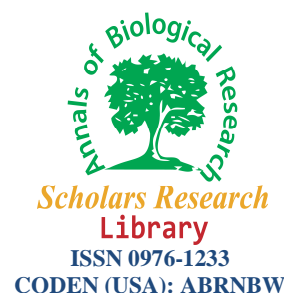




Scholars Research Library

Annals of Biological Research, 2012, 3 (12):5561-5570  
(<http://scholarsresearchlibrary.com/archive.html>)



## Effect of Long-Term Oral Administration of the aqueous and ethanol leaf extracts of *Cymbopogon citratus* (DC. ex Nees) Stapf

<sup>1,2</sup>Protus Arrey Tarkang, <sup>1</sup>G. A. Agbor, <sup>1</sup>N. Tsabang, <sup>1</sup>L. R. Y. Tchokouaha, <sup>1</sup>D. A. Tchamgoue, <sup>3</sup>D. Kemeta, <sup>4</sup>Y. S. N. Mengue, <sup>1</sup>J. R. Mba, <sup>1</sup>F. Weyeye

<sup>1</sup>Centre for the Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plants Studies (IMPM), P. O. Box 6163, Yaoundé, Cameroon.

<sup>2</sup>Department of Pharmacology and Pharmacognosy, University of Nairobi, Kenya

<sup>3</sup>Department of Animal Biology, University of Ngaoundere, Cameroon.

<sup>4</sup>Department of Animal Biology and Physiology, University of Yaoundé I, Cameroon.

### ABSTRACT

*Cymbopogon citratus* is well known for its extensive use in folk medicine. Its essential oils and extracts have been shown to have antimalarial, antileishmanial, hypolipidemic, hypoglycemic, antioxidant, anti-inflammatory, antifungal and antibacterial activities amongst others. This study was carried out to assess the effect of long-term oral administration of the aqueous and ethanol leaf extracts on some biochemical and metabolic parameters in experimental animals. Systemic acute oral toxicity was assessed in mice up to a dose of 5gKg<sup>-1</sup>BW, while the sub-acute and sub-chronic toxicity were studied on wistar rats. Graded doses of 0.25, 0.5 and 1gKg<sup>-1</sup>BW of each extract were administered to experimental groups of rats for a period of 28 days and 90 days respectively, while the control groups received the vehicle. Acute oral toxicity of the extracts did not produce any mortality or adverse signs of toxicity. The extracts did not have any adverse effect in changes in calculated body/organs weights and the hematopoietic system of rats. However, assay on some biochemical parameters revealed mild toxicity of the liver and kidneys at the highest dose for both extracts. The extracts demonstrated strong hypolipidemic and hypoglycemic activities, which could associate it with the management of arterosclerotic diseases and increased blood sugar levels.

**Key words:** *Cymbopogon citratus*, toxicity, blood chemistry, hypolipidemia, hypoglycemia

### INTRODUCTION

*Cymbopogon citratus* (DC. Ex Nees) Stapf, formerly described as *Andropogon citratus* by De Candolle and reclassified by Otto Stapf, is of the Poaceae family [39]. Commonly called West Indian lemon or fever grass, *Cymbopogon citratus* (CC) is of Malaysian origin and has had extensive folk use in the tropical countries and semi-tropical areas of Southeast Asia, for more than 2000 years [43]. It is a perennial robust herb, growing in dense tufts. Its leaves are fragrant, up to 70cm long and 5-15cm broad, with rough margins and a prominent midrib beneath. Its inflorescence is in panicles, 30-60cm long, with sessile spikelets and linear or linear-lanceolate [23]. It is mainly cultivated in Africa, Central America, South East Asia and the Indian Ocean Islands.

*CC* is used medicinally to treat typhoenteritis and to repel mosquitoes [8], flies and other insects. It is also employed as an ingredient in many malaria remedies [17] [34] [1] [5] [19] [35]. It has been shown to have neutralizing effects against snake venom [21] and for the traditional treatment of influenza [24] and jaundice [28]. The leaves of *CC* contain volatile oils which are usually used in the form of tea to serve as a febrifuge while the roots are used as chewing sticks to clean the teeth [15].

Preliminary phytochemical screening revealed the presence of two new triterpenoids, cymbopogone and cymbopogonol, the flavonoids, luteolin and its 6-C and 7-O -glycosides, [11] [39], isoorientin 2'-O-rhamnoside and the flavonoids, quercetin, kaempferol and apiginin. The phenolic compounds, elimicin, catechol, chlorogenic acid, caffeic acid and hydroquinone, were also isolated from the plant. The essential oil of *CC* contains citral  $\alpha$  (~40%), citral  $\beta$  (~32%), nerol (~4.18%), geranicol (~3.04%), citronellal (~2.10%), terpinolene (~1.23%), geranyl acetate (~0.83%), myrcene (~0.72%), terpinol (~0.45%), methylheptenone (~0.2%), borneol (~0.1-0.4%), lanilyl acetate (~0.1%),  $\alpha$ -pinene (~0.07%) and  $\beta$ -pinene (~0.04%) [26] [36] and all are important raw material used in the pharmaceutical and cosmetics industries, especially for the synthesis of Vitamin A and ionones. The constituents of the essential oil; citral, geranial, neral and myrcene have demonstrated antileishmanial activities [30] and other biological activities such as hypoglycemic and hypolipidemic [3], free radical scavenging and antioxidant [22], antibacterial [33], ascaricidal [31], antinociceptive [38], anti-inflammatory [12], antifungal [9], antifilarial [27], antidiarrhoeal [44] amongst others.

*In vitro* cytotoxicity studies revealed that the essential oil is toxic at very high concentrations, though it has the ability to suppress oxidative stress [25].

