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Full Length Research Paper

Toxicological implications of aqueous extract of *Clematis brachiata* Thunb. leaves in male Wistar rats

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Aqueous extract of *Clematis brachiata* Thunb. leaves at the doses of 100, 200 and 400 mg/kg body weight was investigated for its toxic effect in male Wistar rats on days 1, 7 and 21. The extract at all the doses did not significantly (P > 0.05) alter the liver- and kidney-body weight ratio, conjugated bilirubin, total protein, globulin, sodium, potassium, chloride, inorganic phosphorus, calcium ions, LDL-C, RBC, Hb, PCV, MCV, MCH, MCHC and RCDW levels throughout the exposure period. In contrast, albumin, urea, HDL-C, LUC, neutrophils, monocytes, eosinophils and basophils levels increased. The 100 mg/kg body weight of the extract significantly (P < 0.05) reduced the total bilirubin only at the end of the experimental period whereas the 200 and 400 mg/kg body weight increased the bilirubin content of the animals. Whereas the increase in serum GGT activity did not manifest until after the 21 daily doses of the extract, the activities of serum ALP and AST increased throughout the exposure period. In addition, serum ALT activity was not altered by the extract. WBC, platelet, lymphocytes, uric acid, triacylglycerol and the computed atherogenic index were reduced by the extract. The results have suggested that the aqueous extract of *C. brachiata* leaves may be clinically beneficial to the animals as it may not predispose to cardiovascular risk, but has selective toxic effect on the haematological parameters and the functional indices of the liver and kidney of male rats. Overall, the extract of *C. brachiata* leaves is not completely safe when repeatedly consumed for three weeks at the doses investigated for three weeks.

Key words: *Clematis brachiata*, cardiovascular risk, functional indices, haematological parameters, selective toxicity, serum lipids.

INTRODUCTION

Phytomedicines have been used as the mainstay in the treatment of various diseases and preservation of human health since early ages of civilization. For example, Farnsworth et al. (1985) reported that 80% of the population in the developing world still rely on traditional medicine for their primary health care needs. Unfortunately, most of these herbal remedies have not been assessed for their safety as the traditional medicine practitioners believe that herbs are safe simply because they are natural in origin.

Therefore, safety or toxicity in herbal therapy is an important challenge for traditional medicine. *Clematis brachiata* Thunb. (Ranunculaceae), known as traveller's joy (English) is widely distributed in South Africa, Swaziland, Namibia and Botswana. It is a slender, twining, woody deciduous climber that grows up to 5m in length. It bears sasses of small, sweetly scented, creamy white flowers in the late summer and autumn. It is claimed to possess wonderful pain relieving properties and as such used by travellers that embarked on long distance by foot.

The leaves are used to ease headaches, coughs, chest ailments, abdominal upsets and schistomiasis (Roberts, 1990; Spang et al., 2000). The hot decoction of the leaves, stems and roots are separately used to relief cold, malaria, sinus infections and asthma (Chhabra et al., 1991; Koch et al., 2005; Pendota et al., 2008) . Des-pite the various uses of *C. brachiata* in traditional medi-cine of South Africa, there is inadequate information on the toxic implications of aqueous extract of the plant leaves. Therefore, the aim of this study was to evaluate the toxicity risk of the aqueous extract of the plant leaves using male Wistar rats as a model.

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MATERIALS AND METHODS

Plant material

C. brachiata leaves collected in April, 2008 from a natural population growing within the premises of University of Fort Hare, Alice, South Africa, was identified by Prof. D. S. Grierson of the Department of Botany, University of Fort Hare. A voucher specimen (M. Mostafa med., 2008/1) was deposited at the Giffen Herbarium of the University.

Animals

Male albino rats (*Rattus norvegicus*) of Wistar strain weighing 191.21 ± 5.92 g were obtained from the Animal House of the Agriculture and Rural Development Research Institute, University of Fort Hare, Alice. The animals were housed in clean alluminum cages placed in well- ventilated house conditions (temperature 23 ± 1°C; photoperiod: 12 h natural light and 12 h dark: humidity: 45 - 50%). They were maintained on Balanced Trusty Chunks (Pioneer Foods (Pty) Ltd, Huguenot, South Africa) and clean tap water *ad libitum*.

