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ACUTE AND SUB-ACUTE TOXICITY STUDIES OF CHLOROFORM EXTRACTS OF CUCUMIS METULIFERUS AND LIPPIA KITUIENSIS IN MICE AND RATS MODEL

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ABSTRACT

Objective: To evaluate the acute and sub-acute oral toxicity of *Cucumis metuliferus* and *Lippia kituiensis* leaf chloroform extracts in mice and rats model. **Methods**: Acute oral toxicity study of chloroform extracts of *L. kituiensis and C. metuliferus* was carried out by administration of 300, 600, 1500, mg/kg body weight to mice in the respective groups. The LD_{50} of the *C. metuliferus* and *L. kituiensis* extracts was determined to be not greater than 2000 mg/kg body weight. Sub-acute toxicity study was conducted by oral administration of the extracts at daily doses of 150, 300 and 500mg/kg body weight in the respective groups of rats for 28 days, and a positive control consisting of 1% DMSO and 5 mL was given to each. **Results**: In acute toxicity, all treated groups revealed neither mortality nor significant alteration in behavior, body weight, and hematology parameters. However, the significance different was observed in organ weight at a dose of 600mg/kg and 1500mg/kg of the tested plant extract in both plants. In sub acute study the result revealed neither mortality nor significant alteration in behavior between treated and control. The significant different was observed in body weight in all doses in both plants and organ weight and haematological parameter in dose level 300 mg/kg and 500 mg/kg compared to control. Moreover the significant change was observed in biochemical parameters of both sexes in dose of 300 mg/kg and 500 mg/kg body weight of *C. metuliferus* and *L. kituiensis* extract. A significant histological change was observed in liver, kidney, lungs and spleen in all extract of *C. metuliferus* and *L. kituiensis* in a dose of 300 mg/kg and 500 mg/kg body weight. Conclusion: These plants can causes' severe toxicity to animals.

KEYWORDS: *C. metuliferus, L. kituiensis*, toxicity.

1. INTRODUCTION

The search for new drugs which are plant-derived has been receiving renewed interest among researchers throughout the world in view of discovering new drugs that possess potency to combat the menace of drug resistant agent (About *et al.*, 2000: Pimenta *et al*., 2003). Plants can be useful either in their crude or advanced forms, offering a source of drugs in their pure state (Saint *et al*., 2010: Soejarto *et al.*, 2009). According to the World Health Organization's questionnaire, it is announced that 80% of the population in the world are relying on conventional medicine, mainly in plant sources, in the primary health protection (WHO, 2015). Recognized for their ability to produce a wealth of secondary metabolites, many of these natural products have been shown interesting biological and pharmacological activities, which could serve as the starting point in the development of modern medicines (Abubakar *et al*., 2010).

Genus *Lippia* (Verbenaceae, Lamiales/Magnoliopsida) includes about 200 species of herbs, shrubs and small trees mainly distributed in Central and South Americas and in Africa Tropical (Terblanche *et al*., 1996: Santos *et al*., 2009). The species belong to this genus have shown a large number of important usages in folk medicine for various diseases, particularly in the treatment of cough, bronchitis, indigestion, liver, hypertension, dysentery (Pham *et al*., 1988: Pascual *et al*., 2001), and skin diseases (Matos *et al*., 1998). Many *Lippia* species have promising biological activities, including antiviral (Abad *et al*., 1997), antimalarial (Valentin *et al*., 1995), anti-inflammatory, analgesic, antipyretic (Forestier *et al*., 1996: Forestier *et al*., 1996), antimicrobial (Lemons *et al*., 1990), insecticidal (Lima *et al*., 2013), and anticonvulsant (Abena *et al*., 1998) properties. Moreover *Lippia* species revealed *in vitro* antitumor activity on leukemia (K-562, HL-60, and CEM), colon (HCT-116), breast (MCF-7), glioblastoma (U-251), and prostate (PC -3) cell lines (Costa *et al*., 2001: Gonzalez-Guereca *et al*., 2010).

Cucumis metuliferus belongs to the family Cucurbitaceae, and is a monoecious, climbing, annual vine that can be grown practically anywhere, provided the season is warm (Benzion *et al*., 1993). The fruits are ovoid berries of 8-10 cm long and 4-5 cm in diameter with horn-like spines (hence the name horned cucumber), yellow-orange skin and a lime green jelly-like flesh when ripe (Wanning *et al*., 2008). This plant is used by traditional medical practitioners in different place to treat diseases such as peptic ulcer, diabetes mellitus, hypertension,

HIV/AIDS (Wanning *et al*., 2008) and antiviral property (Noel *et al*., 2009). According to (Wanning *et al*., 2007), the fruit pulp is used as a remedy to all diseases hence its local name 'Kanda' which means 'stop it before it comes' or 'a local vaccine. Apart from these traditional medicinal properties of *L. kituiensis* and *C. metuliferus* these two plants have been used traditionally in Tanzania for the management of malaria (Mzena *et al*., 2018). The latest report on evaluation of antimalarial activity of *Lippia kituiensis* and *Cucumis metuliferus* species found in Tanzania revealed a promising result (Mzena *et al*., 2018). However, only little information can be provided regarding the possible toxicity that the plant may cause to the consumers. Thus, acute and sub acute toxicity studies on medicinal plants should be done in order to increase the confidence in their safety to humans. Therefore this study evaluated the acute and sub acute toxicity of *L.kituiensis* and *C.metuliferus.*

2.0. METHOD

2.1. Collection of plant material and extraction

The plants (*C. metuliferus* and *L. kituiensis*) were collected in the month of September 2016 from Ugweno ward within Mwanga District, Kilimanjaro Region in Tanzania. Herbalists residing in Ugweno ward and a taxonomist (Mr Josephat Mboya) were consulted and involved in the identification and collection of plant materials. The voucher specimen coded (Voucher no. 160 and 161) were deposited at Nelson Mandela African Institution of Science and Technology. The plant material were washed and dried for 20 days at room temperature. After shade drying, the leaves were grinded to a course powder using a blender. The powder material of *C.metuliferus* and *L.kituiensis* was extracted at 78^0C for 48 h using chloroform solvent. The extracts were then concentrated under reduced pressure through rotary evaporator (N-10000, Eyela, Japan). Obtained extracts were collected and preserved in a desiccator for further studies.

2.2. Experimental animals

Swiss albino mice weighing 25–30g for male sex and 20-27for female sex and Winstar albino rats weighing 62-96g for male sex and 45-77g for female sex from the College of Veterinary Medicine and Medical Sciences of Sokoine University of Agriculture (SUA) were used in these experiments. All animals were kept in a meshed cage covered with sawdust beddings at room temperature 25°C - 30°C. Different sexes were separated to avoid physiological interaction like mating and fighting. All rats were acclimatized to the working environment for one week before the beginning of the experiment.