Despite the extensive use of *CC* to treat a wide variety of ailments in folk medicine, information of the *in vivo* safety is lacking. In this regard this study aims at evaluating effects of long-term oral administration of *CC* leaf extracts on some biochemical and metabolic parameters in wistar rats.

## MATERIALS AND METHODS

### 2.1 Collection and Extraction of Plant Material

Fresh lemon grass leaves were harvested from their natural habitat in Kombone, Kumba, Cameroon in the month of July 2011. Plant identification and voucher specimen No. TN6228 referencing was done at the Institute of Medical Research and Medicinal Plants Studies (IMPM) herbarium in Yaoundé, Cameroon by a botanist. The freshly harvested leaves were then air dried, pulverized and then weighed quantities were immersed in water and ethanol (80%) respectively for 4 h. Each of the macs was transferred into a conical percolator for 72 h and the extracts were filtered with a sieve of 80 $\mu$ m pore size [42]. The ethanol filtrate was first concentrated using a rotary evaporator and then both filtrates were concentrated in an air oven at 60°C. The extracts were weighed and stored in sealed plastic containers at 4°C for subsequent use.

### 2.2 Animal Husbandry

Male and female Swiss albino mice (25 – 30g) and Wistar rats (170 – 210g) obtained from the animal house of IMPM were used for the acute, sub-acute and sub-chronic toxicity studies respectively. They were housed in stainless steel wire mesh cages up to a maximum of 6 per cage, in a well-ventilated room with 12 h light/dark cycle, with free access to clean drinking water and food (standard rat feed). They were allowed to acclimatize for one week before experimentation. Plant extracts were administered orally. All animals had regular supply of clean drinking water and food [32].

### 2.3 Evaluation of Acute Toxicity

The acute oral toxicity of the aqueous and ethanol leaf extracts of *CC* was evaluated in Swiss albino mice according to the procedures outlined by the Organization for Economic Co-operation and Development (OECD) [45]. Following the fasting period, the mice were weighed and the dose was calculated in reference to the body weight. Volume of the extracts given to the mice was 10mlKg<sup>-1</sup> body weight (*BW*). The crude extract was suspended in a vehicle (distilled water and corn oil for the aqueous and ethanol extracts respectively). The study was initiated with a sighting study, which consisted of a stepwise administration of fixed doses of 0.05, 0.1, 0.3, 1.2 and 2.0gKg<sup>-1</sup> *BW* of the aqueous and ethanol extracts respectively, to single male and female adult Swiss albino mice (25-30g). This was aimed at determining the dose of the acute toxicity, by observation. Since no mortality or signs of toxicity were observed at this dose, an upper limit dose of 5gKg<sup>-1</sup> *BW* was used for the main test; each crude extract was

administered to three male (Test 1) and three female (Test 2) mice in the treatment groups, whereas the control groups received the vehicle. Food was provided to the mice approximately an hour after treatment. The animals were observed 30min after dosing, followed by hourly observation for 8 h and once a day for the next 13 days. All observations were systematically recorded with individual records being maintained for each animal. Surviving animals were weighed and visual observations for mortality, behavioral pattern, changes in physical appearance, injury, pain and signs of illness were conducted daily during the period.

#### **2.4 Evaluation of Sub-acute and Sub-chronic Toxicity**

Sub-acute and sub-chronic toxicity of the aqueous and ethanol extracts of *CC* leaf was evaluated in Wistar rats. For the aqueous extract the rats were divided into 4 groups (A, B, C, D) of 12 rats each, while for the ethanol extract the rats were divided into 4 groups (E, F, G, H) of 6 rats each. Groups A and E served as control and received the vehicle only (water and corn oil for aqueous and ethanol extracts respectively), while groups B, C, D and F, G, H served as test groups and were administered graded doses of 0.25, 0.5 and 1gKg<sup>-1</sup>BW of each extract respectively. At the end of 28 days (sub-acute toxicity), blood was collected through the jugular vein and then 6 rats in each group of A, B, C, D and all the rats of E, F, G, H were sacrificed after an overnight fast, under diethyl ether anaesthesia, whereas the remaining 6 rats of each of groups A, B, C and D were sacrificed in like manner at the end of 90 days (sub-chronic toxicity). The blood collected was used for hematological and biochemical analysis. The liver, kidney and heart were harvested immediately clean of blood using physiological saline and weighed. The liver and kidney were then fixed in 10% formalin for histopathological examination.

White blood cell (WBC), red blood cell (RBC) and platelet (PLT) counts as well as their indices were analyzed using a Hospitex Diagnostics Hema Screen 18 automatic hematology analyzer. Safety endpoints for plasma biochemical analysis included total proteins (TP), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), uric acid (URIC), creatinine (CRE), cholesterol (CHOL), triglycerides (TGY), glucose (GLU) and these were evaluated using standard analytical kits from Fortress Diagnostics Ltd, UK. The fixed organs were dehydrated with 100% ethanol solution and embedded in paraffin. They were then processed into 4-5µm thick sections, stained using hematoxylin-eosin and observed under microscope as earlier described by Gabe [46].

#### **2.5 Statistical Analysis**

All variables were subjected to descriptive data analysis. Continuous variables were expressed as the mean and the standard deviation (SD) from the mean. The results were analyzed statistically using one-way ANOVA and two-tailed Student's *t*-test (IBM SPSS 20 Inc., USA) to identify the differences between treated groups and controls. The data was considered significant at  $P < 0.05$ .

## **RESULTS**

### **3.1 Plant Extraction**

The aqueous and ethanol extraction of *CC* gave yields of 5.8% and 6.7% of the pulverized plant material respectively. Both the plant material and extracts were not contaminated with aflatoxin, pesticide, heavy metal or microbes.

