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Acute and Sub-acute Toxicity Profile of *Carpolobia lutea* Leaf Extract in Rats

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ABSTRACT

Carpolobia lutea (CL) (Polygalaceae) leaf is proven pharmacologically as possessing anti-diarrheal properties. Sub-acute toxicological investigations were executed with the Crude Ethyl Acetate Extract (CEAE) and Ethyl Acetate Fraction (EAF) which earlier reports reveal potent pharmacological activities. The pathological changes were investigated in rodents over a period of 28 days adopting Good Laboratory Practice (GLP) conditions. Thirty adult male and female Wistar rats were randomized to four treatment groups of 6 rats/equal sex/group and were administered orally with three doses of CEAE, 192.5, 385.0, 770 and one dose of EAF 770.0 mg kg^{-1}; while 10 mL kg^{-1} of 20% Tween 80 was used for the control test. Weekly relative body weights were evaluated. Blood samples were collected weekly for hematological examination. At autopsy, the major organs were carefully excised and weighed. Sections were collected for histological analysis. No visible sign of behavioral toxicity were observed during study. The acute toxicity study (LD_{50}) shows that median lethal dose is 3850.0, 3240.4 and 1414.2 mg kg^{-1} for the ethanol fraction, crude ethyl acetate extract and ethyl acetate fraction, respectively. Body weight but not organ weight increased significantly ($p<0.05$-0.001). The levels of Aspartate Amino Transferase (AST), Alanine amino transferase (ALT), Alkaline phosphatase (ALP), total and conjugated bilirubin, total protein, albumin and BUN increased significantly ($p<0.05$-0.001) and dose dependently. The EAF has high acute toxicity and significant biochemical but not haematopoietic sub-acute toxicological effect on oral administration in rats. Its pharmacological and therapeutic effectiveness is not without toxicity implication.

Key words: *Carpolobia lutea*, Polygalaceae, sub-acute toxicity, haematological, biochemical toxicity

INTRODUCTION

Carpolobia lutea G. Don. (Polygalaceae) is a small tree native to West and Central tropical Africa. It is common in rainforest and Guinea savannah of Sierra Leone and Cameroon. This shrub reaches up to 5 m in height and occurs as a dense overgrowth or an evergreen shrub or small tree. *C. lutea* is of good reputation and widely acknowledged among the Efik, Ibibio, Ibo and Yoruba speaking ethnic groups of Nigeria for its anti-inflammatory, anti-arthritic and aphrodisiac potentials. *C. lutea* is called cattle stick in English, Ikpafum in Ibibio, Agba or Angalagala in Igbo

and Egbo Osh unshun in Yoruba tribes of Nigeria (Etukudo, 2003; Muanya and Odukoya, 2008). In addition to its passion power, C. lutea has been used for headaches, general pain and to prevent sleep due to fatigue. The root is reported to have aphrodisiac properties (Walker and Silans, 1961). It has analgesic and androgenic properties; it is reputed to cure rheumatism, insanity, fever, dermal infection, vermifuge, venereal diseases and to combat sterility and promote child birth (Burkill, 1985; Etukudo, 2003; Muanya and Odukoya, 2008). Polyphenolic compounds such E-p-coumaric acid- β-D-2-deoxyglucosyl ester, E-cinnamic acid-β-D-2-deoxyglucosyl ester, E-p-coumaric acid-β-D-glucosyl ester, Z. cinnamic acid-β-D-2-deoxyglucosyl ester, E. cinnamic acid-β-D-glucosyl ester and E. cinnamic acid were isolated and characterised from CL (Nwidu *et al*., 2011).

The use of the leaf and young twigs of CL for treatment of abdominal illness has been reported (Irvine, 1961). It has been pharmacologically established to have antiulcer and antidiarrheal activity in rodents (Nwafor and Bassey, 2007), gastroprotective effects in rodents (Nwidu and Nwafor, 2009), antinociceptive effects in mice (Nwidu *et al*., 2011), anti-inflammatory, antidiarrheal and neuropharmacological activity in rodents (Nwidu, 2010). The screening of the various fractions of the leaf for pharmacological effects reveals the ethyl acetate fraction as the most active. Against this background we investigated the acute and sub-acute toxicological profiles of the crude ethyl acetate extract and fraction which was earlier reported to be effective antinociceptive agent (Nwidu *et al*., 2011).

