Trop J Nat Prod Res, August 2020; 4(8):392-396 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Acute and Sub-Chronic Toxicity Studies on Methanol Stem Bark Extract of *Cussonia barteri* Seeman (Araliaceae) in Wistar Rats

*Galadanchi F. Abdurrahman[1], Aminu Ambi[1], Muhammad Bisallah[2], Abdulhakim Abubakar[3], Abdulhamid Yusuf [4], Usman M. Jajere[5], Idris Z. Yabagi[1]

[1]*Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria, Nigeria*
[2]*Department of Veterinary Pathology, Ahmadu Bello University Zaria, Nigeria*
[3]*Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria*
[4]*Department of Microbiology, Umaru Musa Yar'adua University Katsina, Nigeria*
[5]*Department of Pharmacognosy and Drug Development, Gombe State University, Nigeria*

ARTICLE INFO

Article history:
Received 05 March 2020
Revised 03 July 2020
Accepted 22 August 2020
Published online 28 August 2020

ABSTRACT

Cussonia barteri (Seemann.) belongs to the family Araliaceae. It is widely distributed in the Northern part of Nigeria and is being exploited for its medicinal importance. This study investigated the possible toxicity of the methanol stem bark extract of *Cussonia barteri* in Wistar rats. Methanol extract of *Cussonia barteri* (MECB) was subjected to acute and sub-chronic toxicity studies using the Guidelines of the Organization for Economic Co-operation and Development (OECD 425 and 407, respectively). Limit Test method was employed for acute toxicity using ten male Wistar rats. Group one served as control (5 rats) while group two was administered 5000 mg/kg of MECB and observed for 14 days. The sub-chronic toxicity studies were carried out using 4 groups of rats which were dosed daily for 28 days. The first group was the control, while the rats in groups 2, 3 and 4 were administered MECB (250, 500 and 1000 mg/kg, respectively). The experiment was concluded on the 29th day and the rats were anaesthetized to obtain organs and blood samples for histological, biochemical and haematological investigations. The oral median lethal dose was >5000 mg/kg. In the sub-chronic toxicity studies, there was a significant ($P<0.05$) increase in body weight of the rats at 250 mg/kg in the 4th week compared to control. There were no significant ($P > 0.05$) changes in the renal and haematological parameters compared to control. The results showed that MECB stem bark is non-toxic following acute administration and demonstrated slight sub-chronic toxicity tendency in Wistar rats.

Keywords: *Cussonia barteri*, Acute toxicity, Sub-chronic toxicity, Biochemical parameters, Haematological parameters, Histology.

Introduction

Herbs or medicinal plants have been the commonest and cheapest sources of medicines for preventive, curative or protective purposes from early civilization till date.[1] According to the World Health Organization, a projected 80% of the global population relies on traditional medicine for their health care needs and medicinal plants remain a cornerstone of traditional medicine.[2] There is a general believe that medicinal plants are safer than orthodox medicines which is one of the reasons for their growing popularity but studies have shown that not all natural products are safe.[3] To develop safer natural products from plants, preliminary toxicological studies are necessary to investigate probable risks[4] and as a rationale, many examinations are performed on medicinal plants to avert their toxicities.[5,6] If the toxic effects are not prevented, important organs like the kidneys and liver are susceptible to hazardous effects and this necessitates toxicological evaluations in animals.[7]

*Corresponding author. E mail: fateemahag@gmail.com
Tel: +2348066401994

Citation: Abdurrahman GF, Ambi A, Bisallah M, Abubakar A, Yusuf A, Jajere UM, Yabagi IZ. Acute and Sub-Chronic Toxicity Studies on Methanol Stem Bark Extract of *Cussonia barteri* Seeman (Araliaceae) in Wistar Rats. Trop J Nat Prod Res. 2020; 4(8):392-396. doi.org/10.26538/tjnpr/v4i8.12