### **3.2 Acute Oral Toxicity of *CC* Extracts**

*Effect of oral administration of *CC* extracts on the behavioral pattern and physical appearance:* At the end of 14 days of observation and systematic recording, no mortality or signs of acute toxicity were recorded in both male and female mice treated with the aqueous and ethanol leaf extracts of *CC* up to a fix dose of 2gKg<sup>-1</sup>BW and in the main test, at a dose of 5gKg<sup>-1</sup>BW, as shown in Table 1.

### **3.2 Sub-Acute and Sub-chronic Oral Toxicity of *CC* Extracts**

*Effect of oral administration of *CC* extracts on body and organ weights:* The changes in calculated body and visceral organ weights of the control and treated animals are shown on Tables 2 and 3. There was no significant change in the body and visceral organ weights of experimental animals after short and long term administration of the aqueous and ethanol leaf extracts of *CC* at a dose of up to 1gKg<sup>-1</sup>BW. Change in body and organ weights was normal.

Table 1. Acute systemic toxicity of the aqueous and ethanol leaf extracts of *Cymbopogon citratus*

Extract	Dose (gKg <sup>-1</sup> )	Observations								% Mortality	
		Upon Administration	After								
			½ h	1 h	8 h	24 h	48 h	168 h (7 days)	336 h (14 days)		
Aqueous	0.05	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	0.1	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	0.3	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	1.2	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	2.0	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	5.0	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
Ethanol	0.05	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	0.1	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	0.3	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	1.2	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	2.0	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0
	5.0	Idleness	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0

Table 2. Effect of oral administration of *Cymbopogon citratus* leaf extracts on the body weight of experimental animals.

Extract	Toxicity (days)	Weight (g)	Study Groups (Dose)			
			Control (A, E) (Vehicle)	Test 1 (B, F) (0.25gKg <sup>-1</sup> )	Test 2 (C, G) (0.5gKg <sup>-1</sup> )	Test 3 (D, H) (1gKg <sup>-1</sup> )
Aqueous	Sub-acute (28)	Initial	182.17 ± 3.66	203.17 ± 9.00	194.33 ± 13.11	184.33 ± 13.84
		Final	203.83 ± 3.06	211.67 ± 7.66	212.33 ± 8.87	206.00 ± 11.80
	Sub-chronic (90)	Initial	182.17 ± 3.66	185.50 ± 8.69	185.00 ± 9.01	187.67 ± 11.41
		Final	281.83 ± 10.15	268.17 ± 8.08	269.50 ± 9.63	293.33 ± 12.33
Ethanol	Sub-acute (28)	Initial	178.83 ± 3.66	183.00 ± 5.29	182.33 ± 6.38	182.17 ± 5.67
		Final	203.67 ± 3.33	209.83 ± 8.57	212.33 ± 10.54	213.17 ± 10.72

The data represents the Mean ± SD for each group of rats, n = 6 (number of animals per group).

\*p<0.05 = significant difference and \*\*p<0.001= highly significant difference compared to control (group A & E).

The effect of the aqueous and ethanol extracts of *CC* on the percentage weight gain and relative organ weight (ROW) in experimental animals is presented in Table 4. There was no significant increase in ROW of experimental animals compared to the control. The percentage body weight gain in experimental animals treated with the aqueous extract was lower compared to the control, and increasing normally in a dose-responsive manner. However, in experimental animals treated with the ethanol extracts, the percentage weight gain in experimental animals was higher than that of the control. Increase was in a dose responsive manner as well.

**Table 3: Effect of oral administration of *Cymbopogon citratus* leaf extract on the weight of some visceral organs of experimental animals.**

Extract	Toxicity (days)	Organ	Weight of Organ in Study Groups (g)			
			Control (A, E) (Vehicle)	Test 1 (B, F) (0.25gKg <sup>-1</sup> )	Test 2 (C, G) (0.5gKg <sup>-1</sup> )	Test 3 (D, H) (1gKg <sup>-1</sup> )
Aqueous	Sub-acute (28)	Heart	0.74±0.04	0.75±0.08	0.82±0.09	0.77±0.07
		Liver	6.68±0.29	7.10 ±0.41	6.95 ± 0.14	7.14± 0.20
		Left Kidney	0.66±0.03	0.71±0.08	0.71±0.10	0.75±0.06
		Right Kidney	0.67±0.03	0.72±0.09	0.71±0.06	0.70±0.04
	Sub-chronic (90)	Heart	0.76±0.08	0.72±0.05	0.74±0.04	0.76±0.06
		Liver	6.80±0.21	6.79±0.38	6.86±0.31	7.50±0.71
Left Kidney		0.68±0.07	0.69±0.08	0.67±0.03	0.70±0.06	
Ethanol	Sub-acute (28)	Heart	0.64±0.03	0.68±0.04	0.67±0.02	0.67±0.05
		Liver	6.90±0.51	7.03±0.55	7.22±1.03	7.26±0.83
		Left Kidney	0.66±0.05	0.73±0.09	0.73±0.08	0.73±0.10
		Right Kidney	0.67±0.06	0.72±0.09	0.71±0.08	0.72±0.09

The data represents the Mean ± SD for each group of rats, n = 6 (number of animals per group).

\*p<0.05 = significant difference and \*\*p<0.001 = highly significant difference compared to control (group A & E).

**Effect of long-term administration of *CC* extracts on some hematological parameters:** The effect of long-term administration of the aqueous and ethanol leaf extracts of *CC* on some hematological parameters is shown in Table 5. In rats treated with the aqueous extract for 28 days (sub-acute), we observed a dose-dependent significant increase in LYM # (p < 0.05), HCT (p < 0.001), PLT (p < 0.001); a significant increase (p < 0.05) in WBC (reducing to normal at 1gkg<sup>-1</sup>), HGB (at 1gKg<sup>-1</sup>), MCV (at 1gKg<sup>-1</sup>); a highly significant (p < 0.001) increase in RBC (decreasing dose-dependently) and a dose-dependent significant (p < 0.05) decrease in MCH, when compared to the control. However, after 90 days of treatment with same extract, we observed a significant (p < 0.05) dose-dependent increase in RBC, HCT, PLT (p < 0.001; decreasing) and a significant decrease in MCH (increasing to normal).