This study is significant as it predict the safety associated with the use of isolated bioactive agents from medicinal plant (McNamara, 1976) and justify the execution of toxicological screening of new pharmaceutical product before approval for human use (Echobichon, 1992). This is the first report of toxicological profile of the leaf of CL leaf extract and fraction.

MATERIALS AND METHODS

Laboratory animals: Adult Wistar rats of either sex, aged 6-10 weeks with a weight of 160-230 g were purchased from the University of Jos and Laboratory Animal Center, Vom, Plateau State Nigeria. The experimental protocol was approved by the Faculty of Pharmacy, University of Uyo, Nigeria, Institutional animal Care and Use Committee (UUAEC) on 13/11/2006 via an approval circular No. UUAEC/2006/013 which follows the guidelines of CPSCEA (Committee for the purpose of control and supervision of experimental animals). The animals were kept in an animal room where the temperature was maintained at 22±3°C under a 12 h light-dark cycle. They were provided with food and water *ad libitum* for 1 week to acclimatize them before starting the experiment.

Preparation of plant extract: The leaf of *C. lutea* was collected from Ikot Itak Town in Ibeno Local Government area, Akwa Ibom State, Nigeria. Voucher specimen (UUH 998) was deposited at the University Herbarium, University of Uyo, Nigeria. The extraction of crude extract was performed by macerating air-dried powdered samples (0.75 kg) of *C. lutea* with 2.5 L of ethyl acetate at room temperature for 3 days. After suction filteration through a Buchner funnel, the ethyl acetate filtrates were evaporated by a rotary evaporator (BUCHI, USA) at 40-60°C and then lyophilized to yield crude ethyl acetate extract. The ethyl acetate fraction was prepared as reported earlier by Nwidu and Nwafor (2009). The extract and fraction were kept in desiccators until testing.

Acute toxicity or the median lethal dose (LD_{50}) in mice: The median lethal dose (LD_{50}) was determined by the method of Ellman (1959) with modification. The Swiss albino mice used in this study were starved for 24 h with free access to water except for 2 h prior to experiment. Different doses (100, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000 mg kg^{-1}) of the ethanol fraction (ETF), Ethyl Acetate Fraction (EAF) and the Crude Ethyl Acetate Extract (CEAE) were administered to nine groups of mice (n = 3) to establish the range of doses of the extract that would elicit toxic effects. The acute toxicity profile of crude ethanol extract has already been established (Nwafor and Bassey, 2007). The mice were observed for symptoms of toxicity and mortality intermittently for the next 6 h. Thereafter, it was observed again for 24 h post treatment for physical signs of toxicity. The LD_{50} was estimated by the geometric mean of the highest dose that caused 0% mortality and the lowest dose that caused 100% lethality. The rats were observed and recorded for 24 h after dosing for signs of toxicity such as motor activity, postures and mortality. Further monitoring was done for 7 days. The changes in body weight, food and water intake were monitored including possible signs of toxicity.

Sub acute toxicity studies: The rats were divided at random using random number table into experimental groups and a control group, with 6 animals in each. The control group received 20% Tween 80 and the experimental groups received ethyl acetate crude extract (CEAE) (192.5, 385.0 and 770 mg kg^{-1}) and Ethyl Acetate Fraction (EAF) (770 mg kg^{-1}), administered orally through an orogastric tube for every other day for 28 days to all animals. All animals in each group was weighed on day 0 and then weekly until termination of the investigation on day 28.

Weekly body weight: The body weight of each rat was assessed during the acclimatisation period once before commencement of dosing. Weight of each animal was taken once every 7 days during the dosing period and once at the day of euthanasia. The Relative Body Weight (RBW) of each animal was calculated as follows:

$$\text{RBW} = \frac{\text{Absolute weight of one time interval (g)}}{\text{Body weight of rat on commencement of dosing day (g)}} \times 100$$

Haematology: For haematological studies, blood samples (1 mL each) of these six animals in each treatment group, were taken from the caudal vein puncture, on days 0, 7, 14 and on day 28 from cardiac puncture after autopsy. The blood sample was collected into separate Eppendorf tubes containing heparin (0.125 mg) for hematological analyses using standard procedure (Jain, 1986). Red Blood Cell (RBC), White Blood Cell (WBC) and platelet counts were done electronically using Coulter Counter. The haemoglobin concentration by cyanomethaemoglobin method. The Packed Cell Volume (PCV) was done using capillary method and clotting time by needle streaking of a drop of blood sample on transparent surface. The differential leucocytes count for Neutrophils (N), Eosinophils (E), Basophils (B), Lymphocytes (L) and monocytes were estimated by examination of Giemsa stained blood samples. The Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) were calculated from the data obtained.

