

Phytochemical Screening and Toxicity Study of Methanol Stem Bark Extract of *Prosopis africana* (African Mesquite) In Albino Wistar Rat

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Abstract

Prosopis africana (Guill. & Perr.) is a perennial leguminous tree of the subfamily Mimosoidae and is found to grow in the savanna region of Senegal and Nigeria. Several parts of the plant has been documented to possess ethno medicinal value. The study aimed to determine the phytochemical constituents using methods described by Trease & Evans and toxicity profile (using established toxicity determination protocols) of methanol stem bark extract of *Prosopis africana* in albino Wistar rats. Phytochemical screening of the extract revealed the presence of alkaloids, glycosides, saponins, carbohydrates, terpenoids, flavonoids, and tannins. Acute toxicity test showed that the LD₅₀ of the extract was 3807.88 mg/kg, with only the animal administered with 5000 mg/kg of the extract dying in phase two. The extract did not affect food and water consumption or most hematological parameters, except for red and white blood cell counts. Histological examination of liver, kidney, and heart did not show any signs of malformation. These findings suggest that the methanol stem bark extract of *Prosopis africana* may be safe for use in traditional medicine and could provide a basis for the development of novel drugs for the treatment of various diseases such as malaria fever, wound care and diabetes mellitus.

Keywords: Phytochemical screening, *Prosopis africana*, Toxicity, Wistar rats

INTRODUCTION

Prosopis africana (Guill. & Perr.) Taub (Mimosaceae) is a plant that is commonly found in sub-Saharan Africa, and it has been used in traditional medicine for centuries due to its various therapeutic properties (Ajiboye *et al.*, 2013; Yanda *et al.*, 2022). The stem bark of this plant has been reported to contain several phytochemicals, such as alkaloids, flavonoids, tannins, and saponins, among others, which have been linked to its anti-inflammatory, analgesic, anti-diabetic, and anti-microbial activities (Yanda *et al.*, 2022). Despite its widespread use in

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traditional medicine, however, there is a lack of adequate scientific data on the toxicity profile of *Prosopis africana*.

Therefore, this study was conducted to investigate the phytochemical constituents and toxicity profile of methanol stem bark extract of *Prosopis africana* in albino Wistar rats. The rationale behind this study is that the findings will contribute to the body of knowledge on the safety and efficacy of *Prosopis africana*, and may provide a basis for the development of novel drugs for the treatment of various diseases. Together with efficacy studies, extensive preclinical toxicity studies are *sine-qua-non* for developing any drug candidate (Dorato and Buckley, 2006; Parasuman, 2011). In addition, the toxicity data generated from this study will help to establish safe dosages of *Prosopis africana* extract for subsequent human consumption. Overall, the findings from this study will contribute to the growing body of literature on the safety and efficacy of traditional herbal medicines, and will help to promote their integration into modern healthcare systems.

METHODS

Collection of samples

In July, we collected stem bark of *Prosopis africana* at Gumi local government area of Zamfara state, Nigeria. The stem bark were validated at the herbarium of the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto, Nigeria. For future reference, a voucher specimen was deposited (No. UDUH/ANS/Faba/0854). Prior to usage, the bark was air-dried, crushed, and stored in an airtight plastic bag at 4°C.

Animal handling

Forty albino rats (weighing 180 - 220 g) were purchased from the National Institute of Pharmaceutical Research and Development (NIPRD), Abuja and transferred to the Animal house facility at the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto where the animals were maintained throughout the period of the study. The animals were housed in groups of three in plastic cages and kept at 25-30 °C in a well-ventilated room with a 12/12 h light/dark cycle. The University's ethical committee requirements for the use of animals, as well as the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978), were followed. Before the experiment began, the animals were allowed to acclimate for two weeks on ad libitum mouse chow and free access to water. The University Research and Ethics Committee approved the research protocol and assigned it a reference number UDUS/UREC/2022/019.

Preparation of plant extract and phytochemical screening

Prosopis africana bark (2.5 kg) were pulverized and soaked in methanol (10 L) for 72 hours. Using a rotary evaporator, the extract was collected, filtered, and dried at 40 °C. The extraction process was repeated three times, and the various extracts were combined to yield 220 g of dark green crude methanolic extract. Phytochemical screening of the extracts was performed for the presence of secondary metabolites using standard phytochemical methods as described by Trease & Evans (2002). The extract was screened for the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, proteins and steroids.