In same light, rats treated with the ethanol extract showed a highly significant (p < 0.001) dose-dependent increase in RBC and HCT but a contrary significant decrease in PLT, which increased to normal at 1gKg<sup>-1</sup>.

**Table 4. Effect of oral administration of *Cymbopogon citratus* leaf extracts on the relative organ weight (ROW) per 100 g body weight of experimental animals recorded after 28 days and 90 days.**

Extract	Toxicity (days)	Organ	Weight of Organ in Study Groups (g)			
			Control (A, E) (Vehicle)	Test 1 (B, F) (0.25gKg <sup>-1</sup> )	Test 2 (C, G) (0.5gKg <sup>-1</sup> )	Test 3 (D, H) (1gKg <sup>-1</sup> )
Aqueous	Sub-acute (28)	Heart	0.36±0.02	0.35±0.01	0.38±0.01	0.37±0.01
		Liver	3.28±0.09	3.35±0.05	3.27±0.02	3.46±0.02
		Left Kidney	0.32±0.01	0.34±0.01	0.33±0.01	0.36±0.01
		Right Kidney	0.33±0.01	0.34±0.01	0.33±0.01	0.34±0.01
	<b>% Body wt gained</b>		<b>11.89</b>	<b>4.18</b>	<b>9.26</b>	<b>11.75</b>
	Sub-chronic (90)	Heart	0.27±0.01	0.27±0.01	0.27±0.01	0.26±0.01
		Liver	2.41±0.02	2.53±0.05	2.55±0.03	2.56±0.06
		Left Kidney	0.24±0.01	0.26±0.01	0.25±0.01	0.24±0.01
Right Kidney		0.24±0.01	0.26±0.01	0.25±0.01	0.24±0.01	
<b>% Body wt gained</b>		<b>54.70</b>	<b>44.57</b>	<b>45.67</b>	<b>56.30</b>	
Ethanol	Sub-acute (28)	Heart	0.31±0.01	0.32±0.01	0.32±0.01	0.31±0.01
		Liver	3.38±0.15	3.35±0.06	3.40±0.09	3.41±0.08
		Left Kidney	0.32±0.02	0.35±0.01	0.34±0.01	0.34±0.01
		Right Kidney	0.33±0.02	0.34±0.01	0.33±0.01	0.34±0.01
		<b>% Body wt gained</b>		<b>13.89</b>	<b>14.66</b>	<b>16.45</b>

The data represents the Mean ± SD for each group of rats, n = 6 (number of animals per group).

\*p<0.05 = significant difference and \*\*p<0.001 = highly significant difference compared to control (group A & E).

Table 5. Effect of oral administration of *Cymbopogon citratus* leaf extracts on some hematological parameters in experimental animals.

HEMA PARA.	AQUEOUS EXTRACT ADMINISTRATION								ETHANOL EXTRACT ADMINISTRATION			
	SUB-ACUTE TOXICITY (28 days)				SUB-CHRONIC TOXICITY (90 days)				SUB-ACUTE TOXICITY (28 days)			
	A Control	B 0.25gKg <sup>-1</sup>	C 0.5gKg <sup>-1</sup>	D 1gKg <sup>-1</sup>	A Control	B 0.25gKg <sup>-1</sup>	C 0.5gKg <sup>-1</sup>	D 1gKg <sup>-1</sup>	E Control	F 0.25gKg <sup>-1</sup>	G 0.5gKg <sup>-1</sup>	H 1gKg <sup>-1</sup>
WBC (10 <sup>3</sup> /μl)	11.42 ±2.69	14.47* ±2.01	13.90* ±2.37	13.97 ±2.46	10.75 ±1.54	10.67 ±1.55	11.13 ±0.99	10.65 ±1.59	18.07 ±1.51	16.10 ±1.06	17.93 ±1.94	18.68 ±1.98
LYM # (10 <sup>3</sup> /μl)	7.05 ±1.03	11.33* ±1.80	11.51* ±1.61	11.65* ±2.20	7.17 ±1.21	8.49 ±1.08	8.55 ±0.85	8.95 ±1.05	12.99 ±1.46	13.12 ±0.67	14.86 ±1.38	14.49 ±2.08
LYM % (%)	61.35 ±3.07	70.67 ±1.29	67.95 ±1.29	75.30 ±3.65	64.68 ±8.53	78.21 ±6.81	79.92 ±2.31	82.34 ±4.34	72.73 ±3.16	76.60 ±2.39	77.58 ±1.40	77.80 ±4.17
RBC (10 <sup>6</sup> /μl)	4.48 ±0.23	7.93** ±0.70	7.49** ±0.59	7.46** ±0.51	6.65 ±0.21	8.13* ±0.83	8.19* ±0.67	8.26* ±0.90	5.73 ±0.28	7.97* ±0.67	8.91** ±0.84	9.08** ±0.58
HGB (g/dl)	12.68 ±1.79	14.85 ±2.07	14.68 ±1.28	15.42* ±1.19	12.02 ±1.76	14.40 ±1.05	13.30 ±0.43	13.25 ±0.62	14.73 ±0.93	15.77 ±1.07	15.93 ±0.79	15.95 ±1.16
HCT (%)	29.87 ±0.82	36.28* ±4.10	36.83** ±2.25	37.35** ±0.94	37.03 ±1.51	42.87* ±1.81	43.02* ±1.61	43.27* ±2.02	36.90 ±2.35	36.50 ±1.68	42.72* ±1.84	43.65* ±2.03
MCV (fl)	58.85 ±0.99	54.67 ±3.01	58.00 ±6.42	68.67* ±4.23	60.00 ±1.79	52.17 ±1.17	56.50 ±0.84	52.00 ±1.90	48.50 ±1.97	48.33 ±1.97	48.50 ±3.21	49.50 ±2.35
MCH (pg)	27.88 ±1.60	20.97* ±1.54	21.57* ±1.42	27.00 ±1.25	22.22 ±0.59	16.97* ±0.25	17.28* ±0.98	17.32* ±1.56	16.83 ±0.92	17.32 ±1.91	17.50 ±1.29	18.10 ±1.37
MCHC (g/dl)	43.35 ±0.87	39.07 ±1.95	42.62 ±3.28	45.80 ±3.83	35.02 ±1.86	34.47 ±1.65	35.23 ±1.55	35.83 ±2.24	33.70 ±1.35	35.33 ±2.12	35.50 ±1.08	36.87 ±3.06
PLT (10 <sup>3</sup> /μl)	372 ±14.89	559.50** ±8.31	558.83** ±18.81	579.17** ±12.09	475 ±10.41	521.00** ±8.26	424.00** ±9.08	419.00 ±18.15	490.67 ±9.35	387.50* ±18.62	487.33* ±16.67	507.67* ±21.94