Serum biochemistry: Blood samples for biochemical analyses were centrifuged at 5000×g for 5 min and the plasma collected and stored in Eppendorf tubes at -20°C and used for the analysis of the following parameters: ALT, AST, ALP, urea, creatinine and albumin.

The enzymatic activities of aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were analysed colorimetrically at 546 nm according to standard methods (Reitman and Frankel, 1957; Morgenstern *et al*., 1965). While Alkaline Phophatase (ALP) was analysed calorimetrically at 405 nm using standard procedure (Bassey *et al*., 1946). Serum samples were analysed for the determination of the following: Total protein, urea and creatinine, respectively according to the methods described by Gornall *et al*. (1949) and Hartmann (1971). Serum concentration of glucose and total cholesterol were determined using ACCU-Chek Active test strips and kits, respectively. The effects of extract on triglycerides levels were also examined.

Relative organ weight and macroscopic examination: After taking the blood, organs such as the heart, liver, lungs, spleen, stomach, brain, ovary, testes and kidneys were quickly removed, cleaned with ice-cold saline and weighed. Each organ was examined macroscopically using hand lens for any visible lesions. The Relative Organ Weight (ROW) of each animal was then calculated as follows:

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

Microscopic examination: After euthanising the animals, 0.05 g pieces of liver, kidney, lungs, spleen, heart, testes, stomach, ovary and brain were fixed in 10% neutral buffered formalin before processing for histopathological studies. Tissues were processed by conventional techniques using automatic tissue processor. The paraffin embedded sections of 4-5 μ thickness were prepared with the rotary microtome, stained with haematoxylin and eosin for microscopic examination using optical microscope linked to a computer for pictures and treatment.

Statistical methods: The results are presented as mean and SEM and comparisons among groups for statistical significant differences were done by analyses of variance, followed by Turkey Kramer's multiple comparison test. The p-values of less than 0.05 were considered as indicative of significance.

RESULTS

Acute toxicity (LD_{50}): The acute toxicity study (LD_{50}) shows that median lethal dose is 3850.0, 3240.4 and 1414.2 mg kg^{-1} for the ethanol fraction, crude ethyl acetate extract and ethyl acetate fraction, respectively. The signs of toxicity were observed from 1250, 3000 and 3100 mg kg^{-1} for the EAF, ETF and CEAE, respectively. The mortality rate was observed from 1750, 3600 and 3200 mg kg^{-1} and up to 2000, 4000 and 3500 mg kg^{-1} for the EAF, ETF and CEAE, respectively. The No Observed Adverse Effect Level (NOAEL) for the intraperitoneal dose was 1500 mg kg^{-1} and the Lowest Observed Adverse Level (LOAEL) 3000 mg kg^{-1}. The acute toxicity was more pronounced in the EAF than in the ETF. Signs of toxicity observed, include restlessness, convulsion, salivation, defecation, urination, syncope, asthenia and death under 24 h doses.

Body and organ weights: Graphical representation of the changes in body weight following treatment of rats with CL is shown in Fig. 1. There were significant increases ($p<0.05$-0.001) in body weights from day 7 to 30 for the median dose of CEAE and the highest dose of EAF as in the control group. The baseline mean body weights (g) of control, 192.5, 385.0 and 770 mg kg^{-1} CEAE and 770 mg kg^{-1} CL treatment groups were 185.7±17.36, 193.3±25.3, 207.0±16.6, 194.50±10.7 and

210.2±28.5, respectively. However, there were no significant differences in liver, kidney, lung, pancreas, heart and spleen weights, expressed as percent of body weight, between control and the CL treatment groups at termination of experiment (Table 1).

