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Original Research Article

Toxicity assessment of aqueous extract of *Curtisia dentata* (Burm.f) C.A. Sm: stem bark in male Wistar rats

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Abstract

Purpose: *To investigate the acute and sub-acute toxicity effects of aqueous stem bark extract in male Wistar rats.*
Methods: *For the acute toxicity study, a single dose of 5000 mg/kg body weight of the extract was orally administered to the animals by oral gavage and the rats thereafter were observed for mortality and toxicity signs for 14 days. In the sub-acute toxicity test, the graded doses (50, 100, and 200 mg/kg body weight) of the aqueous extract of CD were given to the animals once daily for 28 days. In each of the experiment, the food and water intake, body weight changes, relative organ weights, hematological, clinical biochemistry and histopathological parameters were evaluated.*
Results: *In both the acute and sub-acute toxicity studies, CD did not show any visible signs of toxicity. There were also no significant differences ($p > 0.05$) between the control and CD-treated rats for all the investigated parameters; no obvious gross pathological features in the kidney, heart and liver of all the experimental animals were observed.*
Conclusion: *The findings indicate that the extract is not toxic when administered at the tested doses and within the exposure period. Thus, the aqueous stem bark extract of CD may be adjudged relatively safe and pharmacologically non-toxic in Wistar rats.*

Keywords: *Curtisia dentata, Hematopoietic, Histopathology, Toxicity*

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INTRODUCTION

Curtisia dentata is a common South African plant belonging to the family Cornaceae. It is known as "Assegaai" by the Afrikaans speaking people while the Xhosa and the Zulu people of South Africa call it "umLahleni". The plant is disseminated in the forests of KwaZulu-Natal down to the Western Cape via the Eastern Cape and up the Limpopo Provinces of South Africa [1]. It is commonly found along the forest margins, on mountains and grassland of southern Africa. *Curtisia dentata* is geographically endemic to southern and eastern coasts of South Africa; extending to Zimbabwe, Mozambique and Swaziland [2,3]. *Curtisia dentata* is one of the most threatened plants of South Africa, as a result of the high trading of the stembark and leaves [4]. Triterpene compounds (2-α- hydroxyursolic acid and lupeol as well as

ursolic acid and betulinic acid) have been isolated from the plant, and these have shown great pharmacological importance [1,3]. The antimicrobial properties of these isolated compounds have been reported [3]. Previous reports indicated that the plant is potent against some pathogenic microbes and free living nematodes [3].The stem bark of CD has also been shown to possess antimicrobial activity and relieving stomach ailments and diarrhea. Its significant therapeutic effects in sexually transmitted infections, as purgative, blood purifier and aphrodisiac have been reported [5-7].The stem bark infusion extract of *C. dentata* has found relevance in livestock for the management of the dreaded heartwater disease of cattle in South Africa [8] and also, it is used in the treatment of pimples in humans, [8,9]. Other pharmacological significance of the stem bark extracts of CD as antioxidant, anti-hyperlipidemic and cytotoxic agent have also been documented [3,10,11].

In spite of the increasing number of reports on the medicinal benefits of CD stem bark, the *in vivo* toxicological implications of treatment with its extracts is yet to be elucidated. It is on this background, therefore, that the present study was conceptualized to investigate the acute and sub-acute toxicity of the stem bark aqueous extract of CD in a Wistar rat model. This study is envisaged to provide significant baseline information for further studies on developing this plant as a viable herbal medicine.