2.3. Acute toxicology assessments

The acute oral toxicity test of chloroform extracts of *L.kituiensis* and *C.metuliferus* were evaluated in swiss albino mice as reported by (Muhammad *et al*., 2015) with little modifications. For *L. kituiensis* extract fifteen (15) mice were grouped into doses 300,600 and 1500mg/kg having five mice in each two females and three males. Similar procedure was repeated for *C.metuliferus* extract and five mice received a vehicle as a positive control. The animals were observed keenly for about 30 min for any signs of toxicity or mortality. The general behavior of the treated animals and control group was observed first for short period intervals of 0, 30 min, 1, 2, 4, 6, 8and 12 h, then daily for a period of 7 days. Behavioral changes and other parameters such as body weight, urinations, food intake, water intake, respiration, convulsion, temperature, changes in eye and skin colors, were determined. Other parameters such as hematological and organ weight were determed.

2.4. Sub-Acute toxicology Assessments

Sub-acute toxicity study (28-day repeated oral toxicity study) was carried out according to Muhammad *et al*., 2015. Both sexes were divided into eight groups, each comprising 5 animals. The 1^{st} group served as positive control, while 2^{nd}, 3^{rd} and 4^{th} were considered as tested groups and orally received *C. metuliferus* and *L. ketuiensis* chloroform extract at dose levels of 150 mg/kg, 300 mg/kg and 500 mg/kg. Before dose administration, the body weight of each animal was determined and the dose was calculated according to the body weight. Group I received 1% DMSO vehicle orally at a dose volume of 5 ml/kg body weight and served as a control group. All the groups of rats were observed once a day for mortality, morbidity, body weight and other clinical signs during the experiment.

2.5. Hematological and Serum Biochemical Examination

On the 29^{th} day of the study, following an overnight fasting, all animals in various groups were anesthetized under chloroform and blood samples were collected by cardiac puncture into heparinised bottles for haematological analysis. Red blood cell count (RBC), white blood cell count (WBC), hemoglobin(HGB), hematocrit (HCT), mean corpuscular volume(MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocytes (LYM), and monocytes were analyzed using *NS4s* auto analyser, Made in Germany. Blood samples without anticoagulant were used for serum biochemistry analysis; the samples were placed at room temperature for 1 h and then centrifuged at 1500 ×g for 10 min to obtain serum. The parameter analyzed were Alanine transaminase (ALT),

Alkaline phosphatase (ALP), Aspartate transaminase (AST), Albumin (ALB), Total bilirubin (TB), Total protein (TP), Creatinine (CREA), urea (UREA), Triglyceride (TG), Total cholesterol (TCHO) and Glucose (GLU).

2.6. Determination of absolute and relative organ weight

Absolute and relative organ weight of vital organs such as liver, kidney, heart, lungs and spleen of a treated animal were determined using electronic balance and compared with that of a control group. Organ-to-body weight ratio was calculated by dividing the weight (g) of each organ by the weight (g) of mice before sacrifice as per given formula.

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Rate body weight}} \times 100$$

2.7. Histopathological examination

For histopathological examinations, organs (liver and kidney) were fixed in 10% formamide before being embedded in paraffin. After routine processing, five micrometers of paraffin sections were prepared and stained with hematoxylin and eosin before microscopic examination. All the sections were examined under a light microscope under different (10x, 20x and 40x) magnifications.

2.8. Ethical consideration

Prior to the experimental work, an ethical clearance with notification number NIMR/HQ/R.8a/Vol. IX/2146 was given by the National Health Research Ethics Sub-Committee (NatHREC) of the National Institute for Medical Research (NIMR) in Tanzania.

2.9. Statistical analysis

Data collected from the biochemical and haematological analyses were expressed as Mean ± SD. One-way ANOVA was used to test the means. Values were considered statistically significant at $P < 0.05$. All results were represented as Mean ± SD ($n = 5$).

3.0 RESULT

The acute toxicity of *C.metuliferus* and *L.kituiensis* extracts was determined as per Muhammad *et al.*, 2015). No treatment related toxic symptom or mortality was observed after oral administration at all doses in both acute and sub acute study. No change in behaviour pattern of the treated animals observed as compared to the control, all the mice given the extracts survived for 14 and 28 days period of observation. The body weights of the male

and female of mice received *C.metuliferus* and *L. kituiensis* at a dose of 300mg/kg, 600mg/kg and 1500mg/kg were observed. No significant differences ($p>0.05$) in the body weight gain between control and treated animals in male and female were observed.

3.1 Effect of *C.metuliferus* and *L.kituiensis* on organ to body weight ratio

For chloroform extract of *C. metulifures* the result showed significant increase of body organs, liver, lungs and kidney in dose level of 600 mg/kg and 1500 mg/kg for both male and female when compared to control (table 11). In the other side the mice treated with *L.kituiensis* chloroform extract reveared significant reduction of kidney and lungs of the male mice treated in a dose of 1500 mg/kg body weight. Female mice, the significant reduction was observed in treatement dose level of 600 mg/kg and 1500 mg/kg of kidney and lungs when compared to control (table 14).

3.2. Effect of plants on hematological parameters

The effects of *C.metuliferus* and *L.kituiensis* extract on haematological parameters were determined. Most haematology measures (white blood cell (WBC), red blood cell (RBC), hematocrit (HCT), haemoglobin (HB), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), lymphocytes (LYM), monocytes (MON), neutrophil granulocytes (NEUT) and RDW were within normal limits at a dose level 300mg/kg, 600mg/kg and 1500mg/kg as compared to control group.

3.3. Sub-acute 28-day oral toxicity study of *C.metuliferus* and *L.kituiensis* in rats

The sub-acute toxicity study of chloroform extract of the tested plants extract were determined as per (Muhammed *et al*., 2015) where the limit test dose of 500 mg/kg was used. No treatment related toxic symptom or mortality was observed after oral administration of the tested plants. No significant change in general behavior of the treated animals and control group was observed. None of the two plant extracts produced mortality after giving a dose of 150 mg/kg/, 300mg/kg and 500mg/kg body weight. All rats given the extracts survived for 28 days period of administration and observation and were sacrificed on day 29.

3.4. Effect of plants on body weight of rats in sub-acute study

Gain in body weight of rats treated with *C.metuliferus* and *L.kituiensis* were recorded and compared with the control group as shown (table 1and 2)**.** The mean body weight of male and

female rats treated with chloroform extract of both plants shown significantly increases in dose level 150mg/kg, 300mg/kg and 500mg/kg as compared to the control.

Table 1: Effect of *C. metuliferous* extract on body weight in sub acute study.

				Body weight(g)				
Sex	**Solvent**	**Dose**	**1st day**	**7th day**	**14th day**	**21st**	**28th**	**Weight gain (g)**
		0	65.43±0.62	72.90±0.34	75.00±0.34	79.33±0.44	82.34±0.35	16.91±0.81
F	**C**hloroform	150	61.09±0.78	67.01±0.21	70.09±0.01	75.02±0.23	81.09±0.21	19.09±0.20*
		300	61.03±0.19	65.01±0.12	72.09±0.22	76.02±0.24	83.07±0.52	22.04±0.11*
		500	61.04±0.80	67.01±0.23	73.07±0.03	76.02±0.25	82.09±0.23	21.09±0.62*
M	Chloroform	0	65.90±0.23	69.90±0.02	69.90±0.02	81.09±0.21	83.09±0.21	19.38±0.12
		150	64.09±0.99	67.20±0.12	73.90±0.22	75.66±0.23	84.09±0.23	24.57±0.33*
		300	65.19±0.100	66.20±0.13	74.90±0.23	78.66±0.24	83.09±0.24	26.37±0.04*
		500	60.09±0.101	68.30±0.14	71.90±0.24	77.66±0.25	84.09±0.25	20.27±0.35

Values are expressed as mean ± SD. P > 0.05 when compared to control group.* = values are significantly different (p < 0.05) from that of the negative control from that of the control.