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Cussonia barteri Seem is a dicotyledonous, medium-sized deciduous tree which grows up to 10-13 m in height. It grows widely in tropical and sub-tropical regions of Sub-Saharan Africa, Yemen and has a convoluted trunk and very compact hard bark.[8] The leaves and flowers spikes cluster at the ends of the thick branches with greenish-white flowers and whitish ripe fruits.[9] The common name is "Octopus cabbage tree" while the local names in Nigeria include: *Gwabsa*, *Sigo*, and *Bumarlahi* in Hausa, Yoruba and Fufulde languages, respectively. It is used in African traditional medicine for the treatment of menstrual pain, gonorrhoea and epilepsy in Ghana and Nigeria.[8] The analgesic, anti-epileptic, anti-diarrheal, anti-malarial, anti-inflammatory, haematopoetic, anti-poison and anti-psychotic activities of *Cussonia barteri* have been reported.[10] There is no scientific information on its safety following prolonged or repeated usage. The present study investigated the safety of *Cussonia barteri* by evaluating its acute and sub-chronic toxicity profiles in Wistar rats.

Materials and Methods

Plant material

The fresh stem bark of *Cussonia barteri* was collected from Basawa, Sabon-gari Local Government Area, Kaduna state, Nigeria in February 2018. It was identified by Namadi Sanusi in the Herbarium Unit of the Department of Botany, Ahmadu Bello University, Zaria, Nigeria. A voucher number of 900287 was given by comparing with an earlier deposited voucher specimen.

Plant extraction
The plant material was air-dried, crushed into coarse powder with pestle and mortar. One kilogram (1 kg) of the powdered plant material was subjected to extraction with 3 L of aqueous methanol (70% v/v methanol and 30% v/v water) using cold maceration for a period of seven (7) days with intermittent agitation. The extract obtained was evaporated over a water bath set at a temperature of 50^{o}C and the dried extract was weighed and labeled as methanol extract of *C. barteri* (MECB). The extract was kept in a desiccator until further use.

Experimental animals
Male Wistar rats (100 - 150 g) were obtained from the Animal House Facility of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. They were kept in well-ventilated cages at room temperature under normal day and night cycle, fed with pelletized animal diet (Vital feed®, Jos) with access to water *ad libitum*. The rats were treated according to NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised, 1996). Ethical approval on the use and care of laboratory animals was obtained from Animal Rights Ethical Committee, Ahmadu Bello University Zaria, Nigeria with the approval number ABUCAUC/2019/005 and the protocols were followed in accordance with the standard laboratory conditions and practices.

Extract preparation and administration
Varying stock solutions of MECB were prepared using distilled water to get the experimental concentrations. Fresh solutions were prepared daily and administered orally with the aid of oral gavages.

Acute toxicity determination
The acute toxicity of MECB was established using the Organization for Economic Co-operation and Development (OECD, 425) Guidelines.[11] The animals were divided into 2 groups of 5 rats each. Group 1 received 1 mL/kg of distilled water and served as the control while group 2 were fasted overnight before dosing with MECB at 5000 mg/kg orally. One rat was initially dosed and was observed for 24 hours and then for 14 days for behavioral pattern, signs of toxicity and mortality. The remaining four rats were also dosed with MECB at 5000 mg/kg orally and observed for 14 days after which the LD_{50} was estimated. The rats were then euthanized on the 15th day following mild chloroform anaesthesia. Their organs (liver and kidneys) were harvested for histological examination.

Sub-chronic toxicity studies
The repeated 28-day daily oral administration was conducted according to OECD 407 Guidelines[12] using twenty four (24) male Wistar rats. The rats were fasted overnight and then divided into four (4) groups of six (6) rats each. Group I received 1 mL/kg of distilled water and served as control while groups II to IV were orally administered graded doses of MECB (250 mg/kg, 500 mg/kg and 1000 mg/kg) body weight, respectively daily for 28 days. General behavioral pattern and mortality of the rats were observed daily and their weights were noted weekly. The rats were euthanized with chloroform on the 29th day of the experiment. Blood samples and organs were collected for haematological, biochemical and histological examinations.