EXPERIMENTAL

Plant material collection, identification and sample extraction

Mature stem barks of *Curtisia dentata* were collected between May and June, 2016 at Ntselamanzi area, Eastern Cape Province, South Africa. The plant was authenticated by Prof DS Grierson, a taxonomist at University of Fort Hare, Alice, South Africa. A voucher specimen was formally prepared and deposited at Giffen's herbarium (no. Win 2014/2). The mature stembark was washed with copious amounts of distilled water to remove dirt and subsequently oven-dried (40 °C) for one week. The stem barks were turned daily to allow for uniform drying. After drying, the sample was pulverised and extracted in sterile distilled water and agitated in a, shaker (Orbital Incubator Shaker, Gallenkamp) for 24 h. The infusion obtained was filtered (Whatman No. 1 filter paper) and the resulting filtrate was freeze-dried (Vir Tis benchtop K, Vir Tis Co., Gardiner, NY) at −40 °C. The crude extract (CDE) obtained was kept air-tight and refrigerated prior to use.

Experimental animals

Thirty-six (36) male Wistar rats weighing 100.0 ± 14.5 g were obtained from the central animal unit of the University of Fort Hare, South Africa. The rats were housed in clean polypropylene cages in a well-ventilated room. They were fed *ad libitum* with rat pellets and tap water freed of contaminants. The animals were allowed to acclimatize for 7 days before the start of the experiment. The rats were maintained at 22 ± 2 ^{o}C on a light/dark cycle for 12 h and 40 – 45 % relative humidity. The cages were cleaned on a daily basis and treatments were in accordance with the guidelines of the Ethics Committee on the use and care of Experimental Animals of the University of Fort Hare, Alice, South Africa. The research also adhered strictly to the Principles contained in the United State National Institutes of Health Laboratory Animal Care (NIH Publication, No. 85–23) [12]. The study was approved by the institutional ethical committee 'of the University of Fort Hare (no. UFH-REC-270710-028-RA WIN001) prior to commence-ment of the study.

Acute toxicity study

This was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines number 420 [13]. Prior to dosing with the extract, the rats were fasted overnight but allowed free access to water. A single dose of 5000 mg/kg body weight (b.w.) of CDE was orally administered to 6 rats and designated as the treatment group. The rats in the control group (n=6) were given sterile distilled water. All the experimental animals were maintained under close observation for any signs of toxicity and mortality immediately after dosing. They were also observed individually during the first 30 min and 4 h post-dosing period and thereafter 24 h for a period of 14 days. On the 15th day, all rats were anaesthetized using halothane and blood samples were collected via cardiac puncture into heparinized and EDTA-containing bottles and subsequently used for biochemical and hematological analysis, respectively using BS-200 Automatic Biochemistry Analyzer (Mindary Co., Ltd) and Automated Hematology System Analyzer (Archem BM240, Turkey). The animals were thereafter humanely sacrificed by cervical dislocation, dissected and the liver, kidney and heart were removed. The organs were cleaned with saline, weighed and preserved in 10% buffered formalin for histopathological

examination [14]. The relative organ weight (ROW) of each organ for each animal was also calculated.

Sub-acute toxicity test

The sub-acute toxicity testing was conducted according to OECD guidelines 407 for testing plant extracts [15]. The 24 rats used were randomized into four groups of 6 rats each. Animals in groups 1-3 were, respectively, administered 1 mL each of CDE at 50, 100 and 200 mg/kg day, while group 4 was given 1 mL distilled water and served as control. The administrations were done only once daily via oral intubation and lasted for 28 days. Probable signs of toxicity, morbidity, and mortality were monitored throughout the investigation period. The feed and water intakes were monitored daily and the average calculated at every 4-day interval, while their body weights were measured at the initial and on weekly basis throughout the period of 28 days [16]. At the end of the experiment, all the rats were anesthetized using halothane and blood samples were similarly collected and processed as detailed in the acute toxicity study. Following this, the rats were humanely sacrificed by cervical dislocation, dissected and the organs harvested and processed as earlier highlighted.

Data analysis

Data are expressed as mean ± standard error of mean (SEM, n = 6) and were subjected to one-way analysis of variance (ANOVA) followed by Duncan multiple range test to determine significant difference for all the parameters. Values were considered statistically significant at $p < 0.05$.