Table 2: Effect of *L.kituiensis* extract on body weight.

				Body weight(g)				
Sex	**Solvent**	**Dose**	**1st**	**7th**	**14th**	**21st**	**28th**	**Weight gain (g)**
		0	65.43±0.62	72.90±0.34	75.00±0.34	79.33±0.44	82.34±0.35	16.91±0.81
F	**Chloroform**	150	61.09±0.78	67.01±0.21	72.09±0.01	75.02±0.23	83.09±0.21	19.09±0.20*
		300	60.03±0.19	65.01±0.12	72.09±0.22	77.02±0.24	82.07±0.52	21.04±0.11*
		500	61.04±0.80	67.01±0.23	72.07±0.03	75.02±0.25	83.09±0.23	20.09±0.62*
M	**Chloroform**	0	65.90±0.23	69.90±0.02	69.90±0.02	81.09±0.21	83.09±0.21	19.38±0.12
		150	65.09±0.99	69.20±0.12	72.90±0.22	75.66±0.23	84.09±0.23	24.57±0.33*
		300	67.19±0.100	66.20±0.13	73.90±0.23	76.66±0.24	82.09±0.24	23.37±0.04*
		500	63.09±0.101	68.30±0.14	72.90±0.24	77.66±0.25	84.09±0.25	24.27±0.35*

Values are expressed as mean ± SD. P > 0.05 when compared to control group.* = values are significantly different (p < 0.05) from that of the control).

3.5. Effect of *C. metuliferus* and *L.kituiensis* extract on hematological parameters in sub-acute test.

The results from haematological parameters of *C.metuliferus* revealed statistically significant increase (p< 0.05) in WBCs, LYMP, MON, NUE at dose level 300mg/kg and 500mg/kg. All RBC, MCV, HCT, MCH, MCHC, RDW and HB were significantly increased when compared to control at dose level of 500mg/kg in male.

For chloroform extract of *C. metulifures* the result showed significant increase in WBCs count, RBCs, HCT, MCV, MCH, MCHC, RDW, and HB of male rats and female rats WBCs

count, HCT, MCV, MCH, MCHC, RDW, and HB in a dose of 300 mg/kg and 500 mg/kg table3.

For *L.kituiensis* plant the rats treated with chloroform extract revealed significant increased of WBCs, RBCs, MCV, HCT, MCH and MCHC in a dose level 500 mg/kg as compared to the with control, and for female rats significant increased of WBCs, RBCs count and HCT, MCV, MCH and MCHC in dose levels of 300 mg/kg and 500 mg/kg was observed when compared to controls Table 4.

Table 3: Effect of chloroform extract of *C.metuliferus* on hematological parameters in rate after 4 weeks of treatment.

Sex	Parameter	Dose mg/kg n = 5				Sex	Parameter	Dose mg/kg n = 5			
		150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**			**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	WBC	85.40±0.06	99.44±0.12*	102.42±0.51*	93.23±0.01	M	WBC	74.12±0.02	85.82±0.02*	89.61±1.02*	75.23±0.02
	LYMP	58.40±0.02	69.00±0.13*	68.02±0.15*	65.00±0.02		LYMP	64.02±0.11	67.00±0.01*	69.00±0.1*	62.0±0.50
	MON	20.00±0.12	24.04±0.14	25.00±0.10	20.03±0.08		MON	14.10±0.12	15.80±0.11*	17.60±0.03*	12.20±1.02
	NEU	7.00±0.02	8.40±0.15	9.02±0.09*	6.20±0.04		NEU	2.00±0.10	3.02±0.05*	3.01±0.12*	1.03±0.03
	RBC	6.57±0.15	7.72±0.11	9.90±0.12*	7.37±0.01		RBC	6.21±0.04	6.92±0.01	7.53±0.01*	5.90±0.03
	MCV	58.7±0.02	59.22±0.17	65.01±0.31*	55.23±0.06		MCV	59.11±0.02	60.23±0.07	66.23±1.07*	58.0±0.03
	HCT	43.9±0.13	43.3±0.12	49.32±0.12*	41.90±0.07		HCT	40.13±0.10	40.12±0.01	45.23±0.09*	39.18±0.03
	MCH	25.4±0.02	26.2±0.19	29.23±0.01*	24.23±08		MCH	19.01±0.01	22.21±0.18	24.22±0.00*	19.31±0.01
	MCHC	34.52±0.02	35.04±0.2	38.05±0.90*	32.40±0.01		MCHC	27.02±0.17	33.21±0.09	35.23±0.03*	30.05±0.02
	RDW	18.34±0.01	18.21±0.21	23.05±0.10*	16.21±1.10		RDW	14.0±0.6	19.81±0.10	22.12±1.10*	16.91±0.05
	HB	15.31±0.71	16.01±0.25	17.13±0.10	14.23±1.01		HB	15.22±0.09	16.8±0.23	19.12±0.01*	13.34±0.01

Key: Values are presented as Mean ± SD; n = 5; * = values are significantly different ($p < 0.05$) from that of the control. White blood cell (WBC), red blood cell (RBC), hematocrit (HCT), he- moglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin con- centration (MCHC), lymphocytes (LYM), monocytes (MON), neutrophil granulocytes (NEUT), RDW.

Table 4: Effect of chloroform extract of *L.kituiensis* on hematological parameters in rate after 4 weeks of treatment.