Evaluation of organ-body weight ratio
The harvested liver and kidneys were trimmed of other tissues and then weighed (paired organs were weighed together). The relative organ-body weight ratio was calculated using the formula below:

$$\text{Relative organ weight (\%)} = \frac{Absolute\ organ\ weight(g)}{Final\ Body\ weight\ of\ rat\ (g)} \times 100$$

Evaluation of haematological parameters
Blood samples for haematological evaluation were collected into sample bottles containing ethylene diamine tetra-acetic acid (EDTA) to prevent coagulation. Thereafter, the levels of white blood cell (WBC), monocytes (MON), granulocytes (GRAN), lymphocytes (LYMP), red blood cell (RBC), haemoglobin (HGB), packed cell volume (PCV) and platelets (PLT) were assayed using automated Haematology Analyzer (Mind-ray-BC-3600).

Evaluation of biochemical parameters
Plain bottles were used to collect blood samples, allowed to clot and then centrifuged at 3500 rpm for 10 min. The serums obtained were investigated to estimate the effect on biochemical indices using photoelectric colorimeter (AC-115 Optima, Japan). The indices evaluated include; alanine aminotransferase (ALT) and aspartate aminotransferase (AST),[13] alkaline phosphatase (ALP),[14] total and conjugated bilirubin,[15] urea[16] and creatinine.[17]

Histology
The harvested livers and kidneys were fixed in 10% formaldehyde for 10 days. They were processed and stained with haematoxylin and eosin. The stained sections were viewed under a microscope for histological changes.[18]

Statistical analyses
The data obtained from the experiments were analyzed using Statistical Package for Social Sciences (Version 20). Descriptive statistics was performed to obtain the mean ± SEM and the data were analyzed using one way and repeated measure Analysis of Variance (ANOVA) followed by Tukey and Bonferroni post hoc tests where appropriate. Statistical significant differences were observed at $P \leq 0.05$.

Results and Discussion

Effect of acute administration of MECB on general behaviour and mortality
The oral administration of MECB produced no visible sign of toxicity and mortality throughout the study period. In addition, no gross pathological changes were observed in the liver and kidney (Plates 1 and 2). The LD_{50} was thus estimated to be greater than 5000 mg/kg. In the acute toxicity study, neither mortality nor any sign of toxicity was observed in animals after oral administration of MECB at the dose of 5000 mg/kg and after a post treatment period of 14 days. Based on the LD_{50}, *C. barteri* could be classified as a low toxic material.[19]

Effect of 28-days daily administration of MECB on general behaviour and mortality
Oral administration of MECB (250 mg/kg, 500 mg/kg and 1000 mg/kg) did not induce any mortality. There were also no visible changes in behaviour throughout the study period when compared to the control group.

Effect of 28-days daily administration of MECB on body weights
The administration of MECB produced a significant ($P < 0.05$) increase in body weight at 250 mg/kg in the fourth week when compared with the control group (Figure 1). Variation in body weights is an indication of toxic effects of chemicals.[20] In this research, administration of *C. barteri* extract did not reduce the body weights of the animals suggesting that it did not exert any deteriorative effect on their growth. Furthermore, the significant increase in the body weights over time showed that MECB did not impair feed utilization of the rats. Similarly, the extract did not alter the organ weights of the rats and could therefore be considered non-toxic because reductions in the weights indicate toxicity.[21]

Effect of 28-days daily administration of MECB on relative organ-body weights
The administration of MECB did not produce a significant ($P > 0.05$) increase or decrease in the relative organ/body weights when compared to control group (Table 1).