RESULTS

There were no signs of toxicity or mortality observed in both the acute and sub-acute toxicity testing in this study. The cage side observation revealed that all the animals were essentially normal in healthy condition, maintained normal behaviors and usual movement patterns throughout the investigation periods. There were no significant ($p > 0.05$) differences in both the body weight changes and the ROW between the control and CDE-administered rats (Table 1 and Table 2).

Similarly, the histopathological examination of the investigated organs did not show any differences in their histoarchitectural features when compared with the control in both the acute

Table 1: Weight gain and relative organ weights (g/100 g body weight) of rats administered 5000 mg/kg b.w. C*urtisia dentata* stem bark aqueous extract

Parameter	Initial body weight (g)	Final body weight (g)	Weight of liver (g)	Weight of kidney (g)	Weight of heart (g)	Relative liver weight	Relative kidney weight	Relativ heart weight
5000 mg/kg extract dose	231.44±3.76	308.52±2.74	8.69±0.30	1.90±0.54	1.12±0.04	2.82±0.05	0.62±0.18	0.36±0.02
Control (distilled water)	135.33±2.72	214.2±2.03	8.53±0.99	1.94±0.14	1.06±0.06	2.90±0.30	0.66±0.05	0.36±0.02

Mean ± SEM. Values without superscripts in the same column for each parameter are not significantly different (p>0.05)

Table 2: Weight gain and relative organ weights of rats administered C*urtisia dentata* aqueous extract for 28 days

Parameter	50 mg/kg	100 mg/kg	200 mg/kg	Control (distilled water)
Initial body weight (g)	95.77±5.74	94.58±4.27	86.00±8.50	94.12±1.42
Final body weight (g)	252.32±4.05	251.00±25.86	246.71±14.88	253.10±10.21
Weight of liver (g)	10.01±1.79	9.07±1.56	9.13±0.52	9.11±0.45
Weight of kidney (g)	2.22±0.04	2.27±0.27	2.25±0.01	2.22±0.07
Weight of heart (g)	1.01±0.08	0.94±0.07	1.04±0.05	0.96±0.05
Liver body weight (%)	3.61±0.67	3.60±0.35	3.65±0.04	3.64±0.10
Kidney body weight (%)	0.83±0.03	0.83±0.06	0.78±0.04	0.78±0.21
Heart body weight (%)	0.36±0.01	0.38±0.02	0.36±0.01	0.35±0.13

Mean ± SEM. Values without superscripts in the same row for each parameter are not significantly different (p>0.05)

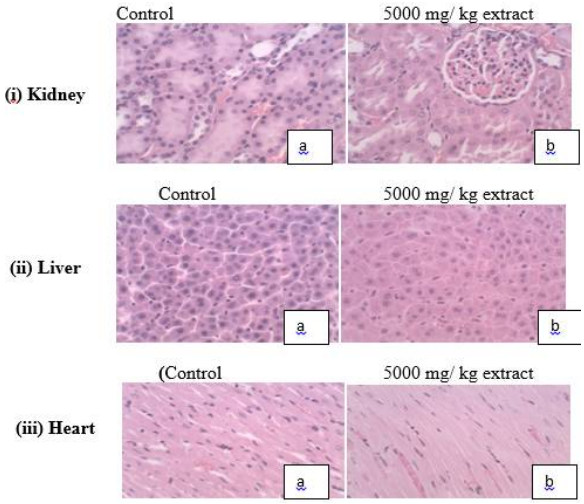

Figure 1: Photomicrographs (400×, Hematoxylin + eosin stained) of tissue sections of the (i) kidney, (ii) liver and (iii) heart of control (a) and (b) 5000 mg/kg body weight aqueous stem bark extract of *C. dentata* treated male rats for 14 days showing well preserved histoarchitectural features of the respective organs

and sub-acute toxicity studies (Figure 1 and Figure 2). In the acute toxicity testing, the liver showed normal architecture, clear lumen of central vein and no evidence of lesion; the kidney showed adequate glomeruli and normal tubules, with no traces of focal intestinal edema and lesion; the heart showed normal architecture of the myocardium (Figure 1). Similar observations were noticed in the sub-acute toxicity study where the liver, kidney and heart of the CDE-treated rats did not show any differences when compared with the control (Figure 2). The data obtained with respect to the amount of food and water consumed also showed no statistical significant ($p > 0.05$) differences between the CDE-treated animals and the control rats in the sub-acute toxicity testing (Figure 3 and Figure 4).