Sex	Parameter	Dose mg/kg n = 5				Sex	Parameter	Dose mg/kg n = 5			
		150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**			**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	WBC	93.70±0.09	99.64±0.12*	103.2±0.51*	93.23±0.01	M	WBC	79.32±0.04	89.72±0.02*	99.91±1.02*	75.23±0.02
	LYMP	67.70±0.02	71.60±0.13*	71.02±0.15*	65.00±0.02		LYMP	61.02±0.11	71.00±0.01*	78.00±0.42*	62.0±0.50
	MON	20.00±0.12	20.04±0.14*	22.00±0.10*	16.03±0.08		MON	15.30±0.12	16.70±0.11	18.90±0.06*	12.20±1.02
	NEU	8.00±0.02	8.40±0.15	8.02±0.09	6.20±0.04		NEU	1.00±0.70	3.02±0.05*	3.01±0.12*	1.03±0.03
	RBC	7.57±0.15	8.72±0.11*	9.90±0.12*	6.3±0.01		RBC	6.71±0.04	6.92±0.01	8.33±0.01*	5.90±0.03
	MCV	58.07±0.2	63.82±0.17*	65.41±0.31*	55.23±0.06		MCV	59.81±0.02	60.83±0.07	63.93±1.07*	58.0±0.03
	HCT	41.9±0.13	44.3±0.12*	48.32±0.12*	41.90±0.07		HCT	40.13±0.10	41.12±0.01*	46.23±0.09*	39.18±0.03
	MCH	20.4±0.02	23.2±0.19	26.23±0.01*	24.23±08		MCH	19.91±0.01	21.81±0.18	23.22±0.0*	19.31±0.01
	MCHC	31.52±0.02	33.04±0.2	36.05±0.90*	32.40±0.01		MCHC	28.02±0.17	30.21±0.09	33.23±0.03*	30.05±0.02
	RDW	12.34±0.01	13.21±0.21	16.05±0.10	16.21±1.10		RDW	12.0±0.6	13.81±0.10	14.62±1.10	16.91±0.05
	HB	14.31±0.01	14.01±0.25	16.13±0.10	14.23±1.01		HB	12.72±0.09	12.48±0.23	14.12±0.01	13.34±0.01

Key: Values are presented as Mean ± SD; n = 5; * = values are significantly different ($p < 0.05$) from that of the control. White blood cell (WBC), red blood cell (RBC), hematocrit (HCT), he- moglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular

volume (MCV), mean corpuscular hemoglobin con- centration (MCHC), lymphocytes (LYM), monocytes (MON), neutrophil granulocytes (NEUT), RDW.

3.6. Chloroform effect of *C.metuliferus* and *L. kituiensis* on absolute (a) and relative organ weight (R %) of rats after 28 days of treatment

The results of absolute and relative organ weight of the animals are shown in (Tables 5and 6). In relative weights there were no statistical difference ($p > 0.05$) in all the excised organs as compared with the control group of both *C.metuliferus* and *L. kituiensis*. But in absolute organ weights significant increase were recorded in liver, heart, kidney and lungs in dose level 500mg/kg as compared to the control.

The liver and heart in male rats treated with chloroform extract of *C.metuliferus* showed significant different in dose levels of 300 mg/kg and 500 mg/kg while kidney and lungs in dose level of 500 mg/kg when compared with control. For female rats, the organ weights of liver, heart, kidney, and lungs showed significantly increase in dose levels of 300 mg/kg and 500 mg/kg when compared with the controls Table 5.

For *L.kituiensis* plant the rats treated with chloroform extract revealed significant reduction of liver, lungs and kidney weight in dose level of 500 mg/kg in male rats and in female rats, the significant reduction of liver in dose of 300 mg/kg and 500 mg/kg was observed while lungs and kidney the significant reduction was recorded in dose level of 500 mg/kg as compared to the control group table 6.

Table 5: Effect of chloroform extract of *C. metuliferus* on absolute (a) and relative organ weight (R %) of rats after 28 days of treatment.

Sex	Organ		Dose mg/kg n=5				Sex	Organ		Dose mg/kg n=5			
			150mg/kg	300mg/kg	500mg/kg	0mg/kg				150mg/kg	300mg/kg	500mg/kg	0mg/kg
F	A(g)	Liver	6.55±0.05	7.90±0.31*	8.43±0.11*	6.51±0.01	M	A(g)	Liver	5.40±0.01	5.70±0.09	7.83±0.16*	5.98±0.02
		Heart	0.97±0.21	1.08±0.41*	1.12±0.01*	0.75±0.21			Heart	0.71±0.01	0.80±0.20	1.30±0.12*	0.82±0.12
		Kidney	1.03±0.11	1.58±0.11	1.93±0.31*	1.35±0.11			Kidney	0.65±0.11	1.48±0.14	1.93±0.06*	1.20±0.21
		Lungs	1.53±0.19	1.80±0.15	1.90±0.10	1.60±0.14			Lungs	1.33±0.01	1.26±0.12	1.94±0.12*	1.32±0.02
		Spleen	0.35±0.05	0.97±0.07*	0.99±0.01*	0.35±0.51			Spleen	0.46±0.10	0.20±0.12	0.90±0.13	0.59±0.14
	R (%)	Liver	5.89±0.56	5.20±0.06	4.69±0.03	5.89±0.03		R(%)	Liver	5.40±0.25	6.02±0.11	6.92±0.14	6.99±0.13
		Heart	1.04±0.02	1.01±0.40	1.09±0.24	1.47±0.04			Heart	1.52±0.14	1.52±0.15	1.63±0.16	1.53±0.12
		Kidney	1.36±0.01	1.48±0.15	1.53±0.25	1.57±0.05			Kidney	1.60±0.17	1.67±0.16	1.61±0.14	1.60±0.17
		Lung	1.42±0.06	1.50±0.11	1.47±0.01	1.60±0.06			Lung	1.77±0.18	1.77±0.54	1.85±0.11	1.75±0.02
		Spleen	0.18±0.07	0.18±0.07	0.18±0.07	0.18±0.07			Spleen	0.42±0.19	0.42±0.15	0.11±0.15	1.18±0.19

Key: Values are presented as Mean ± SD; n = 5; * = values are significantly different ($p < 0.05$) from that of the control. A: absolute organ weight (g); R: relative organ weight (%). * $P < 0.05$ compared with the control (0mg/kg).

Table 6: Effect of chloroform extract of *C. metuliferus* on absolute (a) and relative organ weight (R %) of rats after 28 days of treatment.

Sex	Organ		Dose mg/kg n=5				Sex	Organ		Dose mg/kg n=5			
			150mg/kg	300mg/kg	500mg/kg	0mg/kg				150mg/kg	300mg/kg	500mg/kg	0mg/kg
F	A(g)	Liver	6.55±0.05	7.90±0.31*	8.43±0.11*	6.51±0.01	M	A(g)	Liver	5.40±0.01	5.70±0.09	7.83±0.16*	5.98±0.02
		Heart	0.97±0.21	1.08±0.41*	1.12±0.01*	0.75±0.21			Heart	0.71±0.01	0.80±0.20	1.30±0.12*	0.82±0.12
		Kidney	1.03±0.11	1.58±0.11	1.93±0.31*	1.35±0.11			Kidney	0.65±0.11	1.48±0.14	1.93±0.06*	1.20±0.21
		Lungs	1.53±0.19	1.80±0.15	1.90±0.10	1.60±0.14			Lungs	1.33±0.01	1.26±0.12	1.94±0.12*	1.32±0.02
		Spleen	0.35±0.05	0.97±0.07*	0.99±0.01*	0.35±0.51			Spleen	0.46±0.10	0.20±0.12	0.90±0.13	0.59±0.14
	R (%)	Liver	5.89±0.56	5.20±0.06	4.69±0.03	5.89±0.03		R(%)	Liver	5.40±0.25	6.02±0.11	6.92±0.14	6.99±0.13
		Heart	1.04±0.02	1.01±0.40	1.09±0.24	1.47±0.04			Heart	1.52±0.14	1.52±0.15	1.63±0.16	1.53±0.12
		Kidney	1.36±0.01	1.48±0.15	1.53±0.25	1.57±0.05			Kidney	1.60±0.17	1.67±0.16	1.61±0.14	1.60±0.17
		Lung	1.42±0.06	1.50±0.11	1.47±0.01	1.60±0.06			Lung	1.77±0.18	1.77±0.54	1.85±0.11	1.75±0.02
		Spleen	0.18±0.07	0.18±0.07	0.18±0.07	0.18±0.07			Spleen	0.42±0.19	0.42±0.15	0.11±0.15	1.18±0.19

Key: Values are presented as Mean ± SD; n = 5; * = values are significantly different ($p < 0.05$) from that of the control. Values are expressed as mean± SD. A: absolute organ weight (g); R: relative organ weight (%). * $P< 0.05$ compared with the control (0mg/kg).