Assay kits

The assay kits for creatinine, urea, calcium, sodium, potassium, chloride, phosphorus, albumin, bilirubin, cholesterol, triacylglycerol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were obtained from Roche Diagnostic GmbH, Mannhein, Germany. All other reagents used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

Preparation of extract

The leaves were washed in running tap water, oven-dried at 40°C for 48 h and thereafter pulverized. The powder (200 g) was boiled for 20 min in a litre of distilled water and allowed to cool for 2 h (boiling was done in accordance with the way it is usually prepared in folklore medicine of South Africa). The extract was then suction-filtered with a Buchner funnel and Whatman no. 1 filter paper. The filtrate was later freeze-dried (Savant Refrigerated Vapor Trap, RVT4104, USA) to give a yield of 45 g. This was reconstituted in distilled water to give the desired doses (100, 200 and 400 mg/kg body weight) used in this study.

Animal grouping and extract administration

Seventy-two, male Wistar rats were completely randomized into four groups of eighteen and were orally administered on daily basis using a metal oropharyngeal cannula as follows: Group A (control) received 0.5 ml of distilled water (the vehicle) while groups B, C and D were treated like the control except they received same volume containing 100, 200 and 400 mg/kg body weight of the extract respectively.

Six rats from each group were sacrificed 24 h after their respective 1, 7 and 21 daily doses. The study was carried out following approval from the Ethical Committee on Animal Use and Care of University of Fort Hare, South Africa.

Preparation of serum

The procedure described by Yakubu et al. (2005) was employed in the preparation of serum. Briefly, under ether anaesthesia, rats were allowed to bleed through their cut jugular veins which were slightly displaced (to prevent contamination of the blood with interstitial fluid) into clean, dry centrifuge tubes. An aliquot (2 ml) of the blood was collected into sample bottles containing EDTA (BD Diagnostics, Preanalytical Systems, Midrand, USA) for the hematological analysis. Another 5 ml of the blood was allowed to clot for 10 min at room temperature and then centrifuged at 1282 g × 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300, Hamburg, Germany). The sera were later aspirated with Pasteur pipettes into sample bottles and used within 12 h of preparation for the assay. The rats were thereafter quickly dissected in the cold; the liver and kidney excised, transferred into ice-cold 0.25 M sucrose solution, blotted with clean tissue paper and then weighed.

Determination of biochemical parameters

The levels of sodium, potassium, calcium, magnesium, chloride, inorganic phosphorus, urea, creatinine, total and conjugated bilirubin, albumin, globulin, total protein, ALP, GGT, ALT, AST, cholesterol, triacylglycerol, HDL-C and LDL-C were determined in the serum using assay kits from Roche Diagnostics on Roche Modular System (model P800) Mannhein, Germany, by adopting the standard procedures described by Tietz et al. (1994). The Horiba ABX 80 Diagnostics (ABX Pentra Montpellier, France) was used for the determination of haematological parameters: red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), large unstained cell (LUC), red cell distribution width (RCDW), white blood cell (WBC), neutrophils, monocytes, lymphocytes, eosinophils, basophils and platelets. Organ to body weight ratio was computed using the expression;

Weight of organ / Total weight of the animal) × 100.

Statistical analysis

Data were expressed as means of six replicates ± SD. They were subjected to one way analysis of variance (ANOVA) and means were separated by Duncan Multiple Range Test. Percentage data were arcsine-transformed before analysis. Significant levels were tested at $P < 0.05$.

RESULTS

The extract at all the doses investigated did not significantly ($P > 0.05$) alter the computed liver- and kidney-body weight ratios throughout the exposure period (Table 1).

The total bilirubin content of the animals was not altered significantly ($P > 0.05$) until after 21 daily administration when the 100 mg/kg body weight reduced the level of total bilirubin in the animals. In contrast the 200 and 400 mg/kg body weight of the extract increased the total bilirubin concentration during this period (Table 2). Whereas the levels of conjugated bilirubin, total protein and globulin were not significantly altered by the extract, the albumin level increased significantly. The serum GGT activity of the animals only increased after 21 daily doses of the extract. In addition, the activities of serum ALP and AST of the animals increased throughout the period of exposure, whereas the serum ALT activity was not signi-

Table 1. Effect of aqueous extract of *Clematis brachiata* leaves on the organ- body weight ratios of male Wistar rats. (n = 6, x ± SD).