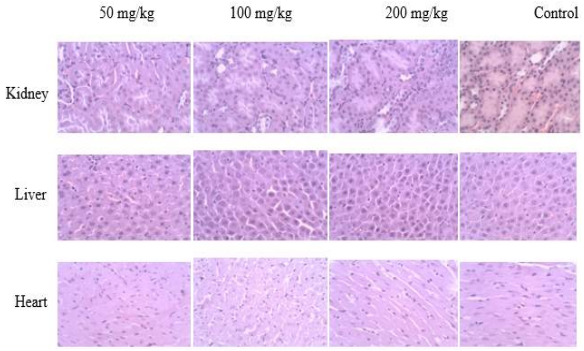

Figure 2: Photomicrographs (400×, Hematoxylin + eosin stained) from the kidney, liver and heart of the 28-day CDE-treated rats and control. There are no treatment-related microscopic changes in all the organs CDE-administered rats compared to the control rats

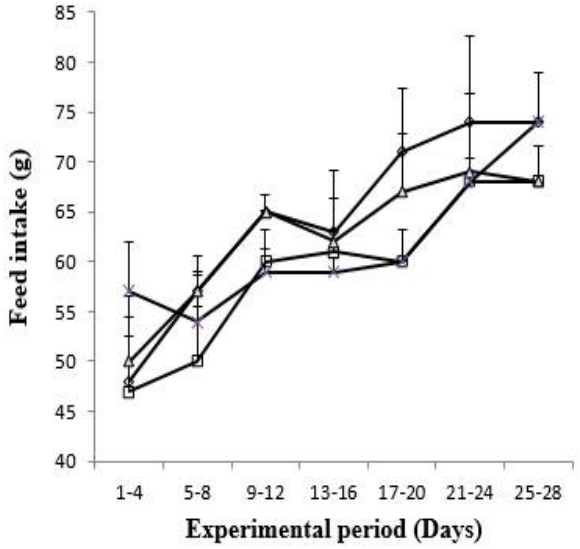

Figure 3: Effect of aqueous stem bark extract of *C. dentata* on feed intake of the animals over 28 days; 50 mg/kg (◊), 100 mg/kg (□), 200 mg/kg (Δ) and control (×)

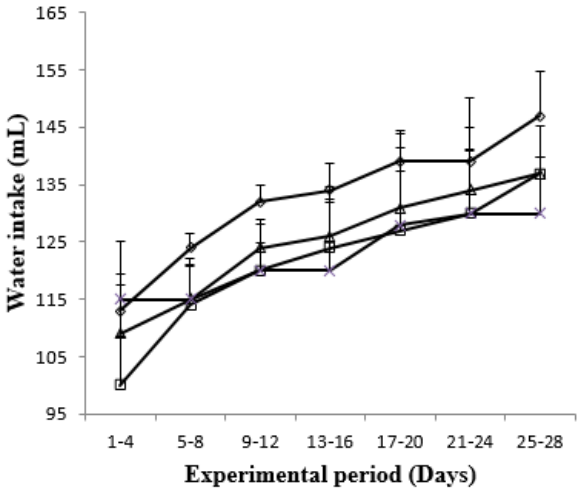

Figure 4: Effect of aqueous stem bark extract of *C. dentata* on water intake of the animals over 28 days; 50 mg/kg (◊), 100 mg/kg (□), 200 mg/kg (Δ) and control (×)

Table 3 to Table 6 show the results of the hematological and clinical biochemistry parameters of the control and extract-treated animals in both the acute and sub-acute toxicity studies, respectively. There were no significant ($p > 0.05$) differences in these parameters for the CDE-administered rats and the respective control in the two experimental protocols.