Acute toxicity study (LD₅₀)

The method of Lorke (1983) was used to determine the LD₅₀ (Table 1). The LD₅₀ value was calculated by taking the geometric mean of the lowest dose that caused death to the animal and the highest dose for which the animal survived after 24 h of administering the compound.

Table 1: Experimental Design of Acute Toxicity Study

PHASE ONE (n = 3)		PHASE TWO (n = 1)	
Route of administration	Dose (mg/kg)	Route of administration	Dose (mg/kg)
Oral	10	Oral	1,600
Oral	100	Oral	2,900
Oral	1,000	Oral	5,000

Sub-acute toxicity test

Twenty-four rats were used in the sub-acute toxicity research. All the mice were orally administered the methanol extract of the bark. The rats were divided into four groups, each containing six rats per group. The first group (control) received the vehicle at a dose of 2 ml/kg body weight, while the second, third and fourth groups received 250 mg/kg, 500 mg/kg and 1,000 mg/kg body weight of methanol extract of *Prosopis africana* respectively. Physiological functions that were monitored included the respiratory rate, occurrence of secretions and excretions, and autonomic activity (such as lacrimation, piloerection, pupil size, and unusual respiratory pattern), changes in skin fur, presence or absence of hair loss (alopecia), eyes and mucous membranes. The rats were weighed weekly to monitor weight changes. They were also monitored for variations in food/water consumption. On day 29 of administration, the rats were fasted for 12 h, euthanized by exposing them to carbon dioxide in a chamber; blood samples were collected by cardiac puncture in plain blood sample and EDTA coated tubes. The serum was separated and quantified for creatinine, aspartate aminotransferase, alkaline phosphatase and alanine transaminase using a Semi Auto Analyser Microlab 300 (Vital Scientific, US) machine. The whole blood was analysed for haematological parameters. Vital organs of interest, including heart, liver and kidney were collected after sacrifice. The organs were rinsed with normal saline and examined macroscopically before being placed in RCI2® for further analysis.

Histopathological studies

The organs were fixed in a 10% v/v neutrally buffered formaldehyde solution for ten days before being treated with paraffin wax to dehydrate, polish, and infiltrate the tissues. They were then inserted, allowing the specimen to be oriented in a 'block' that could be sectioned and stored and handled easily. It was sectioned with a microtome into very thin slices that were then placed on a microscope slide and stained with hematoxylin and eosin before being examined for histopathological characteristics (Rolls, 2011). A Nikon E-600 microscope was used to take bright-field photomicrographs of the rats' heart, liver and kidney tissues using a Retiga 2000R Fast CCD camera (Q-Imaging) (Nikon, Tokyo, Japan). These are shown in figures 2, 3 and 4 below.

Statistical Analysis

The mean and standard error of the mean (SEM) are used to express the results. SPSS version 19 was used to conduct statistical analysis of the data. One-way analysis of variance (ANOVA) was utilized. For post hoc examination of differences found using one-way ANOVA, Tukey's test was utilized. The $p < 0.05$ significance level was chosen. Tables and graphs are used to represent data.

RESULTS

Phytochemical screening result

The percentage yield of the dried stem bark of *P. africana* after extraction with methanol was calculated to be 12.6%. Phytochemical screening of the methanolic extract demonstrated the presence of alkaloids, glycosides, saponins, carbohydrate, terpenoids, flavonoids and tannins.

Acute toxicity study

All animals survived phase one of the acute toxicity test. Only the animal administered with 5000 mg/kg of the stem bark extract died in phase two, therefore the LD₅₀ of the extract was calculated as 3807.88 mg/kg.