		Day 1		Day 7		Day 21	
Test samples	**Doses (mg/kg body weight)**	**Liver-body weight ratio (%)**	**Kidney-body weight ratio (%)**	**Liver-body weight ratio (%)**	**Kidney-body weight ratio (%)**	**Liver-body weight ratio (%)**	**Kidney-body weight ratio (%)**
Distilled water	0.5 ml	3.37 ± 0.68	0.72 ± 0.08	3.40 ± 0.29	0.70 ± 0.05	3.42 ± 0.19	0.69 ± 0.08
Aqueous extract	100	3.40 ± 0.70	0.68 ± 0.01	3.47 ± 0.20	0.65 ± 0.02	3.42 ± 0.32	0.65 ± 0.05
Aqueous extract	200	3.40 ± 0.54	0.70 ± 0.10	3.46 ± 0.17	0.64 ± 0.04	3.40 ± 0.30	0.67 ± 0.05
Aqueous extract	400	3.45 ± 0.43	0.69 ± 0.05	3.47 ± 0.17	0.67 ± 0.06	3.44 ± 0.12	0.69 ± 0.03

Test values are not significantly different (P > 0.05) from their respective control values.

Table 2. Effect of aqueous extract of *Clematis brachiata* leaves on the liver function indices of male Wistar rats. (n = 6, x ± SD).

Parameters	**Day 1**				**Day 7**				**Day 21**			
	Doses (mg/kg body weight)											
	Control	**100**	**200**	**400**	**Control**	**100**	**200**	**400**	**Control**	**100**	**200**	**400**
Total protein (g/L)	67.00 ± 1.73	64.33 ± 5.50	60.66 ± 8.52	66.00 ± 5.64	67.08 ± 0.94	63.66 ± 6.57	64.33 ± 6.08	66.66 ± 4.15	67.00 ± 1.03	67.00 ± 1.06	68.66 ± 1.57	66.00 ± 3.76
Albumin (mmol/L)	15.66 ± 0.81	17.33 ± 1.52*	18.0 ± 1.73*	19.66 ± 0.57*	15.71 ± 0.63	17.94 ± 0.00*	17.99 ± 0.40*	18.33 ± 0.17*	15.48 ± 0.14	19.33 ± 0.15*	18.66 ± 0.15*	19.00 ± 0.00*
Globulin (g)	47.34 ± 1.02	48.00 ± 0.16	46.66 ± 1.41	47.34 ± 0.83	47.40 ± 0.46	46.66 ± 0.44	47.33 ± 0.23	48.33 ± 0.21	47.34 ± 1.01	45.67 ± 3.11	46.00 ± 2.08	47.00 ± 0.86
Total bilirubin (µmol/L)	7.00 ± 0.28	7.16 ± 0.08	7.16 ± 0.12	7.26 ± 0.17	7.07 ± 0.13	7.00 ± 0.23	7.13 ± 0.09	7.09 ± 0.17	7.00 ± 0.30	5.00 ± 0.08*	10.00 ± 0.07*	11.66 ± 0.08*
Conjugated bilirubin (µmol/L)	1.33 ± 0.07	1.33 ± 0.05	1.33 ± 0.07	1.33 ± 0.07	1.34 ± 0.04	1.31 ± 0.08	1.33 ± 0.08	1.35 ± 0.04	1.33 ± 0.07	1.32 ± 0.06	1.29 ± 0.09	1.33 ± 0.07
Alkaline phosphatase (U/L)	315.01 ± 12.23	361.33 ± 9.88*	371.33 ± 7.76*	364.33 ± 9.92	314.20 ± 13.71	398.0 ± 7.44*	394.33 ± 7.61*	342.66 ± 5.73*	313.66 ± 16.96	335.33 ± 8.03*	411.66 ± 19.50*	367.00 ± 8.30*
Gamma glutamyl transferase(U/L)	8.28 ± 0.18	8.00 ± 0.43	8.26 ± 0.15	8.33 ± 0.07	8.30 ± 0.06	8.30 ± 0.02	8.35 ± 0.03	8.22 ± 0.15	8.33 ± 0.08	11.66 ± 0.18*	12.33 ± 0.57*	14.66 ± 0.57*
Alanine aminotransaminase (U/L)	51.00 ± 5.88	52.33 ± 5.29	51.66 ± 6.02	49.00 ± 9.58	51.0 ± 8.88	51.66 ± 6.50	53.66 ± 6.50	50.33 ± 7.71	51.0 ± 8.88	49.00 ± 8.88	51.33 ± 8.11	53.00 ± 8.00
Asparate aminotransaminase (U/L)	168.13 ± 9.13	188.66 ± 7.26*	192.33 ± 6.89*	184.00 ± 4.28*	165.77 ± 11.86	195.0 ± 3.64*	185.00 ± 4.58*	186.33 ± 3.61*	168.13 ± 9.13	182.33 ± 4.09*	197.00 ± 7.21*	195.66 ± 3.08*