DISCUSSION

Phytomedicine has become universally acceptable in primary health care, particularly in developing countries like South Africa. Although, medicinal plants are presumed to be safe without

Table 3: Effect of a single dose (5000 mg/kg body weight) administration of stem bark aqueous extract of *Curtisia dentata* on haematological parameters of male Wistar rats

Parameter	5000 mg/kg extract	Control (distilled water)
Hemoglobin (g/dL)	16.15±0.35	16.45±0.35
Haematocrit (L/L)	0.55±0.02	0.54±0.00
Mean corpuscular volume(fl)	64.65±1.65	61.5±2.12
Mean corpuscular hemoglobin(pg)	18.85±0.15	18.80±0.00
MCHC (g/dL)	32.2±0.50	33.00±1.41
Red blood cell distribution width (%)	11.1±0.20	11.5±0.99
Red blood cell (10^{12}/L)	8.54±0.11	8.48±0.30
Platelets (10^9/L)	778±2.69	769±2.43
White blood cell (10^9/L)	9.15±1.05	9.08±1.56
Neutrophils(10^9/L)	0.44±0.10	0.44±0.09
Monocytes (10^9/L)	2.03±0.40	2.08±0.01
Eosinophils(10^9/L)	0.15±0.20	0.18±0.01
Lymphocytes (10^9/L)	4.61±1.25	4.51±1.62

Data are mean ±SEM. Values without superscripts in the same row for each parameter are not significantly different (p > 0.05). MCHC= mean corpuscular haemoglobin concentration

Table 4: Effect of a single dose (5000 mg/kg body weight) administration of stem bark aqueous extract of *Curtisia dentata* on biochemical parameters of male Wistar rats

Parameter	5000 mg/kg extract	Control
Sodium (mmol/L)	140.50±1.29	140.25±1.89
Chloride (mmol/L)	104.00±2.16	104±0.82
Calcium (mmol/L)	2.37±0.03	2.39±0.03
Magnesium (mmol/L)	1.00±0.02	1.10±0.10
Uric acid (mmol/L)	0.09±0.01	0.11±0.01
Urea (mmol/L)	4.23±0.40	4.13±0.30
Creatinine (umol/L)	40.25±5.44	41.25±5.56
Total protein (g/L)	52.25±2.06	51.00±1.83
Albumin (g/L)	18.25±0.50	17.50±0.58
Total bilirubin (umol/L)	9.00±1.15	9.25±2.06
Conjugated bilirubin (umol/L)	3.50±1.29	3.50±0.96
Alkaline phosphatase(µ/L)	180.50±1.31	183.33±1.01
Alanine aminotransferase (µ/L)	50.50±1.24	52.00±1.83
Aspartate aminotransferase (µ/L)	168.00±3.57	167.50±3.66

Data are mean ± SEM. Values without superscripts in the same row for each parameter are not significantly different (p > 0.05)

any compromising health effects Oladipupo *et al* [17], evaluation of their toxicological implications on key metabolic markers of animals and humans is imperative. Such evaluation will form a crucial part of their assessment for potential toxic effects. To the best of our knowledge, this is the first study reporting the safety profiles of CDE *in vivo*. In the acute toxicity study, a limit dose of 5000 mg/kg of CDE elicited neither signs of toxicity nor mortality during the 14 days observation period. This observation may indicate that its oral median lethal dose (LD_{50}) value is greater than 5000 mg/kgb.w. and could suggest that the extract is non-acutely toxic when orally administered in Wistar rats.

