrow), alveolar sac (black arrow) and alveolar wall (yellow arrow).	Micrograph 348: Lung of male rat administered with SME at 200mg/kg showing normal alveolar wall (yellow arrow) and alveolar sac (black arrow) of the lung.	Micrograph 349: Lung of the male rat administered with SME at 250 mg/kg showing normal alveolar wall (yellow arrow) and alveolar sac (black arrow).

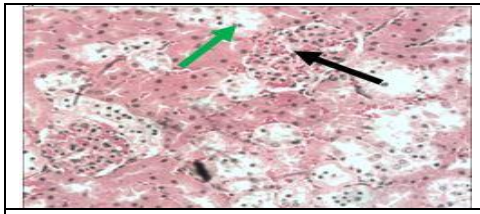
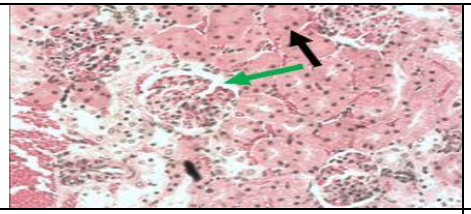
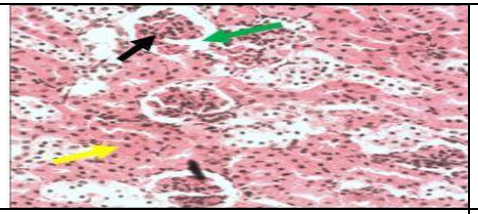
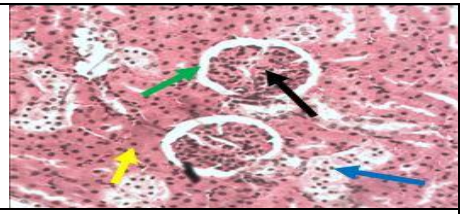
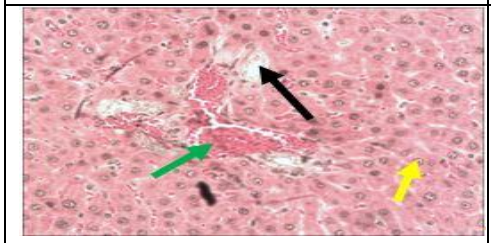
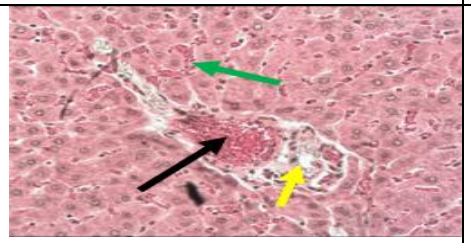
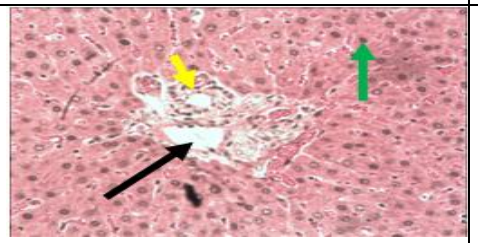
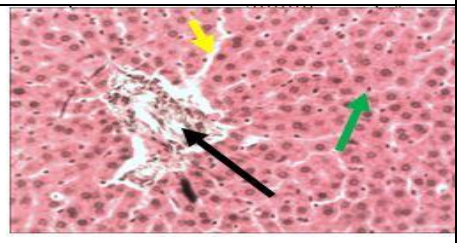
			
Micrograph 350: Kidney of control male rat administered with DMSO showing normal glomeruli (black arrow) and proximal convoluted tubules(green arrow). (H&E stain magnification 40x)	Micrograph 351: Male rat kidney administered with SME at 150 mg/kg showing increased glomerula space(green arrow) and pyknosis (black arrow) (H&E stain magnification 40x)	Micrograph 352: Male rat kidney administered with SME at 200 mg/kg showing widened glomerular space (green arrow), disorganized glomerula and (black arrow) and pyknosis(yellow arrow).	Micrograph 353: Kidney of male rat administered with SME 250 mg/kg showing widened glomerular space (green arrow), disorganized glomeruli (black arrow), collapsed tubular structure (blue arrows) and pyknosis (yellow arrow)
			
Micrograph 354: Liver of control male rat administered with DMSO showing normal bile duct (black arrow), normal hepatocytes (yellow arrow) and normal central vein (green arrow).	Micrograph 355: Liver of male rat administered with SME at 150 mg/kg showing distended sinusoids(green arrow), central vein (black arrow) and bile duct (yellow arrow)	Micrograph 356: liver of male rat administered with SME at 200 mg/kg showing pyknosis (green arrow), wide bile duct (yellow arrow) and central vein (black arrow).	Micrograph 357: Liver of male rat administered with SME at 250 mg/kg showing distended sinusoids (yellow arrow), normal bile duct (black arrow) and pyknosis (green arrow).See disorganized hepatic triad.