Test values carrying asterisks for each parameter and day are significantly different from their controls (p < 0.05) while those without asterisk are not significantly different (P > 0.05).

ficantly altered by the extract throughout the same period (Table 2). The extract did not significantly alter the sodium, potassium, chloride, inorganic phosphorus and calcium ions of the animals (Table 3). Again, the increase in creatinine levels did not manifest until after seven daily doses. The extract at all the doses investigated increased urea levels of the animals throughout the experimental period. In contrast, magnesium ions were reduced only at 200

Table 3. Effect of aqueous extract of *Clematis brachiata* leaves on the kidney function indices of male Wistar rats. (n = 6, x ± SD).

Parameters	Day 1				Day 7				Day 21			
	Doses (mg/kg body weight)											
	Control	100	200	400	Control	100	200	400	Control	100	200	400
Sodium (mmol/L)	140.00 ± 1.00	140.33 ± 1.08	139.33 ± 2.08	138.66 ± 4.57	140 ± 1.00	139.66 ± 1.15	140.00 ± 0.00	139.00 ± 1.00	140.00 ± 1.00	137.00 ± 4.00	139.33 ± 0.57	138.66 ± 1.52
Potassium (mmol/L)	5.50 ± 0.26	5.10 ± 0.15	5.40 ± 0.16	5.50 ± 0.26	5.50 ± 0.26	5.60 ± 0.40	5.40 ± 0.36	5.53 ± 0.32	5.50 ± 0.26	5.36 ± 0.45	5.43 ± 0.15	5.36 ± 0.35
Calcium (mmol/L)	2.52 ± 0.05	2.46 ± 0.05	2.45 ± 0.03	2.43 ± 0.01	2.52 ± 0.05	2.48 ± 0.05	2.43 ± 0.54	2.49 ± 0.04	2.52 ± 0.05	2.48 ± 0.04	2.47 ±0.04	2.47 ± 0.09
Magnesium (mmol/L)	1.24± 0.01	1.23 ± 0.03	0.93 ± 0.02*	0.90 ± 0.06*	1.24± 0.01	1.21 ± 0.02	0.81 ± 0.06*	0.93 ± 0.04*	1.24± 0.03	1.20 ± 0.04	0.74 ± 0.02*	0.76 ± 0.02*
Chloride (mmol/L)	103.00 ± 2.64	104.00 ± 1.73	105.33 ± 1.08	104.00 ± 2.00	103.20 ± 1.75	102.66 ± 4.57	105.66 ± 2.51	104.66 ± 1.52	103.00 ± 2.64	103.66 ± 2.08	102.33 ± 1.52	104.66 ± 0.57
Inorganic Phosphorus (mmol/L)	3.20 ± 0.19	3.20± 0.17	3.03 ± 0.35	2.90 ± 0.46	3.20 ± 0.10	3.33 ± 0.06	2.96 ± 0.40	2.97 ± 0.37	3.20 ± 0.15	3.35 ± 0.04	3.10 ± 0.30	3.00 ± 0.37
Urea (mmol/L)	4.50 ± 0.37	6.13 ± 0.25*	6.36 ± 0.15*	6.60 ± 0.16*	4.53 ± 0.27	6.10 ± 0.10*	5.43 ± 0.10*	5.60 ± 0.19*	4.40 ± 0.24	6.80 ± 0.30*	6.83 ± 0.20*	7.33 ± 0.40*
Creatinine (mmol/L)	44.33 ± 5.77	40.33 ± 9.07	42.66 ± 4.04	46.66 ± 5.50	44.33 ± 5.77	42.00 ± 6.46	45.66 ± 3.78	45.33 ± 4.04	44.33 ± 5.77	52.00 ± 0.00*	51.66 ± 2.08*	58.00 ± 1.60*
Uric acid (mmol/L)	0.11 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.07 ± 0.01*	0.07 ± 0.00*	0.08 ± 0.00*	0.11 ± 0.00	0.08 ± 0.00*	0.07 ± 0.00*	0.07 ± 0.00*