Haematological studies: Results of the haematological studies are presented in Table 2. The data show that Hb, PCV, lymphocytes and granulocytes levels for control rats were not significantly different from those treated with CL during the period of study (Table 2). Baseline haematological parameter levels were similar in all treatment groups as compared to control 0-20th day; except some insignificant increase of platelets and WBC counts up to day 14 with partial recovery at the end of treatment when compared to control (Table 2).

Serum biochemistry: The effect of CL on renal function is summarised in Table 3. There was no significant changes observed for the extract and fraction treated group and the control for

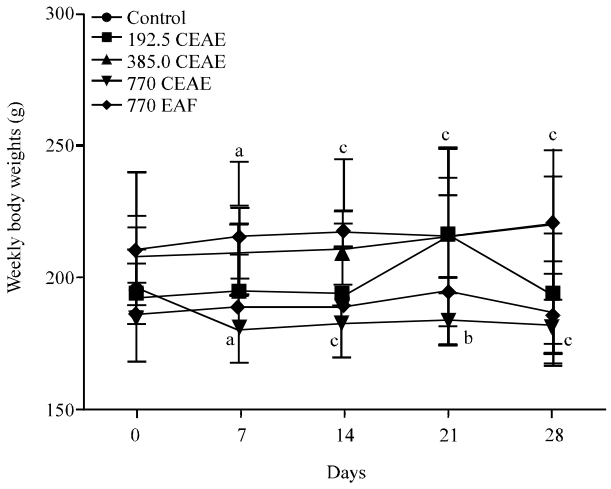

Fig. 1: Effects of *C. lutea* on weekly body weight for 30 days, Significance relative to control: [a]p<0.05, [b]p<0.01, [c]p<0.001, values represent Mean±SEM (n = 6)

Table 1: Effects of *C. lutea* extracts on organ extract relative organ weights after 30 days oral dosing

		Organ weights/% relative organs weights in bracket								
Dose	Mean final									
(mg kg^{-1})	body weight	Stomach	Heart	Kidneys	Lungs	Spleen	Liver	Brain	Ovaries	Testes
Control	186.5	1.21±0.09	0.59±0.05	1.12±0.09	1.47±0.25	0.70±0.16	5.42±0.60	1.72±0.03	0.10±0.71	4.27±0.71
CEAE: 192.5	±20.0	(0.66±0.03)	(0.32±0.02)	(0.61±0.02)	(0.81±0.19)	(0.37±0.05)	(2.92±0.11)	(0.96±0.09)	(0.05±0.01)	(2.55±0.11)
	193.0	1.39±0.17	0.79±0.09	1.42±0.20	1.74±0.53	0.78±0.11	6.37± 1.23	1.83±0.07	0.12±0.01	2.72±0.00
	±17.8	(0.72±0.03)	(0.44±0.01)	(0.73±0.04)	(0.90±0.25)	(0.40±0.05)	(3.27±0.23)	(0.96±0.05)	(0.06±0.00)	(1.74±0.00)
CEAE: 385.0	220.4	1.74±0.26	0.69±0.08	1.42±0.24	1.72±0.16	0.96±0.16	6.87±0.97	1.77±0.08	0.10±0.01	4.42±0.00
	±28.5	(0.79±0.02)	(0.31±0.02)	(0.64±0.03)	(0.80±0.09)	(0.45±0.09)	(3.11±0.12)	(0.83±0.08)	(0.05±0.00)	(1.39±0.00)
CEEA: 770.0	181.5	1.37±0.07	0.60±0.04	1.17±0.12	1.28±0.11	0.70±0.10	4.76±0.36	1.75±0.07	0.09±0.01	4.41±0.00
	±10.5	(0.76±0.03)	(0.34±0.02)	(0.64±0.04)	(0.71±0.05)	(0.39±0.06)	(2.62±0.08)	(0.97±0.04)	(0.05±0.01)	(2.17±0.00)
EAF: 770.0	220.4	1.82±0.15	0.75±0.06	1.55±0.16	1.99±0.23	0.96±0.29	6.67±0.77	1.80±0.05	0.12±0.01	5.12±0.71
	±18.7	(0.83±0.07)	(0.34±0.02)	(0.70±0.02)	(0.92±0.13)	(0.42±0.08)	(3.01±0.13)	(0.83±0.05)	(2.18±0.79)	(0.06±0.01)