Furthermore, the treatment with a single limit dose of CDE also had no significant (deleterious) effect on the hematological, clinical biochemistry, histopathological and ROW parameters of the experimental rats. This is another probable reason buttressing the non-acute toxic effect of CDE. These observations are in agreement with earlier reports of Sabiu and Ashafa [18] and Afolayan *et al* [19]. The authors demonstrated the non-acute toxic potential of some crude plant extract on Wistar rats.

Consequent upon the no treatment-perturbed toxicological effects of CDE in the oral acute toxicity study, the 28-days repeated dosing evaluation was performed to provide detailed toxicological data on CDE. In this study, the repeated graded dosing (50, 100, and 200 mg/kg) of the animals with the extract also had no clinical signs of toxicity and recorded neither morbidity nor mortality across all the treatment groups and could suggest that CDE is not likely to be toxic at these regimens and within the exposure period.

Changes in the body weight of animals could provide vital clue on their well-being and also constitute an important index in toxicological studies [20]. The proportionately increased body weight in both experimental protocols for the controls and the CDE-treated animals in this study could indicate that treatment with the extract had no consequential effect on their normal metabolic processes relating to growth and development. Similarly, the utilization of food and water by the treated animals followed a normal metabolic pattern as elicited by the animals in the control group.

This observation is not only a pointer to the non-toxic tendency of the extract but may also suggests that administration of CDE enhanced normal metabolism of the rats and did not retard their growth as evidently shown in this study.

Table 5: Effect of 28 days administration of stem bark aqueous extract of *Curtisia dentata* on the haematological parameters of male Wistar rats

Parameter	Dose			
	50 mg/kg	**100 mg/kg**	**200 mg/kg**	**Control**
Hemoglobin (g/dL)	14.50±0.20	14.80±0.25	14.60±0.56	14.83±0.42
Haematocrit (L/L)	0.49±0.02	0.51±0.02	0.50±0.02	0.51±0.02
Mean corpuscular volume (fl)	61.20±0.31	60.0±0.87	60.2±0.82	62.68±1.04
Mean corpuscular hemoglobin (pg)	19.50±0.20	20.13±0.31	19.60±0.1	19.25±0.37
MCHC (g/dL)	28.57±0.15	28.87±0.15	28.73±0.40	29.06±0.28
Red blood cell distribution width (%)	11.97±0.03	12.07±0.62	12.05±0.36	11.98±0.15
Red blood cell (10^{12}/L)	7.05±0.03	7.03±0.25	7.08±0.32	7.02±0.34
Platelet (10^9/L)	759±2.00	759±1.39	761.30±3.18	758.67±3.19
White blood cell (10^9/L)	5.82±0.01	5.95±0.63	5.85±0.59	5.74±0.91
Neutrophils(10^9/L)	0.56±0.02	0.54±0.07	0.52±0.61	0.54±0.13
Monocytes (10^9/L)	1.23±0.03	1.23±0.02	1.22±0.23	1.09±0.15
Eosinophils(10^9/L)	0.07±0.02	0.07±0.02	0.07±0.04	0.07±0.02
Basophils (10^9/L)	0.03±0.12	0.02±0.01	0.02±0.01	0.03±0.01
Lymphocytes	2.53±0.03	2.56±0.37	2.54±0.48	2.52±1.11

Data are mean ±SEM. Values without superscripts in the same row for each parameter are not significantly different (p > 0.05). MCHC= mean corpuscular haemoglobin concentration

Table 6: Effect of 28 days administration of stem bark aqueous extract of *Curtisia dentata* on biochemical parameters of male Wistar rats