Figure 12: Micrographs of vital organs after sub-acute exposure of stem bark methanolic extract (SME) (H&E stain and magnification 40x)

3.5 Discussion

Herbal medicines have been used extensively in many African countries including Tanzania as an alternative remedy for different diseases, however their toxicological profiles have not been studied to validate their effects to body tissues. For instance some herbal medicines were made from aristoholic acid led to renal failure, World Health Organisation makes emphasis on herbal medicines quality (WHO, 2002). *Commiphora species* are used in different humans ailments management with different side effects (WHO, 2004). *Commiphora campestris* have been used as medication of various ailments by Pare people since long time, without guarantee by any toxilogical studies. The plant roots, stem bark and leaf were tested for antibacterial effects showed to be effective against several Gram positive and Gram negative organisms Godfrey and Co workers (2016). There are no mortalities that were recorded during sub acute toxicity study at all doses. All rats that received different extracts increased in body weight reveal that the all extracts had no negative impact on the growth.

Triglycerides and Cholesterol make up the body lipids, in this study leaf chloroform extract decreased glucose, cholesterol, total protein, albumin and triglycerides compared to controls, so the plant have hypoglycemic and hypolipidemic effects. Other *Commiphora species* studies have shown antilipidemic effects in animals of both sexes, as supported by studies on *C. mukul* (Strippoli, 2004). Total protein and albumin decreased in tested rats compared to controls, the cause might be due to liver and kidney malfunction after administration of leaf chloroform extract, leading to hypoprotenemia (Burtis *et al.,* 2008), this is also confirmed by aberrations in the kidney glomeruli (Micrograph 171-180) and liver (Micrograph 181-184) interfering protein synthesis and elimination.

There was increase of liver enzymes (ALT, ALP and AST), bilirubin and creatinine in this study. ALT and AST increased in both sexes compared to control groups, this shows that the leaf chloroform has a hepatotoxicity effect and renal cytotoxicity, but in lesser amount these enzymes can originate from damaged heart and skeletal muscles so, ALT results are still not sufficient to conclude the effects of the plant as it could be originating from other tissues (Thapa and Walia, 2007), in this study of the liver (Micrograph 181 – 184 and 197-200) and kidney in (Micrograph 177-180 and 193-196) showed distended liver sinusoids and widened glomerural space a sign of liver and kidney damage, hence increased ALT (Kakadiya, 2009).

Alkaline phosphatase is an enzyme that catalyses transport of fats in the intestine, however increase in levels of this enzyme in blood is associated with bile duct occlusion or bone diseases, caliculi occlusion or inflammation of bile duct, hence increased need for transmission of the bile (Thapa and Walia, 2007). In this study increase of ALP, means the bile ducts were damaged and this is proven by multiplication of bile ducts and disorganization of the bile ducts however the lumen is not affected as seen in liver micrographs (Micrograph 182-184). Studies with other species of Genus *Commiphora* such as *C. opobalsamum* (syn Balesan) have shown that the species are used in treatment of liver disease, wounds, obesity, inflammatory diseases and in renal caliculi to facilitate urine excretion (Shen *et al.,* 2012) so may be this plant also has the same compounds that could facilitate opening of bile duct resulting in urine excretion, however studies are still needed to validate this. Decrease in WBC and RBC and its indices shows that the extract has effect on blood cell production, RDW increase means the RBC produced are changing from their normal size and shape, this has effects on RBC counts this accounts for reduced hemoglobin (Solak *et al.,* 2014).

Red cell distribution width is the erythrocyte size variability caused by nutrition, heart diseases, iron deficiency and chemotherapy and RDW has been used also as a good indicator of renal disease that impairs renal functions (Tekce *et al.,* 2014). Relative organ weight of kidney, liver, heart and spleen were significantly reduced in test rats as compared to control group. Leaf chloroform had effects on the organ weight during sub acute exposure. Kidney increase was observed with increased glomerular space and might have led to reduced renal function hence increased blood creatinine and reduced RBC production.