**Hematological Parameters:** WBC (White Blood Cell Count), LYM # (Lymphocyte number), LYM % (Lymphocyte percentage), RBC (Red Blood Cell Count), HGB (Hemoglobin), HCT (Hematocrit), MCV (Mean Corpuscular Volume), MCH (Mean Cell Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), PLT (Platelet Count).

The data represents the Mean ±SD for each group of rats, n = 6 (number of animals per group).

\*p<0.05 = significant difference and \*\*p<0.001 = highly significant difference compared to the appropriate control (group A and E)

Table 6. Effect of oral administration of *Cymbopogon citratus* leaf extracts on some plasma biochemical parameters in experimental animals.

BIOCH PARA.	AQUEOUS EXTRACT ADMINISTRATION								ETHANOL EXTRACT ADMINISTRATION			
	SUB-ACUTE TOXICITY (28 days)				SUB-CHRONIC TOXICITY (90 days)				SUB-ACUTE TOXICITY (28 days)			
	A Control	B 0.25gKg <sup>-1</sup>	C 0.5gKg <sup>-1</sup>	D 1gKg <sup>-1</sup>	A Control	B 0.25gKg <sup>-1</sup>	C 0.5gKg <sup>-1</sup>	D 1gKg <sup>-1</sup>	E Control	F 0.25gKg <sup>-1</sup>	G 0.5gKg <sup>-1</sup>	H 1gKg <sup>-1</sup>
TP (g/dl)	6.28 ±0.16	9.31** ±0.32	8.70** ±1.31	8.68** ±0.85	6.75 ±1.10	6.36 ±0.16	6.47 ±0.51	6.22 ±0.34	8.23 ±0.70	8.79 ±0.73	9.50 ±1.20	8.71 ±0.45
AST (U/l)	126.93 ±5.58	103.33* ±12.83	112.72* ±10.67	121.58 ±5.87	72.89 ±3.63	75.70 ±3.50	75.18 ±4.72	69.74 ±5.91	41.49 ±2.14	41.33 ±3.10	46.05 ±6.23	51.05** ±1.56
ALT (U/l)	14.70 ±1.27	11.76* ±1.45	12.02* ±1.10	13.57 ±1.23	11.93 ±0.90	13.82 ±1.09	14.28* ±0.54	17.21** ±0.36	14.93 ±1.36	14.14 ±0.61	15.97 ±2.74	17.81* ±2.78
ALP (U/l)	210.13 ±6.43	212.45 ±4.33	214.62 ±8.11	214.96 ±6.29	108.81 ±4.11	106.54 ±6.25	119.83* ±8.75	124.38* ±7.32	67.49 ±3.98	65.83 ±4.29	69.58 ±6.93	85.85* ±7.43
BUN (mg/dl)	61.92 ±4.48	61.87 ±6.13	63.20 ±4.01	65.23 ±3.17	105.91 ±8.30	104.35 ±11.13	94.61 ±5.21	76.83* ±6.13	49.48 ±3.32	56.13 ±5.68	55.58 ±6.39	52.82* ±1.53
URIC (mg/dl)	5.43 ±0.24	4.26* ±0.39	4.28* ±0.37	4.50* ±0.43	2.55 ±0.17	3.43 ±0.14	3.45 ±0.47	3.92* ±0.29	4.09 ±0.46	4.00 ±0.61	4.56 ±0.56	5.09 ±0.82
CRE (mg/dl)	0.73 ±0.16	0.53 ±0.02	0.53 ±0.01	0.80 ±0.03	0.56 ±0.17	0.44 ±0.01	0.44 ±0.01	0.56 ±0.01	0.47 ±0.16	0.40 ±0.01	0.53 ±0.03	0.67 ±0.02
TGY (mg/dl)	87.75 ±6.29	87.25 ±16.07	75.49** ±3.56	72.06** ±4.05	63.38 ±9.04	56.34 ±11.27	71.83 ±10.50	84.98* ±15.47	67.78 ±5.44	75.56 ±5.44	88.89* ±8.07	97.22** ±6.12
CHOL (mg/dl)	59.32 ±5.46	34.22** ±3.79	37.52** ±2.83	56.02 ±5.80	55.05 ±4.63	42.69* ±2.93	54.84 ±5.35	77.10** ±3.97	65.77 ±3.41	54.41* ±1.75	61.69* ±1.35	62.84 ±2.22
GLU (mg/dl)	144.29 ±10.28	87.25** ±12.13	96.30* ±18.24	88.20** ±9.52	128.84 ±8.31	103.19** ±2.57	107.16** ±7.99	100.33** ±4.56	115.22 ±3.36	111.00 ±7.70	115.92 ±10.05	128.10* ±5.04

Biochemical parameters: TP (Total Proteins); AST (Aspartate transaminase); ALT (Alanine transaminase); ALP (Alkaline phosphatase); BUN (Blood urea nitrogen); URIC (Uric acid); CRE (Creatinine); TGY (Triglycerides); CHOL (Cholesterol); GLU (Glucose).