Values represent Mean±SEM (n = 6), CEAE: Crude ethyl acetate extract, EAF: Ethyl acetate fraction

Table 2: Effects of intake of *C. lutea* extracts (mg kg^{-1}) on haematological parameter for 28 days

Parameters/doses	Control	CEAE: 192.5	CEAE: 385.0	CEAE: 770.0	EAF: 770.0
Day 0					
Hb (g dL^{-1})	12.75±0.98	13.75±0.64	13.25±0.63	12.92±0.30	11.22±1.23
Hb (%)	86.83±7.11	94.17±4.51	90.83±4.81	88.17±2.78	76.0±8.500
PCV (%)	46.67±1.38	52.33±1.67	48.33±1.15	48.33±1.29	49.50±2.46
MCV (μm^3)	35.88±5.97	32.90±4.49	66.49±22.20	27.47±1.15	38.32±3.76
MCHC (%)	26.86±2.02	26.37±1.38	27.36±0.77	26.80±0.87	21.85±2.78
MCH (ρg)	9.39±1.380	8.62±1.150	17.94±5.83	7.35±0.310	8.25±1.18
Total WBC×10^9 (N/L)	7.00±1.850	6.63±1.280	7.57±1.350	11.63±1.56	7.23±1.37
Neutrophils (%)	19.67± 1.74	20.00±8.87	23.0±3.780	18.0±2.330	18.33±2.15
Lymphocytes (%)	78.67± 1.93	74.33±8.30	75.0±4.030	77.67±1.83	78.67±2.87
Eosinophils (%)	0.67±0.000	2.67±0.710	3.33±0.820	4.00±0.020	3.33±0.82
Monocytes (%)	1.00±0.030	5.50±1.110	2.00±0.000	4.50±1.130	2.67±0.82
RBC×10^6 (N/L)	14.8±2.140	16.87±1.73	10.80±2.65	16.83±1.08	14.13±1.58
Platelets (×10^3 mm^{-3})	85.33± 21.7	68.00±12.95	118.0±28.66	178.67±39.3	77.33±22.7
Clotting time (min)	1.27±0.070	1.52±0.1400	1.29±0.0800	1.46±0.1800	1.30±0.07
Day 7					
Hb (g dL^{-1})	11.55±0.65	11.33±0.48	11.92±0.09	12.08±0.73	12.08±0.36
Hb (%)	77.00±4.55	76.00±2.93	81.33±1.46	83.33±5.14	82.17±2,83
PCV (%)	53.50±1.44	49.83±0.77	51.50±0.84	51.17±1.43	51.0±2.15
MCV (μm^3)	37.30±8.93	31.56±7.21	52.29±13.64	32.55±6.79	44.26±12.13
MCHC (%)	22.21±1.82	22.78±1.11	23.17±0.50	23.68±1.56	23.88±1.29
MCH (ρg)	7.98±1.800	7.31±1.820	12.01±3.05	7.75±1.900	10.01±2.18
Total WBC×10^9 (N/L)	11.70±2.14	10.80±3.49	8.30±0.980	11.60±2.32	11.55±1.82
Neutrophils (%)	19.33±6.92	22.67±6.32	13.06±2.09	14.33±2.22	18.00±3.30
Lymphocytes (%)	78.00±6.55	76.00±6.40	85.33±2.63	86.67±1.76	81.67±3.46
Eosinophils (%)	2.40±1.670	2.00±1.130	3.33±0.870	2.00±0.200	0.33±0.37
Monocytes (%)	0.67±1.630	-	-	-	-
RBC×10^6 (N/L)	17.93±4.14	18.80±3.67	12.40±2.83	18.20±3.10	14.93±3.47
Platelets (×10^3 mm^{-1})	61.33±15.29	50.67±11.46	38.77±9.97	41.33±17.44	32.00±4.53
Clotting time (min)	1.03±0.2100	1.12±0.1400	1.17±0.090	1.12±0.2500	1.30±0.08
Day 14					
Hb (g dL^{-1})	11.58±0.78	12.42±0.30	12.50±0.28	12.83±0.23	11.17±0.73
Hb (%)	79.00±5.51	84.67±2.63	84.33±2.74	87.67±2.43	74.83±4.69
PCV (%)	52.50±3.00	49.83±1.31	49.83±1.45	52.0±2.530	54.17±1.95
MCV (μm^3)	23.79±2.92	21.29±1.66	28.11±7.27	25.59±4.24	34.50±6.78
MCHC (%)	22.58±2.59	24.96±0.65	25.18±0.96	24.96±1.48	20.70±1.50
MCH (ρg)	5.15±0.470	5.30±0.370	7.08±1.830	6.25±0.900	7.35±1.81
Total WBC×10^9 (N/L)	12.47±2.83	15.83±2.95	9.52±1.500	11.32±3.41	9.70±4.11
Neutrophils (%)	17.00±2.58	21.67±1.32	26.33±3.07	17.67±2.07	19.67±0.88
Lymphocytes (%)	79.33±3.70	73.67±2.29	71.33±3.52	67.33±11.52	76.33±2.22
Eosinophils (%)	1.67±1.430	0.33±0.370	2.33±1.190	2.67±1.4600	3.33±2.01
Monocytes (%)	2.33±0.880	4.33±2.360	0.00	2.33±1.1900	0.67±0.73
RBC×10^6 (N/L)	26.80±2.76	23.87±1.52	21.73±4.37	58.93±39.88	18.00±3.26
Platelets (×10^3 mm^{-3})	213.37±48.88	153.33±58.15	94.00±28.26	257.00±98.15	233.33±69.9
Clotting time (min)	0.51±0.0300	0.29±0.0500	0.45±0.0200	0.40±0.0400	0.39±0.06
Day 28					
Hb (g dL^{-1})	13.18±0.55	13.17±0.34	12.00±0.42	14.35±0.39	13.95±0.28
Hb (%)	91.00±3.92	90.67±2.80	78.33±3.11	99.17±2.52	96.50±1.67
PCV (%)	48.6±1.100	50.7±3.20	50.0±1.700	49.4±1.500	49.6±1.10
MCV (μm^3)	27.85±5.94	23.42±4.51	25.36±5.07	27.76±2.43	27.87±3.23