Effect of 28-days daily administration of MECB on haematological parameters
Administration of MECB produced no significant alteration ($P > 0.05$) in haematological parameters when compared to control group (Table 2).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

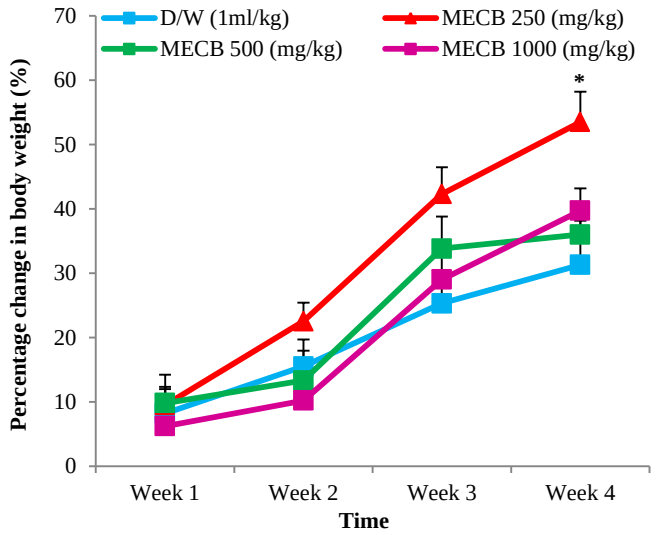

Figure 1: Effect of 28 day daily administration of methanol extract of *C. barteri* on body weights of rats. Values are presented as mean ± SEM; *: $P<0.05$; compared to control. Repeated measure ANOVA followed by Bonferroni post hoc test, n = 6; D/W: Distilled water, MECB: Methanol extract of *C. barteri.*

Table 1: Effect of 28 day oral administration of MECB on relative organ body weights of rats

Treatment (mg/kg)	Mean organ body weights (%)	
	Liver	Kidney
D/W (1 mL/kg)	5.31 ± 0.49	1.12 ± 0.31
MECB 250 mg/kg	4.41 ± 0.53	0.99 ± 0.10
MECB 500 mg/kg	3.61 ± 0.42	1.00 ± 0.08
MECB 1000 mg/kg	4.91 ± 0.75	0.94 ± 0.13

Values are presented as Mean ± SEM, no significant differences compared to distilled water (D/W) control group-One way ANOVA, n = 3; D/W: Distilled water, MECB: Methanol extract of *C. barteri*.

The haematopoietic system is one of the targets of toxic compounds and an important index of physiological and pathological status in man and animals.[22] After 28 days of treatment, there were no significant changes in haematological parameters indicating that the extract did not produce deleterious effects on the haematopoetic system.

Effect of 28-days daily administration of MECB on biochemical parameters in rats

Administration of *C. barteri* extract produced a significant ($P < 0.05$) decrease in the level of ALP at 500 mg/kg when compared to the control group (Table 3).

Damage to the hepatocytes is depicted by elevation of liver transaminases.[23] Serum ALP is a useful diagnostic, screening and follow up index of cholestatic hepatobiliary lesions.[24] Administration of the stem bark extract of *C. barteri* produced a significant ($P < 0.05$) decrease in the level of ALP at 500 mg/kg, this slight variation could not be toxicologically important because it is an elevation in the liver enzymes that is associated with toxicity while low levels are not usually considered.[25] MECB did not produce major effects on the serum levels of other liver enzymes and bilirubin and this indicates that the function of the liver are not affected by the extract after sub-acute exposure. Urea and Creatinine are important indices of renal function.[26] In this research, the absence of significant alterations in renal parameters is a suggestion that MECB has no harmful effect on the kidneys.

Effects of 28-days daily administration of MECB on histology of the livers and kidneys

Histological examination of the liver revealed Slight hepatic nuclei pyknosis and kuppfer cell hyperplasia at the doses of 500 mg/kg and 1000 mg/kg, respectively (Plate 3). The kidney showed slight tubular necrosis and glomerular necrosis at 500 mg/kg and 1000 mg/kg of the extract, respectively (Plate 4). However, there were no histopathological changes in the liver and kidney at 250 mg/kg compared to the control. Finally, significant changes where not observed in the morphology of the kidneys and livers of the rats following 28 days oral administration of *C. barteri.* The slight histopathological alterations observed where not validated by the biochemical findings.