The data represents the Mean ±SD for each group of rats, n = 6 (number of animals per group).

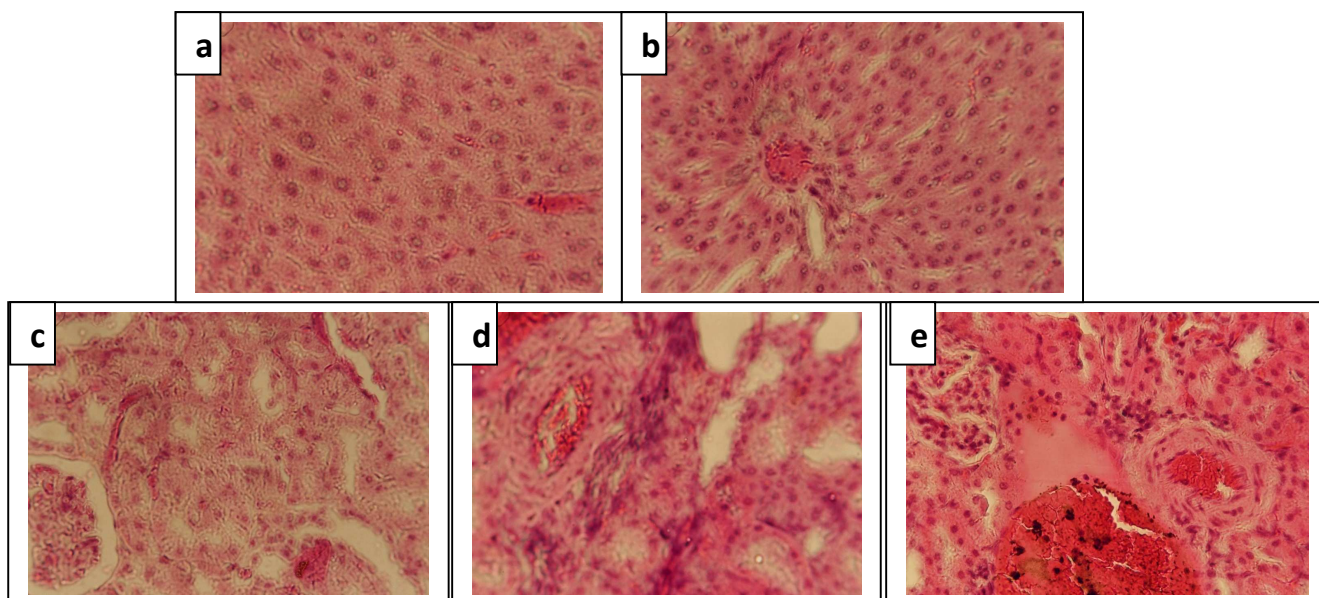
\*p<0.05 = significant difference and \*\*p<0.001 = highly significant difference compared to the appropriate control (group A and E).



*Effect of long-term administration of CC extracts on some biochemical parameters:* The effect of oral administration of the aqueous and ethanol leaf extracts of *CC* on plasma biochemical examination is summarized in Table 6. In rats treated with the aqueous extract for 28 days, we recorded a highly significant ( $p < 0.001$ ) increase in TP (dose-dependently decreasing), a significant ( $p < 0.05$ ) decrease in AST, ALT, URIC, CHOL, which were dose dependently increasing to normal and significantly reduced ( $p < 0.001$ ) values for TGY and GLU, when compared to the control. After treatment with same extract for 90 days, we recorded significantly increased ( $p < 0.05$ ) ALT, ALP, URIC, TGY, CHOL values, at a dose of  $1\text{gKg}^{-1}\text{BW}$  and significantly reduced ( $p < 0.05$ ) values for BUN (at  $1\text{gKg}^{-1}$ ) and GLU, in relation to the control.

Likewise, rats treated with the ethanol leaf extract of *CC* showed significant ( $p < 0.05$ ) increase in AST, ALT, ALP, BUN, TGY, GLU values, at a dose of  $1\text{gKg}^{-1}\text{BW}$  and significant ( $p < 0.05$ ) decrease in CHOL value, which increased to normal at  $1\text{gKg}^{-1}\text{BW}$ , when compared to the control. All other parameters remained normal in when compared to the control.

*Effect of long-term administration of CC extracts on some visceral organs (Histopathological examination):* Gross and microscopic examination of the kidney and liver of experimental animals revealed normal architecture of these visceral organs in rats treated with the aqueous and ethanol extracts at all doses, except for those treated for 90 days with the aqueous extract (Group D) in which we observed vascular congestion in the liver, and in those treated with the ethanol extract for 28 days (Group H), in which we observed scarring of the liver and slight tubular distortion in the liver, at a dose of  $1\text{gKg}^{-1}\text{BW}$ , when compared to the control rats. These abnormal observations are shown in Fig 1.