Test values carrying asterisks for each parameter and day are significantly different from their controls ($p < 0.05$) while those without asterisk are not significantly different ($P > 0.05$).

and 400 mg/kg body weight of the extract. The reduction in serum uric acid became manifest only after seven daily doses and was sustained throughout the exposure period.

Administration of the extract at all the doses for seven days significantly increased ($P < 0.05$) the serum cholesterol concentration of the animals (Table 4). The increase washowever not sustained beyond this point as the cholesterol values of the extract treated animals compared well with the control values. The increase in triacylglycerol content of the animals by single administration of the extract was not sustained beyond the first day as the concentration of the lipid was reduced there-after by the extract. The HDL-C increased throughout the exposure period, whereas the LDL-C levels remained significantly unaltered. In contrast, the extract reduced the computed atherogenic index (Table 4).

Although, the extract did not significantly alter the levels of RBC, Hb, PCV, MCV, MCH, MCHC and RCDW, the levels of LUC, monocytes, eosinophils and basophils increased significantly throughout the exposure period (Table 5). Again, the neutrophils also increased throughout the experimental period except in the animals administered with the single dose of 100 mg/kg body weight. In contrast, the extract reduced the WBC, platelet and lym-phocytes right from the commencement of the administration and was sustained throughout the experimental period (Table 5).

DISCUSSION

The assessment of biochemical parameters such as hematological, liver and kidney function as well as lipid profile of animals following the administration of chemical compounds, including plant extract, plays significant role in the evaluation of toxicity risk or safety of such compounds (Maaroufi et al., 1996; Amresh et al., 2008). Biochemical indices of organ function if altered will impair the normal functioning of the organs (Afolayan and Yakubu, 2009). Organ-body weight ratio can be used to indicate organ swelling, atrophy or hypertophy (Amresh et al., 2008).

Therefore, the lack of an effect on the liver and kidney body weight ratio in this study suggests that the extract did not cause inflammation or constriction at the cellular levels of the rat organs. This agrees with the findings of Amresh et al. (2008) where *Cissampelos pareira* did not produce any

Table 4. Effect of aqueous extract of *Clematis brachiata* leaves on the serum lipid profile of Wistar rats. n = 6, x ± SD.

	Day 1				Day 7				Day 21			
	Doses (mg/kg body weight)											
Parameters	**Control**	**100**	**200**	**400**	**Control**	**100**	**200**	**400**	**Control**	**100**	**200**	**400**
Cholesterol (mmol/L)	1.23 ± 0.15	1.80 ± 0.20*	1.56 ± 0.08*	1.66 ± 0.05*	1.20 ± 0.15	1.43 ± 0.07*	1.43 ± 0.05*	1.53 ± 0.05*	1.23 ± 0.15	1.26 ±0.15	1.23 ± 0.11	1.20 ± 0.10
Triacylglycerol (mmol/L)	1.06 ± 0.04	1.30 ± 0.05*	1.80 ± 0.12*	1.43 ± 0.09*	1.00 ± 0.06	0.76 ± 0.05*	0.80 ± 0.00*	0.66 ± 0.04*	1.01 ± 0.03	0.73 ± 0.03*	0.80 ± 0.03*	0.68 ± 0.08*
High-density lipoprotein (mmol/L)	0.83 ± 0.05	1.43 ± 0.04*	1.20 ± 0.03*	1.20 ± 0.06*	0.84 ± 0.05	1.30 ± 0.07*	1.30 ± 0.00*	1.17 ± 0.06*	0.80 ± 0.05	1.20 ± 0.07*	1.20 ± 0.03*	1.10 ± 0.06*
Low-density lipoprotein (mmol/L)	0.51 ± 0.05	0.53 ± 0.02	0.52 ± 0.06	0.50 ± 0.08	0.51 ± 0.06	0.50 ± 0.03	0.53 ± 0.02	0.51 ± 0.06	0.52 ± 0.03	0.52 ± 0.06	0.51 ± 0.07	0.53 ± 0.04
Atherogenic index (LDL-C/HDL-C)	0.61 ± 0.04	0.37 ± 0.06*	0.43 ± 0.02*	0.42 ± 0.02*	0.61 ± 0.07	0.38 ± 0.04*	0.41 ± 0.07*	0.51 ± 0.02*	0.65 ± 0.05	0.43 ± 0.03*	0.43 ± 0.05*	0.48 ± 0.01*