Table 2: Effect of 28 days Daily Administration of Methanol extract of *C.barteri* on Haematological Parameters in Rats

Treatment (mg/kg)	WBC ($\times10^3$/µL)	RBC ($\times10^6$/µL)	PCV (%)	PLT (U/L)	LYMP (%)	MON (%)	GRA (%)
D/W(1 mL/kg)	4.87 ± 0.07	4.43 ± 0.03	31.00 ± 2.00	282.67 ± 16.33	59.67 ± 0.03	4.53 ± 0.03	35.50 ± 0.00
MECB 250 mg/kg	4.63 ± 0.03	4.00 ± 0.00	43.67 ± 0.17	185.67 ± 0.33	65.40 ± 0.20	6.77 ± 0.03	28.77 ± 0.23
MECB 500 mg/kg	3.33 ± 0.17	4.40 ± 0.00	36.67 ± 5.67	215.73 ± 13.97	64.17 ± 2.37	4.60 ± 0.00	31.27 ± 2.33
MECB 1000 mg/kg	3.87 ± 0.07	4.13 ± 0.13	45.67 ± 0.33	300.93 ± 0.43	59.33 ± 0.33	5.70 ± 0.10	32.87 ± 0.87

Values are presented as mean ± SEM, and no significant difference compared to distilled water (D/W) control-One way ANOVA followed by Tukey test, n = 6; WBC = White blood cells, LYMP = Lymphocytes, MON = Monocytes, GRAN = Granulocytes, RBC = Red blood cells, HGB = Haemoglobin, PCV = Packed cell volume, PLT = Platelets. D/W: Distilled water. MECB: Methanol extract of *C.barteri*.

Table 3: Effect of 28 days Administration of Methanol extract of *C.barteri* on Biochemical Parameters in Rats

Treatment (mg/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)	TB (U/L)	CB (µmol/L)	Urea (mmol/L)
D/W (1 mL/kg)	89.83 ± 0.44	65.07 ± 2.98	44.77 ± 2.72	12.37 ± 2.82	6.00 ± 1.45	49.50 ± 3.25
MECB 250 mg/kg	93.17 ± 0.17	65.10 ± 0.06	60.30 ± 0.15	10.07 ± 0.03	8.53 ± 0.03	36.23 ± 0.15
MECB 500 mg/kg	85.33 ± 2.84	56.87 ± 3.64	17.57 ± 1.48[a]	9.57 ± 1.02	6.16 ± 1.42	59.00 ± 1.53
MECB 1000 mg/kg	88.17 ± 0.17	70.10 ± 0.06	30.17 ± 0.03	9.40 ± 0.10	4.07 ± 0.03	60.17 ± 0.17

Values are presented as mean ± S.E.M. Significant [a] = $p < 0.05$ compared to control group – One way ANOVA followed by Tukey test, n = 6. ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase, TB = Total bilirubin, CB = Conjugated Bilirubin. D/W: Distilled water. MECB: Methanol extract of *C.barteri*.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