**Fig 1. Photomicrograph ( $\times 40$ ) of kidney and liver of experimental rats treated with *Cymbopogon citratus* leaf extracts: (a)-Normal tubular architecture of the kidney in control. (b)-Normal architecture of the liver in control. (c)-Vascular congestion of the liver in rats treated with the aqueous extract at  $1\text{gKg}^{-1}\text{BW}$  for 90 days. (d) – Tubular distortion in the kidney of rats treated with the ethanol extract for 28 days at  $1\text{gKg}^{-1}\text{BW}$ . (e) – Edema and scarring of the liver in rats treated with the ethanol extract for 28 days at  $1\text{gKg}^{-1}\text{BW}$ .**

## DISCUSSION

In acute oral toxicity, the aqueous and ethanol leaf extracts of *CC* did not cause any mortal or adverse effect on the male and female experimental mice during the observation period of 14 days, up to a dose of  $5\text{gKg}^{-1}\text{BW}$ . Changes in the parameters recorded are usually the first signs of toxicity [41] and none of these were recorded. And in accordance with the OECD Guidance Document on Acute Oral Toxicity Testing [45] and an earlier study carried out by Kennedy *et al.*, [20],  $\text{LD}_{50}$  values of test compounds greater than  $5\text{gKg}^{-1}\text{BW}$  are considered to be safe. As such, we can conclude that these extracts are non-toxic and safe at single dose oral toxicity.



The record of the calculated body and visceral organ weights did not reveal any effect from the administration of both the aqueous and ethanol leaf extracts of *CC*. This shows that the extracts did not have any adverse effect on the experimental animals [6]. However, the percentage body weight gained in the rats treated with the ethanol extracts is higher than that of the control, revealing either increase in appetite or the effect of the corn oil. This was not adverse as it did not effect a significant change in body or organ weights.

The hematological parameters WBC, RBC and platelets are mediators of immunity and play a vital role in immune-protection and tissue repair [10] [40]. The general increase and/or normal values of these parameters and their indices when compared to the control indicate that there was no observed adverse effect of these extracts on the hematopoietic system, which serves as an important index of the physiological and pathological status [4]. Hence, these extracts acted like a boost to the immune system. This correlates with earlier studies by Carbajal *et al.* [12] and Chee *et al.* [22], which demonstrated the anti-inflammatory, free radical scavenging and antioxidant activity of this plant.

Serum transaminase (AST, ALT) and phosphatase (ALP) are indicators of hepatic function [47]. Increased ALT and ALP after long term (90 days) administration of the aqueous extract at a dose of  $1\text{gKg}^{-1}\text{BW}$ , may be indicative of a hepatic tissue or cellular damage [10]. The same observation was made after administration of the ethanol extract for 28 days, at same dose. This was reflected in the histopathological examination of the liver from both groups, which revealed vascular congestion and scarring respectively. Hence an observed adverse hepatic effect of these extracts at high doses. However, at lower doses, the hepatic protection of these extracts was confirmed by normal values in experimental animals when compared to the control.

URIC is the end product of purine metabolism [10] and it is an indicator of cardiovascular and renal diseases [29], while BUN and creatinine are indicators of glomerular filtration rate (GFR), which is an indicator of the renal function [13]. Increased URIC, TGY and CHOL values in rats treated with the aqueous extract for 90 days at a dose of  $1\text{gKg}^{-1}\text{BW}$  might be indicative of liver tissue damage as a result of increased production and secretion of TGY by the liver, because BUN and CRE were normal when compared to the control. This is evident in the vascular congestion observed in the histo-architecture of the liver at this dose.

The observed increased BUN, TGY and GLU after administration of the ethanol extracts for 28 days at  $1\text{gKg}^{-1}\text{BW}$  might be indicative of renal toxicity, as evidenced in the scarring of the liver and mild tubular distortion observed in the kidney in rats treated with the extract at the same dose. However, the normal creatinine level when compared to the control indicated that this toxicity is of non-renal cause [10]. Most plant extracts are known to produce degenerative changes to renal architecture [18] due to the presence of certain secondary metabolites, which might be the case.

A general assessment of the lipid and glycemic profile indicated significantly reduced and/or normal values for TGY, CHOL and GLU after long-term administration of the aqueous and ethanol extracts at doses lower than  $1\text{gKg}^{-1}\text{BW}$ . This might be indicative of hypolipidemic and hypoglycemic activities, as earlier reported. The hypolipidemic activity might be as a result of the antioxidant property of the extracts, which lowers the level of cholesterol in the blood by increasing LDL catabolism. The extracts might also inhibit cholesterol synthesis and delay its absorption [37] at low doses. The hypoglycemic effects may be due to the presence of insulin-like substance in the plant extracts [14] [7], stimulation of  $\beta$  cells to produce more insulin [2], increasing glucose metabolism or regenerative effect of the extracts on pancreatic tissue [16]. Hence, these plant extracts could be used in the regulation of blood sugar levels and management of arterosclerotic diseases.

## CONCLUSION

The findings of this study indicate the relative safety and important biological activities of the aqueous and ethanol leaf extracts of *CC* but long term use and high doses should be discouraged.

## REFERENCES

- [1] A Caraballo, B Caraballo, A Rodriguez-Acosta. *J. Braz. Soc., Trop. Med.*, **2004**, 37(2): 186-188.
- [2] A Khan *et al.* *Biol. Trace Elem. Res.*, **1990**, 24:183-188.
- [3] AA Adeneye and EO Agbaje. *J. Ethnopharmacol.*, **2007**, 112: (3) 440-444.