Test values carrying asterisks for each parameter and day are significantly different from their controls ($p < 0.05$) while those without asterisk are not significantly different ($P > 0.05$).

Table 5. Effect of the aqueous extract of *Clematis brachiata* leaves on the haematological parameters of male Wistar rats. (n = 6, x ± SD).

Parameters	Day 1				Day 7				Day 21			
	Doses (mg/kg body weight)											
	Control	**100**	**200**	**400**	**Control**	**100**	**200**	**400**	**Control**	**100**	**200**	**400**
WBC (x 10^9/l)	11.80 ± 0.05	8.13± 0.12*	6.89 ± 0.06*	8.60 ± 0.10*	11.72 ± 0.10	7.55 ± 0.06*	7.35 ± 0.07*	9.14 ± 0.01*	11.58 ± 0.10	8.18 ± 0.06*	7.25 ± 0.05*	7.50 ± 0.03*
RBC (x 10^{12}/l)	7.50 ± 0.31	7.54 ± 0.18	7.68 ± 0.04	7.47 ± 0.29	7.57 ± 0.26	7.51 ± 0.27	7.38 ± 0.37	7.48 ± 0.28	7.50 ± 0.31	7.55 ± 0.20	7.41 ± 0.29	7.48 ± 0.22
Hb (g/dl)	13.83 ± 0.35	13.43 ± 0.70	13.5 ± 0.81	13.73 ± 0.47	13.83 ± 0.35	14.26 ± 0.41	13.9 ± 0.43	14.56± 0.45	13.83 ± 0.35	13.76 ± 0.46	14.33 ± 0.49	14.66± 1.15
PCV (l/l)	0.47 ± 0.005	0.45 ± 0.03	0.47 ± 0.02	0.47 ± 0.02	0.47 ± 0.005	0.49 ± 0.01	0.47 ± 0.01	0.48 ± 0.005	0.47 ± 0.01	0.46 ± 0.01	0.47 ± 0.02	0.48 ± 0.02
MCV (fl)	63.23 ± 2.98	63.30 ± 3.13	62.50 ± 2.04	62.73 ± 0.66	63.23 ± 2.98	63.36 ± 1.02	62.13 ± 1.33	61.46 ± 3.40	63.23 ± 2.98	63.60 ± 0.87	64.63 ± 0.40	62.90 ± 1.86
MCH (pg)	18.46 ± 1.10	18.86 ± 1.13	17.6 ± 0.43	18.36 ± 0.15	18.46 ± 1.10	18.5 ± 0.43	18.8 ± 0.43	18.46 ± 0.25	18.46 ± 1.10	19.06 ± 0.23	19.06 ± 1.2	18.56 ± 1.00
MCHC (g/dL)	29.20 ± 0.36	29.90 ± 0.43	28.60 ± 0.75	29.26 ± 0.40	29.20 ± 0.36	29.13 ± 0.28	29.30 ± 0.10	30.03 ± 0.17	29.20 ± 0.36	29.96 ± 0.27	30.03 ± 0.41	29.46 ± 0.43
RCDW (%)	14.03 ± 1.30	14.09 ± 1.01	14.03 ± 0.95	14.03 ± 1.22	14.03 ± 1.30	14.56 ± 0.76	14.26 ± 1.00	14.23 ± 0.80	14.03 ± 1.30	14.96 ± 0.58	14.76 ± 0.77	14.33 ± 0.45
Platelet (x 10^9/l)	829.33± 15.73	446.66 ± 4.77*	702.66 ± 5.61*	706.00 ± 4.34*	827.45± 15.73	709.00 ± 5.86*	673.66 ± 8.14*	729.00 ± 7.09*	836.25± 8.73	659.66 ± 8.85*	683.66 ± 7.79*	722.00 ± 4.53*

Table 5 Contd.