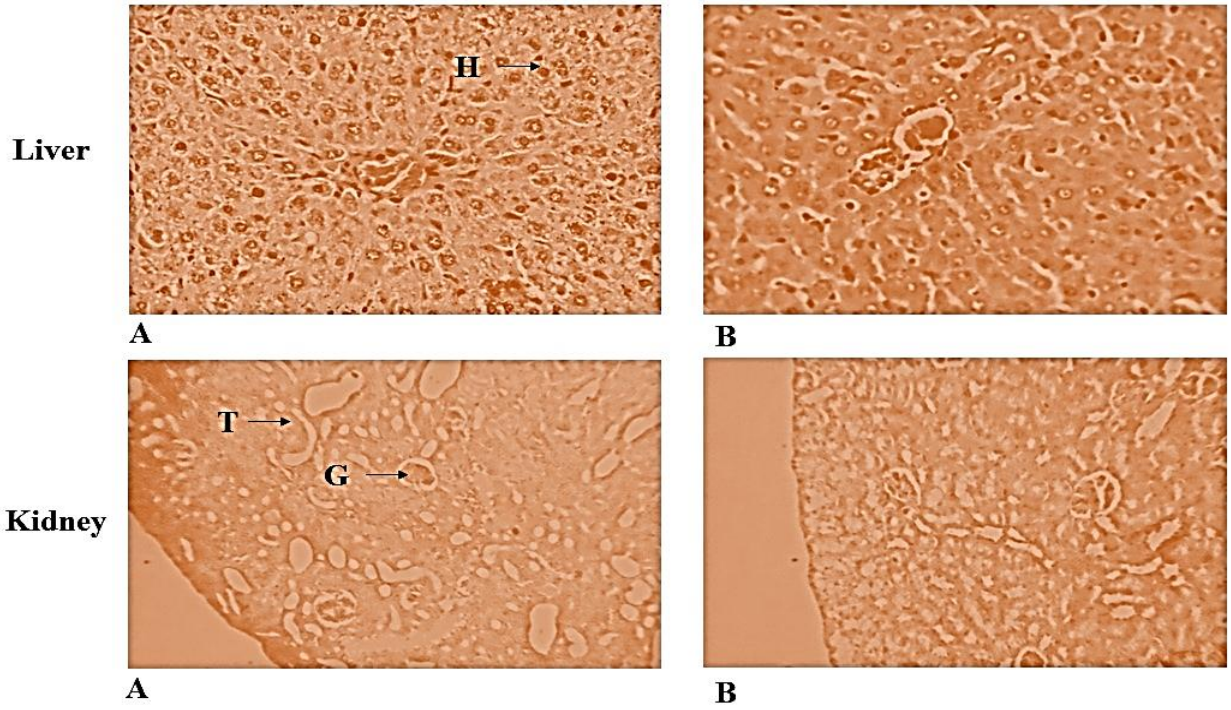

Plate I: Photomicrograph of the liver and kidney sections of rats administered distilled water (**A**) and MECB 5000 mg/kg (**B**) for 14 days (H and E, magnification x250). H = Normal hepatocytes, G = Glomerulus, T = Tubules