The kidney is a good source of erythropoietin hormone essential for stimulation of erythrocytes production from hemopoietic organs, swelling might have altered hormone quantity resulting to low RBC production in the male rats. Increased liver relative organ weight might have led to increased levels of AST, ALT, ALP and increased urea levels. Impaired deamination might have caused low urea production (Burtis *et al.,* 2008). The spleen is involved in production of RBC, destruction of damaged RBC and lymphocytes (Burtis *et al.,* 2008). Leaf chloroform might be directly associated with splenomegaly (increased spleen size), that caused reduction in circulating RBC seen in male rats, because spleen plays a role in control of amount of RBC in circulation. Female rats showed increased RBC levels with increasing doses, this indicates that there was no effect on the hematopoietic organs. However, reduced spleen weight might have contributed to decreased hemoglobin

levels, due to spleen failure to maintain RBC in circulation, although the micrographs shows increased follicular cells than red pulps for RBC (Elmore, 2016).

Rats that received leaf ethyl acetate extract caused decrease in WBC, RBC, RDW and [HB] this shows that the extract interfered the hematopoietic organs, this is seen in association with increased in relative organ weight especially for kidneys and decreased relative organ weight of the liver. Increased relative weight of kidneys shows that the extracts caused increased activity of the kidney hence it increased in relative organ weight so as to compensate excretion of the compounds where as liver atrophy may have resulted from detoxification of extract. Kidney might have decreased secretion of erythropoietin an important hormone for blood cell production (Fisher, 2003) and liver decrease in relative organ weight might have caused decrease in total protein production that led to decrease in synthesis of essential protein for blood cell synthesis (Al- saeed and Alawami, 2016). Creatinine increase is associated with failure of elimination by the kidneys, this shows the extract caused damage to the kidneys that resulted to accumulation of creatinine. Unlike in females there was increased RBC production with increased total protein level and decreased relative organ weight of the kidney, liver and spleen which are important organs for blood cell production. The extract caused increase in RDW which is a good indicator for changes in RBC structure that is related in decrease of haemoglobin.

Male rats that received leaf ethyl acetate had increase in glucose, albumin, triglyeride, urea, Bilirubin, ALT, ALP and Cretinine, this shows the extract has interference with such parameters this is linked to renal diseases (McClellan *et al.,* 2004), however the extract showed decrease in cholesterol, total protein and AST while female rats had increase in glucose, total protein, triglycerides, bilirubin, ALT, AST and ALP and decrease in cholesterol, albumin urea and creatinine. Liver enzymes increase (ALT and ALP) this is a good indicator, of an extract to cause liver (Micrograph 213 – 216, 229 - 232) and kidney damage (Micrograph 209 – 212, 225 - 228).

Creatinine is a byproduct of muscle cellular metabolism of creatine for energy production and is removed by kidneys. Creatinine accumulation in the blood it indicates renal impairment, these results show that leaf ethyl acetate extract caused kidney damage, glomerula filtration rate seems to have been impaired and resulted to creatinine accumulation. Renal micrographs show that glomerular space has widened as seen in (Micrograph 213 – 216 and 228 – 231), this could be the reason for insufficiency of creatinine clearance (Burtis *et al.,* 2008).

BUN is blood urea nitrogen, this is formed from breakdown of protein in the gastro intestinal tract content by bacterial protease, urease and amine oxidases. In this study urea was high in both sexes of tested rats compared to control group, this increase may be aggravated by dysfunctional renal function as seen in (Micrograph 213 – 216 and 228 – 231) (Biosystem, 2014).