Neutrophils (%)	9.26 ± 0.50	10.86 ± 0.05	15.40± 0.13*	13.36 ± 0.06*	9.26 ± 0.44	11.9 ± 0.09 *	13.33 ± 0.04*	12.13 ± 0.07*	9.26 ± 0.42	16.86 ± 0.18*	14.30 ± 0.18*	18.53 ± 0.15*
Monocytes (%)	14.36 ± 0.76	20.10 ± 1.67*	29.2 ± 1.32*	25.73 ± 1.80*	14.30 ± 0.69	22.76 ± 1.40*	26.33 ± 1.80*	20.20± 1.69*	14.46 ± 0.46	21.33 ± 2.60*	20.00 ± 1.42*	19.86 ± 1.15*
Lymphocytes (%)	66.43 ± 2.41	50.63 ± 1.42*	48.4 ± 2.20*	45.5 ± 2.21*	63.43 ± 4.41	50.56 ± 3.51*	47.03 ± 2.33*	48.16 ± 1.17*	66.61 ± 1.41	52.0 ± 0.50*	47.13 ± 1.81*	48.26 ± 1.35*
LUC (%)	7.03 ± 0.26	10.96 ± 0.14*	14.40 ± 0.22*	12.43 ± 0.14*	7.05 ± 0.24	12.00 ± 0.09*	10.86 ± 0.07*	11.13 ± 0.11*	7.07 ± 0.21	11.76 ± 0.28*	10.76± 0.05*	10.10 ± 0.10*
Eosinophils (%)	0.60 ± 0.03	2.56 ± 0.08*	1.83 ± 0.03*	2.63 ± 0.07*	0.63 ± 0.03	2.33 ± 0.06*	1.53 ± 0.05*	1.76 ± 0.25*	0.61± 0.02	1.56 ± 0.15*	2.40 ± 0.02*	2.76 ± 0.08*
Basophils (%)	0.26 ± 0.02	0.90 ± 0.03*	0.36 ± 0.02*	0.36 ± 0.00*	0.22 ± 0.04	0.46 ± 0.03*	0.86 ± 0.08*	0.70± 0.02*	0.25 ± 0.02	0.43 ± 0.05*	0.40 ± 0.07*	0.46 ± 0.02*

Test values carrying asterisks for each parameter and day is significantly different from their controls ($p < 0.05$) while those without asterisk are not significantly different ($P > 0.05$).

effect on the organ-body weight ratio of the animals. Albumin, total bilirubin, globulin and the totality of the molecules can be used to assess the secretory ability of the liver (Yakubu et al., 2003). The extract exhibited selective toxicity on the liver functional endpoints since only albumin and total bilirubin were affected whereas total protein, conjugated bilirubin and globulin were not altered significantly. Bilirubin is an important catabolic product of blood with biological and diagnostic values (Chowdhury et al., 1989). The alterations in the extract in the levels of total bilirubin prior to the end of experimental period implies that adverse effect have occurred on the normal functioning of the liver. The elevated levels of serum albumin also suggest adverse effect of the extract on the secretory ability of the liver and hence the normal functioning of the organ.

There are many enzymes found in the serum that did not originate from the extracellular fluid. During tissue damage, some of these enzymes find their way into the serum through leakage arising from altered membrane permeability (Wills, 1985). Serum enzyme measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. ALP and GGT are ectoenzymes of the plasma membrane where-as ALT and AST are of the cytosol (Shahjahan et al., 2004). Damage to structural integrity of tissues is always reflected by increase in some of these enzymes in the serum. Therefore, the increase in serum ALP and GGT activity in this study could possibly imply damage to the plasma membrane. This was however supported by similar increase in the serum AST. The lack of an effect on the activity of serum ALT implied selective toxicity of the extract on the cytosolic enzyme. Alteration in the level of ALP, GGT and AST will have consequential effect on the adequate transportation of required ions across the cell membrane, glutathione metabolism as well as metabolism and regulation of amino acids respectively.