3.7. Effect on biochemical parameters of *C.metuliferus* in sub-acute study

Liver parameter**:** The administration of chloroform extract of *C.metuliferus* showed a dose dependent increase in Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and Alanine aminotransferase (ALT) at a dose of 300mg/kg and 500mg/kg of male and female as compared to the control. The result in kidney parameters (blood urea) of rats treated with chloroform extract of *C. metuliferus* revealed significant increase in dose level 500 mg/kg in male rats, while in dose level 150 mg/kg and 300 mg/kg remain normal as control. And in female rats significant increase was observed at a dose of 150 mg/kg, 300 mg/kg and 500 mg/kg (Table 7). For creatinine level increases significantly in a dose level 500mg/kg male and in female the significant increase was observed in dose level 300mg/kg and 500mg/kg. Albumin blood serum is another kidney parameter tested in this study, for the rats treated with chloroform extract of *C. metuliferus* revealed significant decreases in dose level 500 mg/kg for male and female when compared with control. More over the study tested for lipid parameters**,** for triglyceride level the animal treated with chloroform, ethyl acetate and methanolic extract of *C. metuliferus* revealed significant decrease in dose level 150 mg/kg, 300 mg/kg and 500 mg/kg when compared with control. The cholesterol level of treatment dose150 mg/kg, 300 mg/kg and 500 mg/kg decreases significantly in rats treated with chloroform, ethyl acetate and methanolic extract when compared to control both male and female rats. Total protein was another parameter tested in this study, the rats treated with chloroform extract revealed no significant change in male while in the female the significant decrease was observed in dose level 300 mg/kg and 500 mg/kg. Glucose level is another parameter tested and the animal treated with chloroform extract of *C. metuliferus* reveared

significant increase in dose 500 mg/kg both for male and female rats. No significant different in total bilirubin was observed Table 7.

3.8. Effect on biochemical parameters of *L.kituiensis* in sub acute study

Liver parameter: The administration of chloroform extract of *L.kituiensis,* serum aspartate aminotransferase (AST), alkaline phosphatase (ALP) and Alanine aminotransferase (ALT)activities significantly decreases (P <0.05) in a dose-dependent level 300mg/kg and 500mg/kg as compared to the control.

The kidney parameters of male and female rats of chloroform extract of treated and control groups showed that blood urea for the rats treated with chloroform extract of *L. kituiensis* revealed significant increase at a dose of 500 mg/kg of male and female rats significantly increase was observed at 300 mg/kg and 500 mg/kg when compared to control (Table 8). The rats treated with chloroform extract of *L. kituiensis* in dose level 300 mg/kg and 500 mg/kg in the female (Table 8), while in male no significant changes were observed as compared to control of creatinine level. Albumin blood serum decreases significantly in dose level 300mg/kg and 500mg/kg for male and female as compared with the control. The result of lipid profile of male and female treated and control group shows that triglyceride level of treatment dose 150mg/kg, 300mg/kg and 500mg/kg decreases significantly chloroform extract as compared with control. The cholesterol level of treatment dose150, 300 and 500mg/kg decreases significantly as compared to the control in male and female rats. The total protein revealed no significant different as compared with the control. The glucose level in treatment dose 500mg/kg significant decreases in dose level 500mg/kg in male and 150mg/kg 300mg/kg and 500mg/kg in female and as compared with the control. No significant different in total bilirubin was observed (Table 8).

Table 7: Effect of chloroform extract of *C.metuliferus* on biochemical parameters in rate after 4 weeks of treatment.

Sex	Parameter	Dose mg/kg n = 5				Sex	Parameter	Dose mg/kg n = 5			
		150mg/kg	300mg/kg	600mg/kg	0mg/kg			150mg/kg	300mg/kg	600mg/kg	0mg/kg
F	ALP(U/L)	37.5±1.20*	49.3±1.05*	55.5±1.02*	46.8±1.0	M	ALP (U/L)	53.3±1.10	58.4±0.10	65.3±1.0*	59.6±1.0
	AST(U/L)	44.6±0.70	52.6±0.06*	55.8±1.10*	48.5±0.01		AST (U/L	57.2±0.90	62.1±0.10*	68.9±1.0*	55.3±0.13
	TP(g/L)	0.13±0.01	0.11±0.01	0.01±0.01	0.11±0.01		TP (g/L	0.18±0.11	0.18±0.11	0.18±0.11	0.18±0.11
	ALB(U/L	2.60±0.20	2.00±0.40*	1.70±1.10*	2.80±0.30		ALB (U/L)	3.20±0.50	2.6±0.10*	2.4±0.10*	3.2±0.50
	ALT(U/L)	52.9±0.70*	54.6±0.60*	57.0±0.50*	31.1±0.09		ALT (U/L)	35.1±1.50	39.4±0.70*	43.3±0.10*	35.4±0.9
	GLU (mmoI/L	59.5±0.8*	62.8±1.50*	69.0±1.07*	54.8±1.0		GLU (mmoI/L)	59.2±0.9	60.8±1.1	68.4±0.15*	57.0±0.12
	CREA (µmol/L	31.0±0.50*	32.6±0.12*	34.7±0.02*	25.7±0.9		CREA (µmol/L	25.2±1.10	29.5±0.15*	33.7±0.40*	28.4±0.15
	TCHO(mmol/L	72.7±0.16*	65.4±0.6**	52.8±1.3**	117.2±2.9		TCHO(mmol/L)	97.4±0.12	77.1±0.9*	65.1±0.50**	96.1±0.8
	TG (mmol/L	65.5±1.14*	53.9±1.4**	46.2±0.9**	94.1±1.6		TG (mmol/L	85.5±0.01*	84.0±1.50*	78.3±0.10*	94.0±2.4
	UREA (µmol/L	28.44±0.4	24.39±1.0*	20.58±1.0*	28.06±0.2		UREA (µmol/L	22.58±0.6*	21.67±0.9*	18.29±0.8*	29.73±0.4
	TB(mg/dl)	0.8±0.1	0.8±0.10	0.9±0.1	0.7±0.0		TB (mg/dl)	0.60±0.20	0.8±0.1	0.80±0.20	0.70±0.20

Key: Values are presented as Mean ± SD; n = 5; * = values are significantly different ($p < 0.05$) from that of the control. Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Albumin (ALB), Total bilirubin (TB), Total protein (TP), Creatinine (CREA), urea (UREA), Triglyceride (TG), Total cholesterol (TCHO) and Glucose (GLU.