Leaf methanolic extract caused increase in glucose, triglycerides shows that the extracts do not interfere with metabolism of carbohydrates, this shows that the glucose and triglycerides are absorbed into blood stream. Excessive uptake of glucose and triglycerides can be seen in liver diseases, diabetes and kidney dysfunction (Biosystem, 2011). Increased urea, albumin, bilirubin, ALT, ALP, AST and cretinine shows that there is a damage to the organs that are involved with metabolism of protein which are essentially liver and kidneys, liver enzymes increases in the blood due to damage to liver as seen in micrograph 245 – 248 and 261 - 264, heart or kidneys as seen in (Micrograph 241 – 244 and 257 – 264). Increase of glucose, albumin, bilirubin, ALT, AST and ALP in females indicate damage to liver, kidneys and heart., Damage of liver and kidneys is related to decreased excretion of urea, creatinine and decrease protein levels (due to liver failure to produce proteins). Diminishing cholesterol levels is associated with breakdown of lipids in both sexes. Defects in vital organs are associated also with decreased in weight because of shrinkage of the organs in both sexes. Hematological changes in males shows that leaf methanolic extract interferes with hematopoietic processes, increased RDW reflects abnormal RBC which in turn results to decreased RBC and its indices. Increased hematological parameters in females is associated with decreased relative organ weight, this may be aggravated by organ to compensate lost blood in circulation.

Increase in glucose, triglycerides shows that the stem chloroform extracts do not interfere with metabolism of carbohydrates. Increased carbohydrate (glucose) uptake and conversion to triglycerides is reflected in body weight increase during the course of the study also increased blood glucose might have been caused by moderate exercise that provoked stress response that led to increased blood glucose at times of draining blood (Burtis *et al.,* 2008). Increased urea, albumin, bilirubin, ALT, AST and cretinine shows that there is a damage to the organs that are involved with metabolism of protein which are essentially liver and kidneys, liver enzymes increases in the blood due to damage to liver as shown in Micrograph 277 – 280 and 293 - 296, heart or kidneys as seen in (Micrograph 273 – 276 and 289 – 292) (McClellan *et al.,* 2004 ; Kakadiya, 2009). Decrease in cholesterol, albumin and ALP shows

the liver was damaged such that failed in synthesis of albumin but the bile system were not affected. Damage to females liver, kidneys, heart and decreased protein levels is due to liver failure to produce proteins (Thapa and Walia, 2007) where diminishing cholesterol levels is associated with breakdown of lipids in both sexes. Defects in vital organs is seen where the organs are decreased in weight may be because the extract causes shrink of the organ in both sexes. Liver enzymes ALT, ALP and AST increase shows that the extract caused tissue damage in the liver, kidney and possibly heart (Kakadiya, 2009). Hematological changes in males shows that the extract interferes with hematopoietic processes. Increase in RDW causes production of abnormal RBC which in turn results to decreased RBC and its indices as well as hemoglobin. Increased hematological parameters in females is associated with decreased row, this may be aggravated by organ to compensate lost blood in circulation.

Increase in glucose, triglycerides shows that the stem ethyl acetate extract do not interfere with metabolism of carbohydrates, might have been caused by moderate exercise that provoked stress response that led to increased blood glucose at times of draining blood (Burtis *et al.,* 2008). Increased urea, albumin, bilirubin, ALT, AST and cretinine in males and females shows that there is a damage to the organs that are involved with metabolism of protein which are essentially liver and kidneys, liver enzymes increases in the blood due to damage to liver, heart or kidneys (Thapa and Walia, 2007). Diminishing cholesterol levels is associated with breakdown of lipids in both sexes is important as the extract might be very important in management of cholesterol. Defects in vital organs are seen where the organs are decreased in relative organ weight may be because the extract causes shrink of the organ in both sexes. Liver enzymes ALT, ALP and AST increase shows that the extract caused tissue damage in the liver as seen in (Micrograph 309 – 312 and 325 – 328), kidney as seen in (Micrograph 305 – 308 and 321 – 3324) and possibly heart (Thapa and Walia, 2007). Hematological changes in males shows that the extract interferes with hematopoietic processes, increase in RDW causes production of abnormal RBC which in turn results to decreased RBC and its indices. Increased hematological parameters in females is associated with decreased relative organ weight, this may be aggravated by organ to compensate lost blood in circulation.