- [4] AA Adeneye *et al.* *J. Ethnopharmacol.* **2006**, *105*, 374-379.
- [5] AA Aiyelaja, and OA Bello. *Edu. Res. and Rev.*, **2006**, *1*, 16–22.
- [6] AC Winder, LA Lembke and MD Stephens. *Arthritis Rheumatism*, **1969**, *12*: 472 – 482.
- [7] AM Gray and PR Flatt. *Br. J.Nutr.*, **1999**, *81*: 203-208.
- [8] AO Oyedele *et al.* *Phytomedicine*, **2002**, *9*, 259–262.
- [9] B Wannissorn *et al.* *Phytotherapy Research*, **1996**, *10*, 551-554.
- [10] BM Cavanaugh. *Nurse's Manual of Laboratory and Diagnostics Tests*. 4<sup>th</sup> Ed. *F. A. Davis Company*, Philadelphia. **2003**; 688pp.
- [11] CBG Guanasingh and S Nagarajan. *Ind. J. of Pharm. Sc.*, **1981**, *43*, 115.
- [12] D Carbajal *et al.* *J.Ethnopharmacol.*, **1989**, *25*, 103-107.
- [13] DC Eaton and JP Pooler. *Vander's Physiology*. 7<sup>th</sup> Ed. *McGraw-Hill Lange*, USA. **2009**; 230pp.
- [14] E Collier *et al.* *J. Biol. Chem.*, **1987**, *262*: 6238 -6241.
- [15] E Sawyer. *Nigerian J. Pharm.*, **1982**, *13*:28-33.
- [16] ER Shanmugasundaram *et al.* *J.Ethnopharmacol.*, **1990**, *30*:265 -269.
- [17] F Tchoumbounganget *et al.* *PlantaMedica*, **2005**, *71*: 20-23.
- [18] FA Khorshid. *Int. J.Pharmacol.*, **2008**, *4*:443 – 451.
- [19] G Bidla *et al.* *Indian J.Pharmacol.*, **2004**, *36*, 245-246.
- [20] GL Kennedy, RLJ Ferenz and BA Burgess. *J. Appl.Toxicol.*, **1986**, *6*, 145-148.
- [21] IK Makhija and D Khamar. *Der Pharmacia Lettre*, **2010**, *2(5)*: 399-411.
- [22] J Cheele *et al.* *J. Agric. Food Chem.*, **2005**, *53*, 2511-2517.
- [23] JE Adjanohoun *et al.* Contribution to Ethnobotanical and Floristic Studies in Cameroon. *Scientific, Technical and Research Commission, Organization of African Unity*. Addis Ababa, **1996**; 641pp.
- [24] JRS Tabuti, KA Lye and SS Dhillion. *J. Ethnopharmacol.*, **2003**, *88*, 19–44.
- [25] K Koba *et al.* *Bangladesh J.Pharmacol.*, **2009**, *4*: 29-34.
- [26] LCA Barbosa *et al.* *Molecules*. **2008**, *13*, 1864-1874.
- [27] M Suresh and RK Rai. *Curr. Sci.*, **1990**, *59*, 477-479.
- [28] ME Bassey and EO Effiong. *J. Nat. Prod. Plant Resour.*, **2011**, *1 (3)*: 33-42.
- [29] MK Kutzing and BL Firestein. *J.Pharmacol. Exp. Therap.*, **2007**, *324*, 1.
- [30] MR Santinet *et al.* *Paras. Res.*, **2009**, *105*, 1489–1496.
- [31] N Chungsamarnvart and S Jiwajinda. *Kasetsart J Nat Sc.*, **1992**, *26*, 46-51.
- [32] National Academies Press. *Guide for Care and Use of Laboratory Animals*. 8<sup>th</sup> Ed. *Nat. Res. Council of the Nat. Academies*. **2010**; 248pp.
- [33] OO Ojo and II Anibijuwon. *Adv. Nat. and Appl. Sc.*, **2010**, *4(1)*: 93-98.
- [34] P ArreyTarkanget *et al.* *J. Nat. Prod. Plant Resour.*, **2012**, *2 (3)*:372-380.
- [35] P Saotoinget *et al.* *J. Ecol. Nat. Environ.*, **2011**, *3(3)*: 104-117.
- [36] R Kumar *et al.* *Der Pharmacia Lettre*, **2010**, *2(2)*: 181-189.
- [37] R Rajendran and E Krishnakumar. *Avicenna J. Med. Biotech.*, **2010**, *2*, 4.
- [38] RB Pedrosa *et al.*, *Actaprotozoologica.*, **2006**, *45*, 231-240.
- [39] RRB Negrelle and EC Gomes. *Rev. Bras. Pl. Med., Botucatu*, **2007**, *9*, 1, 80-92.
- [40] S Tripathy, D Pradhan and M Anjana. *Int. J.Pharma. Bio. Sci.*, **2010**, *1*.
- [41] SA Carol. Acute, subchronic and chronic toxicology. In: Derelanko MJ, Hollinger MA, editors. *CRC Handbook of Toxicology*. U.S.A.: *CRC Press*. **1995**. p 51-104.
- [42] SS Handa, SPS Khanuja, G Longo and DD Rakesh. *Extraction Technologies for Medicinal and Aromatic Plants. ICS-UNIDO International Centre for Science and High Technology*, Trieste, Italy. **2008**; 266pp.
- [43] The East-West School for Herbal and Aromatic Studies (EWSHAS) *Comprehensive Monographs. Essential oil MateriaMedica; Lemongrass: Cymbopogon citratus (DC Ex. Nees) Stapf syn. Andropogon citratus DC ex. Nees.* **2012**. [theida.com](http://theida.com) consulted on the 15/10/2012.
- [44] V Tangpu and AK Yadav. *Pharmacologyonline.*, **2006**, *2*, 290-298.
- [45] OECD. *OECD Guidance Document on Acute Oral Toxicity Testing*; Organization for Economic Co-operation and Development: Paris, France, **2001**.
- [46] M Gabe. *Techniques Histologiques. Mason, 120, Boulevard Saint Germain, Paris.* **1968**; 128-243.
- [47] Konan *et al.* *J. Ethnopharmacol.*, **2007**, *110*: 30 – 38.