Renal function indices such as serum electrolytes, urea, creatinine and uric acid can be used to evaluate the functional capacity of the nephrons of animals (Yakubu et al., 2003). In this study, the levels of sodium, potassium, chloride, inorganic phosphorus and calcium ions were not signifycantly altered. This may suggest selective and dose specific effect of the extract of *C. brachiata* leaves. Creatinine, urea and uric acid are major catabolic products of muscle, protein and purine metabolism respectively. The increase in the serum creatinine and urea contents of the animals may be attributed to compromising the renal functional capacity. The extract might have interfered with creatinine and urea metabolism leading to increased synthesis or the tissue might have compromised all or part of its functional capacity in tubular and glomerular excretion (Zilva et al., 1991). Similarly, the reduction in the serum urea content of the animals suggests abnormality in the physiological excretion of urea.

Alterations in the concentration of major lipids of animals such as cholesterol, high and low-density lipoprotein cholesterols, and triglycerides can give useful information on the lipid metabolism as well as predisposition of the animals to cardiovascular risk (Yakubu et al., 2008). The elevated level of serum cholesterol following the administration of the extract may be attributed to increase in the concentration of acetyl CoA arising probably from enhanced -oxidation of fatty acid (Rang et al., 1995). High blood cholesterol concentration is one

one of the important risk factors of cardiovascular disease (Treasure et al., 1995). In this study, the levels of serum cholesterol which compared well with the control by the end of the experimental period suggests that the extract may not pose threat to the well being of the animals notwithstanding the initial increase. Similarly, the reduction in the levels of triacylglycerol may be adduced to reduced lipolysis. HDL-C is considered to have anti-atherogenic properties. Therefore, increase in HDL-C may be clinically beneficial to the animals since it will reduce cardiovascular risk. The lack of an effect on the serum LDL-C of the animals further buttress the selective effect of the extract. It has been reported that cardio-vascular diseased patients had markedly elevated levels of triacylglycerol but reduced HDL-C. Since the elevation in triacylglycerol in this study was not accompanied by reduction in HDL-C, it is unlikely that the extract may potentate cardiovascular risk. In addition, the reduction in the computed atherogenic index also lends support to the non-cardiovascular risk of the extract.

The lack of an effect on the RBC suggests that the extract did not alter the balance between the rate of production and destruction of the blood corpuscles. Since MCHC, MCH and MCV relate to individual red blood cells while Hb, RBC, PCV and RCDW are associated with total population of red blood cells, the lack of an effect on these indices implies that neither the incorporation of haemoglobin into red blood cells nor the morphology and osmotic fragility of the red blood cells was affected (Adebayo et al., 2005). Therefore, it is unlikely that the extract will affect the oxygen-carrying capacity of each of the RBC as well as the total population.

Interestingly, WBC and indices relating to it were significantly altered during the experimental period. The decrease in WBC may be due to impairment in the rate of entrance of the haematological parameter into the blood from the bone marrow and an enhanced rate of removal from circulation. The elevated level of neutrophils may stimulate phagocytosis in the animals. In contrast, reduction in lymphocytes observed in this study suggests adverse effect on the effector cells of the immune system. Since monocytes, eosinophils and basophils are necessary for normal functioning of the immune system, the alterations in these blood indices has suggested challenges on the immune system. Platelets when pre-sent in sufficient size, number and function are involved in the process of normal coagulation of the blood (William and Levine, 1982). Therefore, the reduction in platelet levels by the extract may be attributed to diminished effect on thrombopoietin (Li et al., 1999). The altered levels of WBC and factors relating to it by the extract, accompanied with lack of effect on RBC and factors relating to it suggest selective and localized stimulatory effect on the bone marrow. This may be an indication of localized systemic toxicity of the extract which may adversely affect the normal functioning of the WBC and its related indices. This agreed with the findings of Adebayo et al. (2005) on ethanolic extract of *Bougain-villea spectabilis* leaves.

Our present findings have shown that aqueous extract of *C. brachiata* is not completely 'safe' at the doses investigated. While it appears to be clinically beneficial to the animals as it may not predispose to cardiovascular risk, it has the tendency to selectively affect the normal functioning of the liver and kidney of the animals. In addition, the heamatotoxic effect of the extract may be localized to the WBC and factors relating to it.

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