Table 2: Continued

Parameters/doses	Control	CEAE: 192.5	CEAE: 385.0	CEAE: 770.0	EAF: 770.0
MCHC (%)	25.03±1.77	25.98±1.07	23.33±0.93	28.52±1.69	25.62±1.06
MCH (ρg)	6.63±1.050	6.13±1.330	9.33±4.100	7.99±1.040	7.06±0.68
Total WBC×10^9 (N/L)	5.68±1.340	6.82±1.730	7.95±1.570	5.98±1.490	5.53±0.93
Neutrophils (%)	15.33±2.87	19.33±2.87	23.33±5.33	27.00±6.57	22.00±1.79
Lymphocytes (%)	82.67±3.13	78.00± 2.65	77.00±3.86	71.00±6.57	77.33±2.01
Eosinophils (%)	0.00	1.00±0.750	0.00	2.00±0.890	0.33±0.37
Monocytes (%)	2.00±1.130	1.67±0.880	1.33±0.7300	0.00	0.33±0.37
RBC×10^6 (N/L)	23.07±4.87	25.07±4.45	25.85±5.260	16.27±3.410	20.67±1.02
Platelets (×10^3 mm^{-3})	46.17±34.26	35.67±7.40	22.83±21.32	60.17±18.63	66.83±24.33
Clotting time (min)	1.05±0.1600	1.17±0.150	1.53±0.1900	1.09±0.1300	1.19±0.10

Values represent Mean±SEM (n = 6), CEAE: Crude ethyl acetate extract, EAF: Ethyl acetate fraction

Table 3: Effect of Sub-acute intake of CL leaf extract and fraction on renal functions