Parameter	Dose			
	50 mg/kg	***100 mg/kg***	***200 mg/kg***	***Control***
Sodium (mmol/L)	143.00±2.00	145.67±8.50	145.70±0.88	147.80±0.84
Magnesium (mmol/L)	1.35±0.20	1.38±0.05	1.38±0.05	1.38±0.07
Calcium (mmol/L)	2.47±0.21	2.47±0.02	2.40±0.67	2.47±0.01
Potassium (mmol/L)	3.73±0.21	3.77±0.67	3.79±0.35	3.77±0.25
Chloride (mmol/L)	103.07±3.51	99.00±2.65	99.01±1.00	103.09±1.22
Uric acid	0.20±0.02	0.23±0.05	0.25±0.21	0.23±0.01
Total protein (g/L)	55.33±1.15	53.67±2.04	55.70±2.08	52.20±2.05
Albumin (g/L)	19.33±0.58	19.30±0.58	19.30±0.58	19.83±0.45
Total bilirubin (umol/L)	14.87±1.53	14.83±1.04	14.90±1.61	14.80±1.15
Conjugated bilirubin (umol/L)	7.03±0.58	6.97±1.40	7.00±1.00	7.00±1.00
Alkaline phosphatase (µ/L)	308.00±1.00	305.33±1.45	304.70±1.28	305.04±1.0 0
Alanine aminotransferase (µ/L)	66.00±2.31	69.67±1.03	65.00±1.42	65.08±1.63
Aspartate aminotransferase (µ/L)	328.67±1.06	323.67±1.53	324.09±1.76	324.06±2.89

Data are mean ± SEM Values without superscripts in the same row for each parameter are not significantly different (p > 0.05)

ROW is more viable and sensitive index of toxicity than absolute organ weight as it relates the overall wellbeing of the animals to each of their organ [21]. The non-significant changes in the ROW of the CDE-administered animals relative to the control rats in both the acute and sub-acute toxicity studies are indicative of the well preserved functionality of the investigated organs. These were further supported by the non-toxic infiltrations of the extract on the overall histoarchitectural features of the kidney, heart and liver of the treated rats as shown in this study.

The haematological and clinical biochemistry parameters investigated in this study are useful indices to ascertain the safety profiles of plant extracts in metabolically active experimental animals [16]. The non-significant differences in the hematological indices for the control rats and the extract-treated animals in the acute toxicity test following the 28-days repeated dosing treatment with CDE could be suggestive of its non-toxic effect on the hematopoietic system. Specifically, the non-significant effect on the hemoglobin (Hb), red blood cell (RBC) counts and their related parameters (heamatocrit, red blood cell distribution width, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentra-tion) may indicate that, the morphology as well as either incorporation of Hb into the RBCs or osmotic fragility of the RBCs was not affected subsequent to CDE administration. It could also imply that treatment with CDE both acutely and in the 28-days repeated daily dosing did not predispose the rats to anaemic condition within the evaluation period.

These findings corroborate previous reports [18,19], where similar assertions were made while evaluating the toxicological implications of treatment with plant extracts on the blood

systems of Wistar rats. The unaltered white blood cell (WBC) and platelet counts subsequent to treatment with CDE may also be a further probable reason that the vascular permeability and immune system of the treated rats were adequately maintained and corroborated the non-haematotoxic effect of the CDE at the investigated doses and within the period of experimentation.

The kidney and liver function parameters are important biomarkers and any damage to these organs has often been associated with alteration in their serum concentrations [22,23]. Therefore, the non-significant effects observed in all the renal and liver function indices investigated in this study following treatment with CDE for 28 days at the tested doses is a further confirmation of its non-toxic effect. Hence, it could be logically inferred that the extract is unlikely to be toxic to the liver and the kidney at the tested doses and within the exposure period as evidently supported by the photomicrographs of the investigated organs.

CONCLUSION

CDE has been established to have a LD_{50} value > 5000 mg/kg in rats. Although, further studies to determine the long-term toxicity effects of CDE in animals is imperative to establish its complete safety profile. The aqueous stem bark extract can be considered relatively safe at the tested doses and over the period of exposure used in this study.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this study.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. OAW and AJA conceived and design the study, OAW prepared the extracts and carried out the study. AJA helped in the coordination and revise of the manuscript. All authors read and approved the final manuscript.

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