Table 8: Effect of chloroform extract of *L.kituiensis* in biochemical parameters in rate after 4 weeks of treatment.

Sex	Parameter	Dose mg/kg n = 5				Sex	Parameter	Dose mg/kg n = 5			
		150mg/kg	300mg/kg	500mg/kg	0mg/kg			150mg/kg	300mg/kg	500mg/kg	0mg/kg
F	ALP(U/L)	40.7±1.10*	37.3±1.01*	36.6±1.0*	46.8±1.1	M	ALP (U/L)	45.6±0.10*	43.6±0.09*	40.9±1.01*	59.6±0.1
	AST(U/L)	45.3±0.10	35.5±1.0*	38.4±0.9*	48.5±0.1		AST(U/L	53.4±1.10	48.4±1.10*	48.4±0.90*	55.3±0.10
	TP(g/L)	7.26±0.10	6.88±0.61*	6.08±0.1*	7.4±0.21		TP (g/L	6.34±0.41	6.06±0.21	5.21±0.61*	6.18±0.11
	ALB(U/L	3.3±0.200	2.60±1.10	1.40±0.4*	2.8±0.30		ALB (U/L)	3.10±0.20	2.90±0.20	2.10±0.80*	3.2±0.50
	ALT (U/L)	29.4±0.11	25.3±0.41*	23.5±0.9*	31.1±0.9		ALT (U/L)	32.4±0.80	35.8±0.90	39.3±0.60*	35.4±0.90
	GLU (mmoI/L	54.4±1.10	58.7±1.2*	63.6±1.5*	54.8±0.2		GLU (mmoI/L)	54.3±1.40	57.0±1.17	63.10±1.0*	57.0±0.60
	CREA (µmol/L	25.4±1.01	29.4±1.09*	32.2±1.4*	25.7±0.90		CREA (µmol/L	24.9±1.10	28.9±0.60	35.6±0.10*	28.4±1.01
	TCHO(mmol/L	85.2±1.10*	72.3±0.90*	67.3±1.50*	117.2±2.9		TCHO(mmol/L)	65.4±1.50*	58.0±0.10**	43.3±0.80**	96.1±0.31
	TG (mmol/L	77.5±1.01*	66.0±0.70*	54.2±0.30	94.1±1.60		TG (mmol/L	79.3±0.90*	72.6±0.80*	60.9±0.10**	94.0±0.17
	UREA (µmol/L	23.31±0.1	22.25±0.8*	18.74±0.9*	28.06±0.2		UREA (mmol/L	27.07±0.8	25.86±0.9*	18.81±1.0*	29.06±0.2
	TB(mg/dl)	0.5±0.1	0.8±0.1	1.9±0.40*	0.7±0.01		TB(mg/dl)	0.60±0.10	0.70±0.10	1.8±0.01*	0.70±0.20

Key: Values are presented as Mean ± SD; n = 5; * = values are significantly different ($p < 0.05$) from that of the control. Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Albumin (ALB), Total bilirubin (TB), Total protein (TP), Creatinine (CREA), urea (UREA), Triglyceride (TG), Total cholesterol (TCHO) and Glucose (GLU).

3.9. Histopathological Examination.

Histological examinations of liver, kidney, lungs, heart and spleen organs were performed at the end of the experiment. Below are the figures that shows the representative photomicrographs of organs from the rats of control and *C.metuliferus* and *L.kituiensis* treated groups.

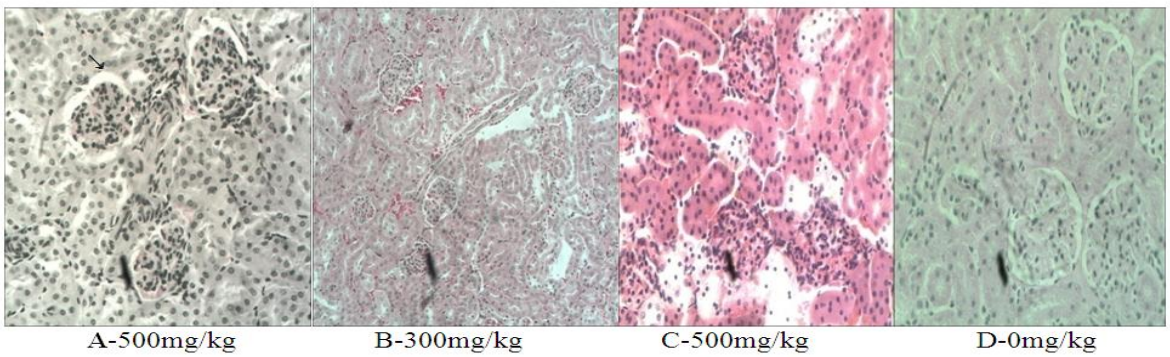

Figure 1: Histology of kidney from control& treated rat (male and female) 40x magnifications.

The results of histopathological examination of kidney section in rats treated with chloroform extract of *C.metuliferus* plant are shown in (Figure 1). The kidney in rats administered with 300mg/kg of chloroform extract presented the intact glameli and distraction of tubules (Figure1B). For rats administered with 500mg/kg body weight showed mild tubular necrosis and distraction of glomerula and bowman capsule was observed as shown in (figure1C).While 500mg/kg body weight of chloroform extract showed shrinkage of glomerula, inflammations and degeneration of tubular cells as shown in (figure1A).

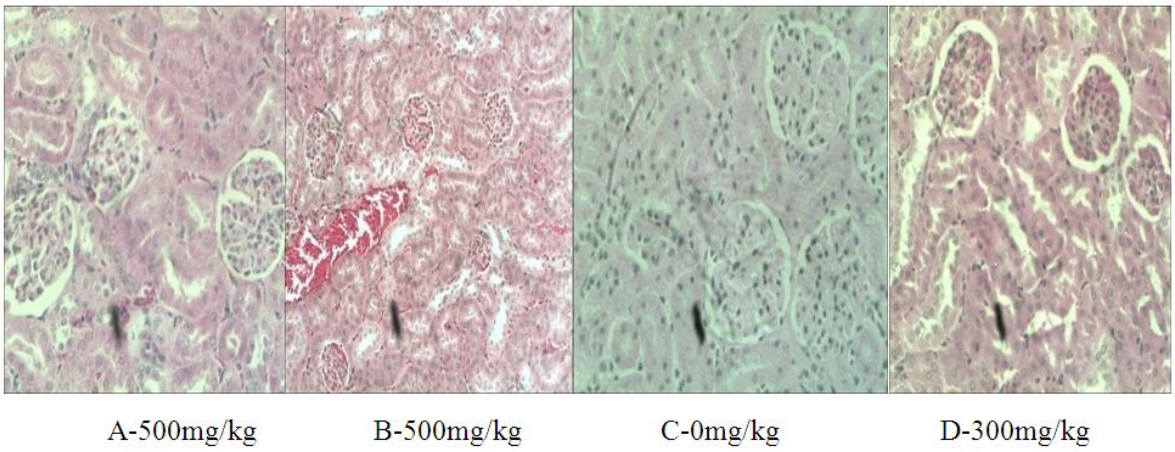

Figure 2: Histology of kidney of *L.kituiensis* from control & treated rat (male and female) 40x magnifications.