Parameters	Na^+ (mmol L^{-1})	K^+ (mmol L^{-1})	Cl^- (mmol L^{-1})	HCO_2^-	BUN (mmol L^{-1})	Creatinine (µmol L^{-1})	Glucose (mmol L^{-1})
Control	136.50±0.99	11.58±0.83	106.17±0.96	27.5±0.910	18.50±0.91	134.50±0.25	5.34±0.39
192.5 CEAE	141.75±0.53^c	9.85±0.37	110.78± 0.23^c	26.25± 0.02	22.50±0.02^b	64.75±0.12^c	4.48±0.59
385.0 CEAE	130.0±0.000^c	13.74±0.44	112.2±0.5200^c	26.80± 0.07	21.60±0.53^a	18.60±0.79^c	3.84±0.92
770.0 CEAE	133.0±0.580^b	10.87±0.81	110.0±0.2600^c	29.0±0.170	26.83±0.58^c	34.67±0.42^c	3.73±0.67
770.0 EAF	134.20±0.54	9.36±0.10	111.0±0.1200^c	27.8±0.290	24.4±0.920^c	86.40±0.05^c	2.37±0.19^a

Values represent Mean±SEM (n = 6), BUN: Blood urea nitrogen, Significance relative to control: ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001, CEAE: Crude ethyl acetate extract, EAF: Ethyl acetate fraction

Table 4: Effect of sub-acute intake of CL leaf extract and fraction on Liver functions

	Total proteins	Albumin	Globulin	ALT	AST	ALP
Parameters	(mmol dL^{-1})					
Control	58.80±0.82	38.8±0.420	19.60±0.57	16.8±0.960	15.40±0.27	19.60±0.84
192.5 CEAE	54.5±0.330^b	37.00±0.47	16.50±0.33^a	16.0±0.470	16.50±0.75	17.25±0.44^a
385.0 CEAE	60.5±0.650	41.33±0.10^a	20.83±0.83	24.83±0.14^c	19.50±0.62^c	25.83±0.50^c
770.0 CEAE	60.83±0.34	39.5±0.680	21.5±0.930	17.17±0.11	26.84±0.18^c	26.83±0.34^c
770.0 EAF	56.75±0.99	41.25±0.99	16.25±0.29^b	22.5±0.120^c	20.0±0.47^c	19.25±0.55

Values represent Mean±SEM (n = 6), Significance relative to control: ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001, CEAE: Crude ethyl acetate extract, EAF: Ethyl acetate fraction

potassium and bicarbonate ions (Table 3). However, a significant (p<0.001) decrease in creatinine in all treatment groups; significant (p<0.05) decrease in glucose for the 770 mg kg^{-1} of EAF and a significant (p<0.01-0.001) decrease in sodium ions in all treatment groups when compared with the control group. Besides, there were significant (p<0.01) increase in chloride ions in all treatment group and significant (0.05-0.001) increase in BUN for the entire treatment group when compared to control.

The total proteins, globulin and alkaline phosphatase deceased significantly (p<0.05-0.01) while there was a significant (p<0.05-0.001) increase in albumin, alanine amino transferase, aspartate aminotransferase and alkaline phosphatase in moderate and highest dose of the extracts and fraction (Table 4).

The effects on lipid profile are summarised in Table 5. The data show that Total Cholesterol (TC), triglycerides (TG), High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL) and Uric Acid (UA) levels for control rats were not significantly different from those treated with CL during the study period (Table 5).

Table 5: Effects of sub-acute intake of CL leaf extract (mg kg^{-1}) and fraction on lipid profile

	Parameters				
Treatments	TC (mmol dL^{-1})	TG (mmol dL^{-1})	HDL (mmol dL^{-1})	LDL (mmol dL^{-1})	UA (mmol dL^{-1})
Control	1.68±0.16	1.20±0.04	0.95±0.18	0.53±0.16	0.33±0.13
CEAE: 192.5	1.50±0.24	1.75±0.21	0.88±0.14	0.31±0.14	0.20±0.04
CEAE: 385.0	1.83±0.25	1.10±0.21	0.57±0.01	0.75±0.20	0.21±0.05
CEAE: 770.0	1.80±0.09	1.35±0.13	0.93±0.16	0.38±0.08	0.28±0.05
EAF: 770.0	1.58±0.08	1.07±0.13	1.17±0.15	0.28±0.13	0.24±0.04

Values represent Mean±SEM (n = 6), Significance relative to control: Not significant, TC: Total cholesterol, TG: Triglycerides, HDL: High density lipoproteins, LDL: Low density lipoproteins, UA: Uric acid, CEAE: Crude ethyl acetate extract, EAF: Ethyl acetate fraction