Triglycerides and cholesterol make up the body lipids, in this study stem bark methanolic extract decreased cholesterol and triglycerides compared to controls, so the plant have hypolipidemic effects. in other studies of *Commiphora species* have shown antilipidemic effects in animals of both sexes, as supported by studies on *C. mukul* (Strippoli, 2004). Total

protein decrease in tested rats compared to controls, the cause might be due to liver and kidney malfunction due to extract, leading to hypoprotenemia (Burtis *et al.,* 2008), this is also confirmed by aberrations in the liver and kidney glomeruli interfering protein synthesis and elimination. In females, increased protein was observed (Table 23), which is an indication that the organs were not affected, however, this could have been influenced by other female physiological processes which influence rise in total protein levels like estrus cycles (Yaqub, 2013). Albumin is a serum protein that is produced from the liver and is excreted through the kidneys (Townsend, 1990). In this study, decrease of albumin can directly be linked to liver and kidney dysfunction. The effect of stem bark methanolic extract in test animals could be a reason for liver failure to synthesize proteins (Thapa and Walia, 2007). Liver damage as seen in Micrograph 341-344 and 357 – 360) and kidney damage as seen in (Micrograph 337 – 340 and 353 – 356) might have aggravated albumin decrease, this decreased is also seen in hepatitis and liver cirrhosis (Burtis *et al.,* 2008). Bilirubin is a yellow pigment that is produced after breakdown of the RBC hemoglobin in the liver resulting in protein separation into globin and heme which is further broken to biverdin that is reduced into bilirubin, this occurs in the reticuloendothelial system of the liver, spleen and bone marrow (Burtis *et al.,* 2008).

Increased bilirubin is a result of liver or kidney disease and is related to decreased levels of RBC in males suggesting excessive breaking down of RBC (hemolysis) releasing its hemoglobin that gives rise to total serum bilirubin (Burtis *et al.,* 2008). There was increase of two liver enzymes ALT and AST. Increased ALT levels was observed in treated rats of both sexes compared to control groups, indicating hepatotoxicity effect, this enzyme however is not only found in the liver it could originate from other organs like kidneys, heart and skeletal muscles (Thapa and Walia, 2007).

Liver micrographs as seen in (Micrograph 341-344, 357 – 360) shows pyknosis and distended sinusoids suggesting liver damage, hence increase of ALT (Ezeja *et al.,* 2014). Aspartate Amino Transferase is found in large amounts in the muscles, liver and heart, elevated AST in blood occurs due to damage to cells of muscles, heart muscles and liver and reduced clearance of abnormal compounds in blood plasma and when there is increased hemolysis of RBC (Burtis *et al.,* 2008). Significant rise in the AST, may be due to liver damage as supported by liver micrographs seen in (Micrograph 341-344 and 357 – 360). Alkaline Phosphatase is an enzyme that catalyses transport of fats in the intestine, however increase in levels of this enzyme in blood is associated with bile duct occlusion or bone diseases, caliculi

occlusion or inflammation of bile duct, hence increased need for transmission of the bile (Burtis *et al.,* 2008) in this study there is significant decrease of ALP, this means the bile ducts were not damaged and this is proven by liver micrographs as seen in (Micrograph 341-344 and 357 – 360) shows normal architecture of the bile ducts. Studies with other species of Genus *Commiphora* such as *C. opobalsamum* (syn Balesan) have shown that the species are used in treatment of liver disease and in renal caliculi to facilitate urine excretion (Shen *et al.,* 2012), so maybe this plant also has the same compounds that could facilitate opening of bile duct resulting in urine excretion, however studies are still needed to validate this.

There was a rise in creatinine levels of test rats compared to the control group. Creatinine is a byproduct of muscle cellular metabolism of creatine for energy production and is removed by kidneys and remains at levels that are fairly reasonable (Papakadis and Arief, 1987). If creatinine accumulates in the blood it indicates renal impairment, these results show that stem bark methanolic extract caused kidney damage, glomerula filtration rate seems to have been affected as creatinine accumulated in blood. In this study tested rats renal showed disorganised glomerulus (Micrograph 337 – 340 and 353 – 356), possible reason for insufficiency creatinine clearance (Burtis *et al.,* 2008).

BUN is blood urea nitrogen, this is formed from breakdown of protein in the gastro intestinal tract content by bacterial protease, urease and amine oxidases. In this study urea was lower in tested rats compared to control group, this may be due to the influence of liver cell damage caused by extracts which led to reduced metabolism of ammonia by hepatocytes resulting in lower urea production. The most common cause of failure to metabolize ammonia is impairment of hepatocyte functioning that is due to liver disease (Burtis *et al.,* 2008). Lowering of blood urea has been observed in human studies with cirrhosis without azotemia (McClellan *et al.,* 2004).