The results of histopathological examination of kidney section in mice treated with chloroform extract of *L.kituiensis* are shown in (Figure 2). The kidney rats administered with 300mg/kg chloroform extract showed degeneration of tubular cells (Figure2A). In rats administered with 500mg/kg body chloroform extract distraction of glomerula and bowman capsule and congestion was observed as shown in (figure 2B). While 500 mg/kg body weight of chloroform extract shown shrinkage of glomerula, inflammations and degeneration of tubular cells as shown in (figure2B) as compared to control (figure 2C).

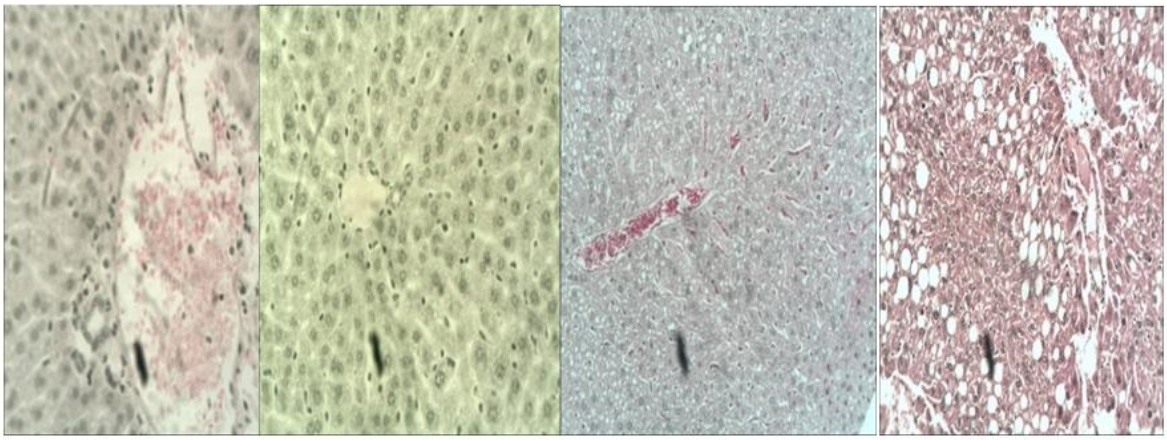

Figure 3: Histology of liver of *C.metuliferus* from control& treated rat (male and female) 40x magnifications in chloroform extract.

The results of histopathological examination of liver section in rats treated with chloroform extract of *C.metuliferus* are showed in (figure 3). The liver in rats administered with dose level 300mg/kg in all extract showed mild necrosis, portal congestion and portal dilation as shown in (figure 3c). For the rats administered with 500 mg/kg body weight of chloroform, extract showed hepatic necrosis, bile lakes and shrinkage of cell in the liver was observed in both male and female and vacuolation (figure 3D). While the mice treated with dose level 500 mg/kg body weight of chloroform showed and bile duct hyperplasia in portal triad, moderate hepatic necrosis, and portal congestion as shown in (figure3A) as compared to control (figure 3B).

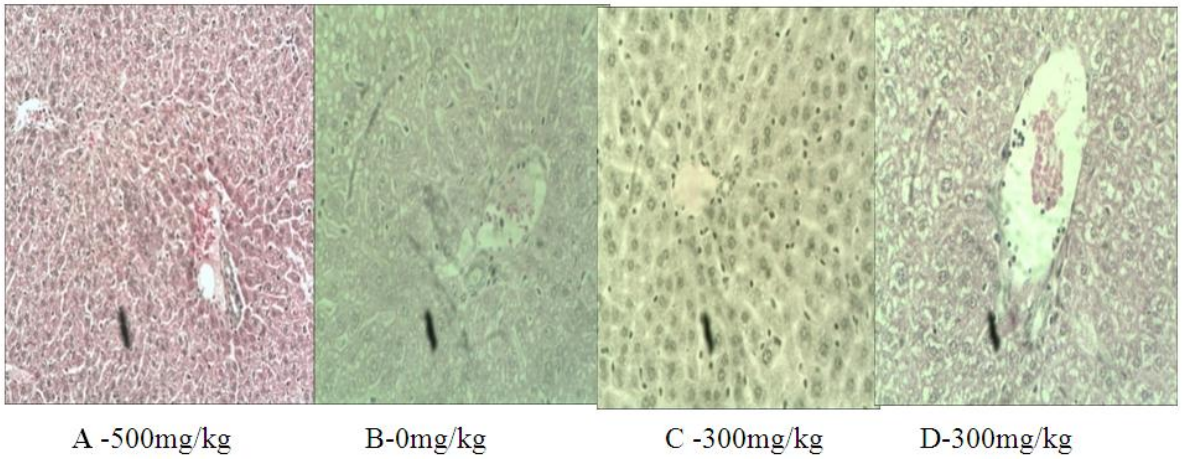

Figure 4: Histology of liver of *L.kituiensis* from control& treated rat (male and female) 40x magnification.

Histopathological section of liver treated with *L.kituiensis* at a dose of 300mg/kg treated to male and female rats in chloroform extract showed normal portal triad with mid necrosis as show in (figure 4D). For the rats administered with 500 mg/kg body weight of chloroform extract showed mild hepatic necrosis, vacuolation and normal portal triad as shown in (figure 4D), while 500 mg/kg body weight showed hepatic necrosis and congestion as shown in (figure 4A).

4.0 DISCUSSION

The acute toxicity study in mice was performed according to (Muhammed *et al*., 2015) to assess the safety profile of *C.metuliferus* and *L.kituiensis*. The oral acute toxicity study of the tested plants extract ware carried out on Swiss albino mice at a dose of 300mg/kg, 600mg/kg and 1500 mg/kg body weight. No major changes in behavior and mortality were observed in all groups throughout the 14 days of study. No significant changes were found in Salvation, piloerection, drowsiness, fur, food intake, water intake, mortality, breathing, diarrhea, urination, coma and death. This report is in agreement with the work reported by (Clarke*et al*., 2010). Also no significant change observed in body weight, relative organ weight (organ-to-body weight ratio) and hematological parameters in all animals treated with *C.metuliferus* and *L.kituiensis* at a dose of (300mg/kg, 600mg/kg and 1500mg/kg. The result is in agreement with the result reports by (sellers' *et al*., 2007). However, in absolute organ weight the result revealed significant increases in liver and kidney of mice at a dose of 600 and 1500 mg/kg as compared to control. And female shows the significance increases of liver, lung and kidney at a dose of 600mg/kg and 1500mg/kg of *C.metuliferus* extract as compared to control. The mice treated with *L. kituiensis* no significant different was observed at a dose of 300mg/kg, 600mg/kg and 1500mg/kg as compared to control in acute study.