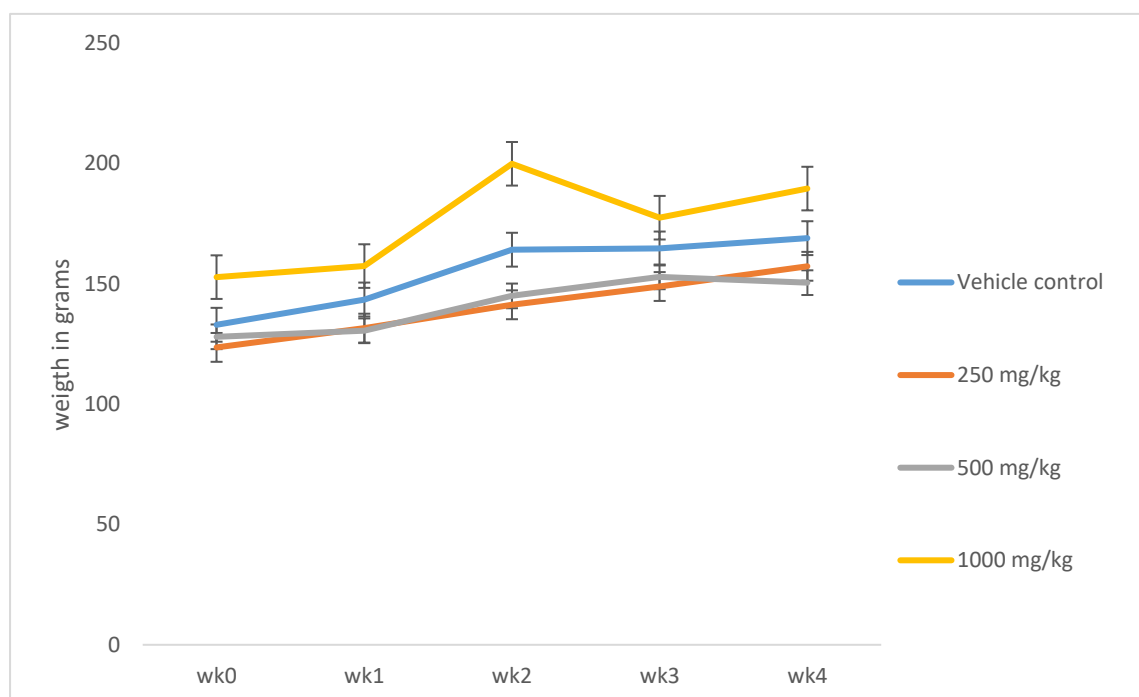


Figure 1: Weight changes in rats following oral administration of graded doses of methanolic stem bark extract of *P. Africana*

Sub-acute toxicity test

There was no variations in food/water consumption among the various interventions. The rats administered 1000 mg/kg of the extract showed weight change compared to the control rats. All other treatment groups did not show any weight change compared to the control group (Figure 1). Except for red blood cell and white blood cell counts, all other haematological parameters were not affected by the varying dose of *P. Africana* extract (Table 2).

Table 2: serum haematological parameters in Wistar rats following oral administration of graded doses of methanolic stem bark extract of *P. africana*

Intervention →	Vehicle control	250 mg/kg	500 mg/kg	1000 mg/kg
Hematological parameters ↓				
WBC *10 ⁹ /L	4.7 ± 2.0 ^a	7.03 ± 1.8 ^a	10.4 ± 2.8 ^b	7.04 ± 2.1 ^a
RBC *10 ¹² /L	3.92 ± 1.2 ^a	4.82 ± 0.6 ^a	5.27 ± 0.3 ^a	5.66 ± 0.5 ^b
HGB g/dL	9.52 ± 36.88 ^a	11.9 ± 0.85 ^a	12.85 ± 0.07 ^a	14.04 ± 0.93 ^a
PLT *10 ⁹ /L	259.66 ± 297.9 ^a	680.66 ± 313.7 ^a	764.5±34.64 ^a	739.6±240.23 ^a
MCHC g/dL	35.52 ± 1.72 ^a	38.95 ± 0.95 ^a	37.3 ± 3.32 ^a	37.62 ± 1.63 ^a
MCH pg	24.08 ± 1.17 ^a	24.83 ± 1.90 ^a	24.45 ± 1.90 ^a	24.86 ± 0.99 ^a
MCV fL	67.95 ± 4.87 ^a	66.7 ± 6.27 ^a	62.9 ± 0.42 ^a	65.46 ± 3.91 ^a
MPV fL	6.97 ± 0.78 ^a	21 ± 0.46 ^a	6.65 ± 0.21 ^a	6.46 ± 0.40 ^a
HCT %	26.9 ± 9.19 ^a	31.9 ± 1.41 ^a	33.2 ± 2.69 ^a	37.04 ± 3.83 ^a
RDW-CV %	15.07 ± 0.88 ^a	15.07 ± 0.49 ^a	15.00 ± 0.28 ^a	14.46 ± 0.48 ^a
RDW-SD fL	39.47 ± 5.17 ^a	38.93 ± 4.38 ^a	35.55 ± 0.21 ^a	44.90 ± 3.54 ^a
PDW %	2.77 ± 2.40 ^a	13.00 ± 0.6 ^a	12.55 ± 0.49 ^a	12.54 ± 0.76 ^a
PCT%	0.247 ± 0.19 ^a	0.477 ± 0.23 ^a	0.509 ± 0.04 ^a	0.475 ± 0.15 ^a
P-LCR%	8.98 ± 4.25 ^a	9.3 ± 4.28 ^a	7.4 ± 1.27 ^a	6.46 ± 2.76 ^a