DISCUSSION

The acute toxicity profile was in the order EAF>CAEA>ETF. The pre-concentration of polyphenolic glucosides in EAF may in part be responsible for acute toxicity profile. Animals in the treatment groups gained weight during the 28 day period, although, the rate was significantly slower for the 192.5 mg kg^{-1} group but it was higher in all the other treatment groups as in the control group. However, the CEAE 770 mg kg^{-1} group showed marked reduction in weights when compared to the control group. Unlike the body weight, no treatment-related significant changes in organ weights occurred suggesting that CL did not promote cell proliferation or cause cellular damage and hence edema.

Various medicinal herbs or conventional drugs/chemicals adversely affect certain blood components (King and Kelton, 1984). Haemolytic anaemia and thrombocytopaenia is known to be induced by flavonoids from herbs (Gandolfo *et al*., 1992). CL extract also contains polyphenolics glucosides which did not reveal any significant impact on the haematopoietic system. The effects on Hb and other haematological indices were not significant. This may suggest no adverse effects on bone marrow, a source of reticulocytes. It is likely that preconcentration of polyphenolic content by ethyl acetate fraction of CL administered is too high and did reach plasma threshold concentration that produce toxicity in the rats hence the high LD_{50}.

Though, initially an insignificant increase in blood platelet counts were observed, thrombocytosis; however, this effect was normalized at 28th day of treatment. CL treatment increased WBC counts and slight decrease of lymphocyte and granulocyte counts all of no statistical significance. This observation suggests that the elevation of WBCs caused by CL was compensated for by the decreased bone marrow production of granulocytes, the precursors of WBCs. Thus, the report of WBC counts could be evidence of the balance between the rate of granulocyte production and that of WBC destruction as a result of direct actions of CL.

Some herbal medicines have hepatotoxic and nephrotoxic effects (Lin *et al*., 2003; Akdogan *et al*., 2003). Damage to these organs often results in elevation in clinical chemistry parameters (Stonard and Evans, 1995) such as serum enzymes like AST and ALT and analytes like total and conjugated bilirubin, BUN and creatinine (Akdogan *et al*., 2003). CL contains polyphenolic glucosides (Nwidu *et al*., 2011). Flavonoid-containing herbal medicines have been implicated in the impairment of kidney and liver functions, an increase of some clinical chemistry parameters in the treatment group that would suggest that CL treatment had adversely compromised the integrity of the liver or kidney was observed. For example, the fractions and extract did reduce total plasma proteins and globulin levels but albumin levels were slightly increased; this might indicate inhibition of protein biosynthesis and invariably reduction of oncotic pressure (Whitby *et al*., 1987). Hypoproteinemia is a common feature of most liver damage (Kaneko, 1989). Rise in BUN as observed could imply kidney damage. The significant rise of plasma

ALP levels may indicate that CL caused damage to cardiac or skeletal muscle and impacted on hepatic excretory function (Stonard and Evans, 1995; Gaw *et al*., 1998). A typical myocardial infarction gives an AST/ALT ratio greater than 1; however, AST/ALT ratios of less than 1 are found due to the release of ALT from the affected liver (Hawcroft, 1987). Since, the results gave an AST/ALT ratio to be less than 1, the extract is less likely to lead to myocardial infarction if large doses are taken over a long period of time. Moreover, the standard range for plasma ALT levels for rats is 21-52 UI L^{-1} (Coimbra *et al*., 1995), present results provide evidence of no hepatic overload.

Traditionally, phytomedicinal application of the plant is in either cold or hot aqueous extract and not ethyl acetate extract. The HPLC profiling of the cold aqueous extract unlike the ethyl acetate fraction and extract did show low level of polyphenolic contents. This may unlikely exhibit gross biochemical impact as observed in this study.

These results indicate that EAF has the highest acute toxicity. Oral administrations of CEAE and EAF at sub-acute dosing do not exhibit significant toxicological effects in rats. The insipient toxicological effect is more significant in the biochemical rather than in the haematopoietic system of rats. The reported pharmacological and therapeutic effectiveness of the ethyl acetate extract and fractions are not without toxicity implication.

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