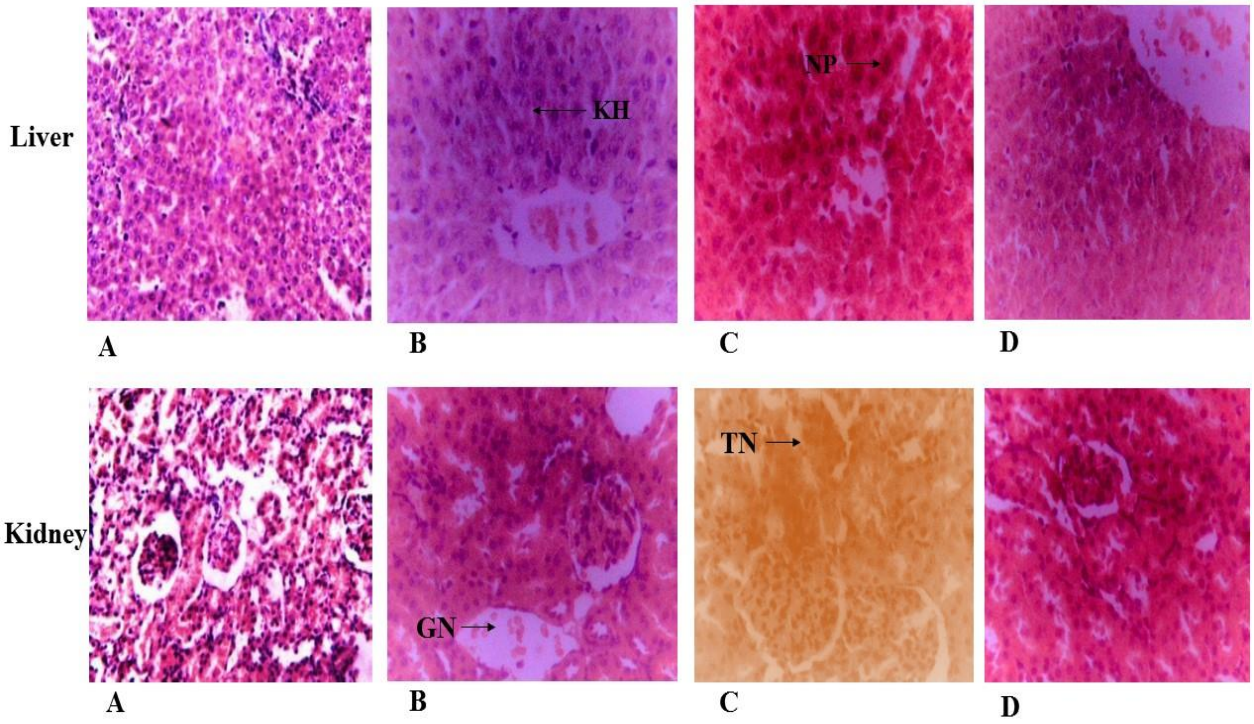

Plate II: Photomicrograph of the liver and kidney sections of Wistar rats administered distilled water and MECB for 28 days. **A:** Distilled water treated group, **B:** MECB 1000 mg/kg treated group, **C:** MECB 500 mg/kg treated group, **D:** MECB 250 mg/kg treated group. (H and E, magnification x250). KH = Slight Kuppfer cell hyperplasia. NP = Slight nuclei pyknosis, TN = Slight tubular necrosis, GN = moderate glomerular necrosis.

Conclusion

The single oral dose of 5000 mg/kg of the methanol stem bark extract of *C. barteri* caused no mortality, no behavioral changes and any treatment-related signs of toxicity. Generally, the findings of the present study suggest a very low potential of *C. barteri* to produce adverse effects after 28 days repeated administration in rats.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors wish to acknowledge the guidance of Dr. (Mrs.) Hadiza Nuhu of Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria as well as the staff and management of Pharmacology and Therapeutics and Pharmacognosy and Drug Development.