Glucose increased in the blood of tested rats as compared to control group. Glucose increase might be due to the stimulation of insulin as result of rat struggling exercise at times of blood sample draining (Burtis *et al.,* 2008).

3.6 Conclusion

In sub acute toxicity no mortality that was recorded. Major effects were rise of glucose levels, decreased vital organs size, and thickened female lung alveolar walls that probably increased RBC counts. Reduced cholesterol levels in leaf and stem extracts suggest antilipidemic effect of *C. campestris*. More research is needed to profile the chemical compounds in the extract and validation of safety doses in humans.

CHAPTER FOUR

4.1 General discussion

Use of traditional medicines in Tanzania started long years ago by inheriting the medications from the ancestors (Augustino and Gillah, 2005). Plants have shown increasing improvement on different disease conditions. In Pare mountains, use of traditional medicines has been common even before introduction of conventional medicines. The medicines are used without prior assessment of the effect it pose to the body.

Commiphora campestris has shown to have observable effects in acute studies to the tested mice and no observable effects seen during sub acute tests in rats. In acute study with mice the extracts have shown some toxic effects and resulted to mortalities. This was also associated with changes in microscopic appearance of the organs. Assurance of safety and efficacy validation is a concern of WHO, this led us to conduct this study to ascertain toxicity of plant to body tissues (WHO, 2002). In the acute study, mice were subjected to 300 mg/kg, 600 mg/kg and 1200 mg/kg for each extract, extracts that caused mortalities were used to calculate lethal dose 50 of the extract which was found to be 424 mg/kg using method described by Lorke (Lorke, 1983), this dose was used to calibrate dose range to be used in sub acute toxicity.

Lethal Dose 50 (LD_{50}) was 424 mg/kg) that was obtained from acute study was used to calibrate doses to be use in sub acute toxicity study. The used doses of 150, 200 and 250 mg/kg did not cause any mortality throughout the experiment which implies that the extracts are toxic at doses above 424 mg/kg. Hematological assessment showed that the extract had interference with levels of blood cell counts and non blood cell parameters. Liver enzymes amount changes were noted, this means the extracts resulted to damage of vital internal organs like liver and kidneys which is reflected by widened sinusoids of the liver and renal glomeruli (Thapa and Walia, 2007).

Decrease in levels of cholesterol in some extracts, this shows that to some extend the extracts have antilipidemic effect. Other biochemical parameters like creatinine and urea were increased due to poor elimination an indication of liver and kidney damage (Kakadiya, 2009). The extracts also showed not to interfere with carbohydrate metabolism as the levels of glucose remained high in most of the tests rats, this might have been because of the stimulation of insulin at times of draining the samples (Burtis *et al.*, 2008).

Anatomical structures are important components in assessment of the toxicity. Liver and kidney play role in detoxification and elimination of different compounds, in this study kidneys showed widening of the glomeruli and where as the liver had distended sinusoids. This is means the extract at higher doses is toxic to body tissue, as at acute study mice mortalities and tissue microscopic changes.

4.2 General conclusion

The extracts in high doses in acute toxicity causes detrimental effects to the mice and in sub acute the effects are highly limited to internal organs and hematological parameters. The extracts cause the changes in liver and kidneys which are key organs for excretion of the extracts metabolite. The extract causes changes in blood parameters which imply that the extract has effects on hematopoietic organs like bone marrow and kidneys. Further studies are needed to identify the compounds and organs that are interacted by the compounds in the extract.

4.3 Recommendations

Following reported antibacterial and antifungal effects of *Commiphora campestris* (Godfrey, 2016), use of the plant by Pare community in management of human infectious diseases and findings of this research the following are recommendations that are put forward for further investigate the plant:

(i) The extracts that caused mortalities was in dose depended fashion this signify that the extracts are toxic with increasing doses, further studies are to be conducted to identify the compounds present in toxic extracts and their effects.

(ii) Although the plant extracts showed to be with toxic, this plant is still a potential drug template for antibacterial, antifungal compounds and anticancer drug.

(iii) Currently there is increased use of herbal medication that lead to extreme plant cut off, this is posing a danger of extinction of the plant, so it is time to preserve the species for further studies and future generation.

(iv) Most of the anticancer drugs have a tendency of increasing the white blood cells, this was congruent with findings of this study, further studies are need to compare the activity of the *C. campestris* extract (screened compounds) with conventional anticancer drugs.

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