For sub-acute toxicity, the study was therefore carried out with doses of 150mg/kg, 300mg/kg and 500mg/kg of extract to assess the safety profile of *C.metuliferus* and *L.kituiensi*. No mortality was observed for male and female rats with the maximum dose of 500 mg/kg throughout 28 days of study. No significant changes were found in Salvation, piloerection, drowsiness, fur, food intake, water intake, mortality, breathings, diarrhea, urination, coma and death. This report is in agreement with the work reported by (Clarke *et al*., 2002).

Decreases or increases in the body weights are associated with toxic effects of chemicals and drugs (Sireeratawong *et al*., 2008). However, scientific evidence confirmed that increases or decreases in the body weights are accompanied with accumulation of fats and physiological

adaptation responses to the plant extracts rather than to the toxic effects of chemicals or drugs that lead to decrease appetite and, hence, lower caloric intake by the animal (Arsad *et al*., 2013). In sub acute study the result revealed significant increases in body weight in all dose level 150mg/kg, 300mg/kg and 500mg/kg in all animals treated with *C.metuliferus* and *L.kituiensis*. This suggests that the extracts did not interfere with normal body metabolism of the animals as the increment in food and water intake is synonymous to an increase in body weight this suggests that the plant extract might not have altered food intake through any mechanisms of appetite suppression. Also this suggests that the extract did not course depressant action on the appetite of the animals, this result is in agreement with the result reported by (Michael, *et al*., 2007).

Organ weight is one of the most sensitive drug toxicity indicators as significant differences in organ weight between treated and control animals may occur in the absence of morphological changes (Piaot *et al*,. 2013). Based on the results, significant increase was recorded in liver, heart, kidney and lungs in dose level 500mg/kg as compared to the control in male. And in female rats, the results shows significantly increase in liver and heart in dose level 300mg/kg and 500mg/kg and kidney and spleen in dose level 500mg/kg as compared to the control. For rats treated with *L. kituiensis* the significant reduction was observed in liver, kidney and lungs in dose level 500mg/kg as compared to a control ($p< 0.05$). As to female rats, the results were similar to those of male rats.These alterations in liver weight may suggest treatment-related changes including hepatocellular hypertrophy (enzyme induction or peroxisome proliferation or lipidosis) however elevation of liver weight signifies enzyme-induction which is one marked activity of a terpene. Changes in kidney weight may reflect renal toxicity. Evaluation of liver and kidneys are very important because of their greater sensitivity to predict toxicity, frequent target organs of toxicity and there is little inter animal variability, and is often reflective of physiologic perturbations. In general, the presence of significant differences in the liver, kidney, heart and lungs weight reflects toxicity proper of these two plants.

After 28 days of treatment with tested plant extract, the hematological parameters showed significance change ($P > 0.05$) when compared to control group. The results from haematological parameters of *C.metuliferus* revealed statistically significant increase ($p< 0.05$) in WBCs, LYMP, MON, NUE in dose level 300mg/kg and 500mg/kg. All RBC, MCV, HCT, MCH, MCHC, RDW and HB were significantly increases as compared to control in dose level 500mg/kg in male. While in female statistically significant increase were recorded

in WBCs and LYMP at a dose of 300mg/kg and 500mg/kg and all RBCs and HCT, MCV, MCH, MCHC and RDW were significantly increases as compared to control in dose level 500mg/kg. In *L.kituiensis* the hematological result revealed significant increase in WBC, LYMP, MON and NEU in dose level 300mg/kg and 500mg/kg and RBC, MCV, HCT MCH and MCHC in dose level500mg/kg in male as compared to control. While in female significant increase was observe in WBC, LYMP, MON in dose level 300mg/kg and 500mg/kg and all RBC, MCV, HCT, MCH and MCHC in dose level 500mg/kg. From this result, it is possible that the extract contains agents that stimulate the bone marrow to produce neutrophils and release them into the blood. Neutrophils are the major granulocytes to be activated when the body is invaded by bacteria and they provide the first line of defense against invading microorganisms. The granules of the neutrophil contain many enzymes which makes it a powerful and effective killer machine. This effect on neutrophil count may be partly responsible for the claim that *C.metuliferus* and *L.kituiensis* has antibacterial actions. There was also some significant rise in lymphocytes. The results from the current study are in agreement with those of (Kinney *et al.,* 1999), where he associated the significant rise in lymphocytes to the potential usefulness of the plant as immune system stimulant, a factor that may justify the use of the plant in treatment of malarial in Tanzania (Bruneton *et al*., 1995; Hoffmann *et al.,* 2003). More over this result suggests that the extract did not change the haemopoietic sites that produce white blood cells in treated groups (Lissoni *et al*. 2006).The bone marrow is responsible for the production of the blood cell and some phytochemicals isolated from plant have affected red blood cell levels(Govid *et al*., 2012); hence, the tested plant extract may not have harmful effects on bone marrow. Similarly, estimation of serum biochemical parameters in treated animals showed significance ($P > 0.05$) compared to control group. The assessment of clinical biochemistry provides an insight to possible damage brought about by the extract in the hepatic and renal functions. In toxicity studies, assessment of liver and kidney functions is germane because both organs are essential for the survival of an organism (Olorunnisola *et al*., 2012). Alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) are sensitive enzymes used in assessing the severity of liver damage (Ramaiah *et al*., 2011). Elevated activities of these enzymes are associated with liver or heart damage (Wasan *et al*., 2001: Brawtbar *et al*., 2002). The significant increases in ALT and AST of the treated animals in both sexes compared with normal control could suggest that C.metuliferus may have hepatotoxic effect and while the increase in ALP might suggest obstruction of the biliary tract which may be present in the liver. While in *L.kituiensis*. The significant reduction in ALT, AST and ALP of

the treated animals in both sexes compared with normal control could suggest *L.kituiensis* that may not have hepatotoxic effect (Crook *et al*., 2006).The above mentioned biochemical investigations were in correlation with the histopathological studies. Section of the liver and kidney of the control and *C.metuliferus* and *L.kituiensis* treated animals showed abnormality as shown in Figures above, Inflammation, necrosis, duct hyperplasia in portal triad, moderate hepatic necrosis, and portal congestion were observed in the histological study of liver in rats tested with *C.metuliferus* and *L.kituiensis* at a dose of 300 mg/kg and 500mg/kg. In kidney shrinkage of glomerula, inflammations, degeneration of tubular cells and distraction of glomeruli were observed indicating that *C.metuliferus* and *L.kituiensis* extract causes kidney damage.

CONCLUSION

The present findings have shown that chloroform extracts of *C.metuliferus* and *Lippia kituiensis* when used in these doses for 28 days are most likely to produce severe toxicological risk which is demonstrated by LD_{50} values. Based on the findings of the present study, the plants seem to induce some hazardous effects. Hence detailed toxicity studies including the study at the cellular level in a chronic study and their underlying mechanism of changes is highly recommended.

Competing Interests

The authors declare no competing interests.

Authors' Contributions

Hulda Swai and Musa Chacha contributed equally to this work.

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