References

1. Jayachitra A, Rajalakshmi A, Gopal P, Krithiga N. Toxicity Analysis of different medicinal plant extracts in Swiss Albino Mice. Pharmacol Toxicol Res. 2014; 1(1):1-6.
2. Chanda S, Parekh J, Vaghasiya Y, Dave R, Baravalia Y, Nair R. A Review: Medicinal Plants - From Traditional Use to Toxicity Assessment. Int J Pharm Sci Res. 2015; 6(7):2652-2670.
3. Ansar JA and Indamar NN. The Promise of Traditional Medicine. Int J Pharmacol. 2010; 6(6):808-812.
4. Li Yihang, Li Guang, Song Meifang, Li Xuelan, Zhan Xia, Lu Juan, Chen Xi. Acute toxicity study of *Aspidopterys obcordata* aqueous extract in Sprague-Dawley rats**.** J Trad Chin Med. 2016; 36(3):377-381.
5. Subramanian K, Sankaramourthy D, Gunasekaran M. Toxicity studies related to medicinal plants. In: Mandal SC, Mandal V, Konishi T, (eds). Natural products and drug discovery: An integrated approach. UK: Elsevier; 2018. 491-505 p.
6. Patil UH and Gaikwad DK. Phytochemical profile and antibacterial activity of stem bark of *Anogeissus latifolia*. Pharmacog J. 2010; 2(17):70-73.
7. Ekeanyanwu CR and Njoku OU. Acute and subacute oral toxicity study on the flavonoid rich fraction of *Monodora tenuifolia* seed in albino rats. Asian Pac J Trop Biomed. 2014; 4(3):194-202.
8. Burkill, HM. The useful plants of west tropical Africa, Vol 1, London, U.K: Royal Botanical Gardens, Kew, Richmond Surrey, U. K. 1985. 443-44 p.
9. Michel A. Trees Shrubs and Lianas of West African dry zones: Cambridge University Press, U.S.A. 2015. 172 p.
10. Igbe I, Edosuyi, O, Okhuarobo A. Harnessing the medicinal properties of *Cussonia barteri* seem. (Araliaceae) in drug development. A review. Herbal Polon. 2018; 64(3):50-61.
11. Organization for Economic Cooperation and Development OECD guideline for testing of chemicals. Test No: 425: repeated dose 28days oral toxicity in rodents OECD publishing Paris. 2001.
12. Traesel GK, de Souza JC, de Barros AL, Souza MA, Schmitz WO, Muzzi RM, et al. Acute and subacute (28 days) oral toxicity assessment of the oil extracted from Acrocomia aculeata pulp in rats. Food Chem Toxicol 2014; 74:320-325.
13. Reitman S and Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am J Clin Pathol. 1957; 28(1):56-63.
14. Belfield A and Goldberg D. Colorimetric determination of alkaline phosphatase activity. Enzyme 1971; 12:561-566.
15. Walters MI and Gerard HW. An ultramicro method for the determination of conjugated and total bilirubin in serum or plasma. Microchem J.1970; 15(2):231-243.
16. Weatherburn MW. Phenol-hypochlorite reaction for determination of ammonia. Anal Chem. 1976; 39:971-974.
17. Bartels H, Bohmer M, Heierli C. Serum creatinine determination without protein precipitation. Clin Chim Acta. 1972; 37:193-197.
18. Arthur SJ and John B. A colour Atlas of Histopathological Staining Techniques. Wolf MED.Pub. Ltd. London, 1978. 14-20 p.
19. Ou´edraogo S, Som´e N, Ouattara S, Kini FB, Traore A, Bucher B. and Guissou IP. Acute toxicity and vascular properties of seed of *Parkia biglobosa* (JACQ) R. Br Gift (Mimosaceae) on rat aorta. Afr J Trad Compl Altern Med. 2011; 9(2):260-265.
20. Hayelom K, Mekbeb A, Eyasu M, Wondwossen E, Kelbesa U. Effect of methanolic root extract of *Clerodendrum myricoides* on blood, liver and kidney tissues of mice. Afr Health Sci 2012; 12: 489-497.
21. Waller DG and Sampson A. Medical pharmacology and therapeutics. 5th ed. China: Elsevier; 2017;1-744.
22. Adeneye AA, Ajagbonna OP, Adeleke TI, Bello SO. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. J Ethnopharmacol. 2006; 105:374-379.
23. Wolf PL, Williams D, Tsudaka T, Acosta L. Methods and Techniques in Clinical Chemistry. John Wiley & Sons, USA. 1972. 1-417 p.
24. Wolf PL. Clinical significance of an increased or decreased serum alkaline phosphatase; Arch Pathol Lab Med. 1978; 102:497-501.
25. Deeb A and Elfatih A. Could Alerting Physicians for Low Alkaline Phosphatase Levels Be Helpful in Early Diagnosis of Hypophosphatasia? J Clin Res Pediatr Endocrinol. 2018; 10:19-24.
26. Gowda S, Desai PB, Kulkarni SS, Hull VV, Math AA, Vernekar SN. Markers of renal function tests. N Am J Med Sci. 2010; 2:170-173.