Data represent mean ± SD (n=6). Row superscripted by the same letter are not significantly different at p < 0.05 in Tukey's multiple comparison. WBC: white blood cell; RBC: red blood cell; HGB: haemoglobin; PLT: platelet; MCHC: Mean corpuscular hemoglobin concentration; MCH: Mean corpuscular hemoglobin; MCV: Mean corpuscular volume; MPV: mean platelet volume; HCT: haematocrit; RDW-CV: Red cell distribution width; RDW-SD: Red cell distribution width standard deviation; PDW: Platelet distribution width; PCT: Plateletcrit; P-LCR: Platelet larger cell ratio

Figure 2 (a-d) showed the histology of the heart of rats administered graded doses of methanol extract of *P. africana*. The photomicrographs showed normal and centrally arranged nucleus. The connective tissue also appeared normal. The cardiac muscle fibers are well arranged.

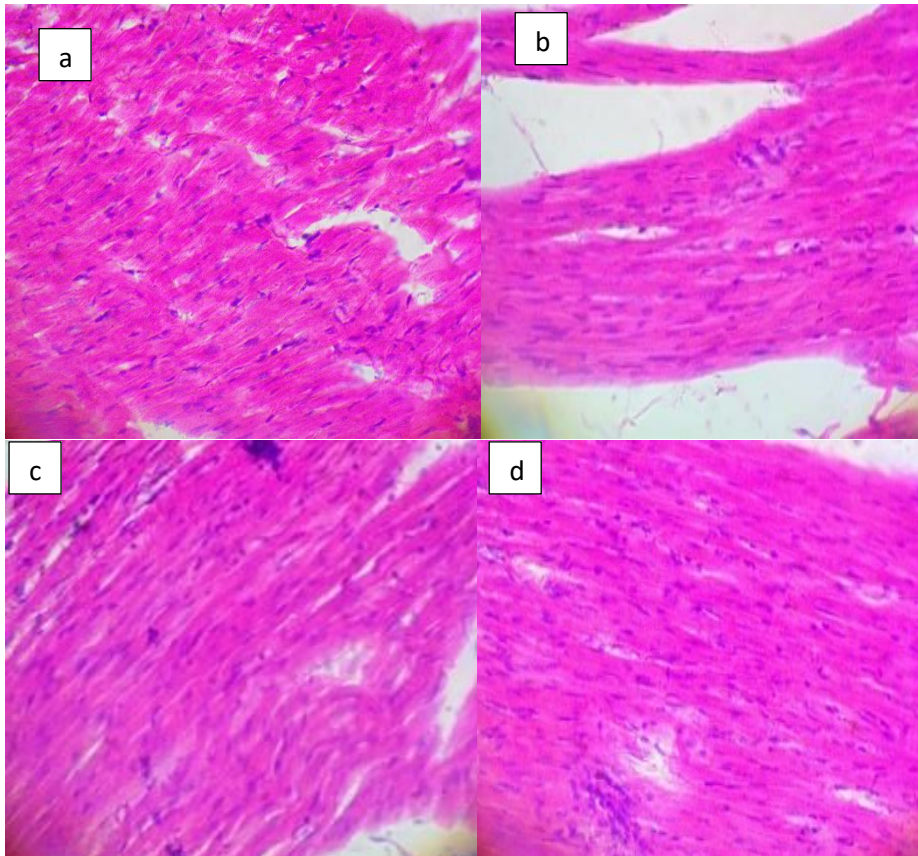


Figure 2: Photomicrograph of sections of heart of Wistar rats administered graded doses of *P. africana* for 28 days. a= vehicle control; b= 250 mg/kg; c= 500 mg/kg d= 1000 mg/kg

Figure 3 (a-d) showed the histology of the liver of rats administered graded doses of methanol extract of *P. africana*. Figure 3d showed progression in distortion of the hepatic architecture of the liver tissues of rats administered the highest dose of the extract. The central vein appears congested.

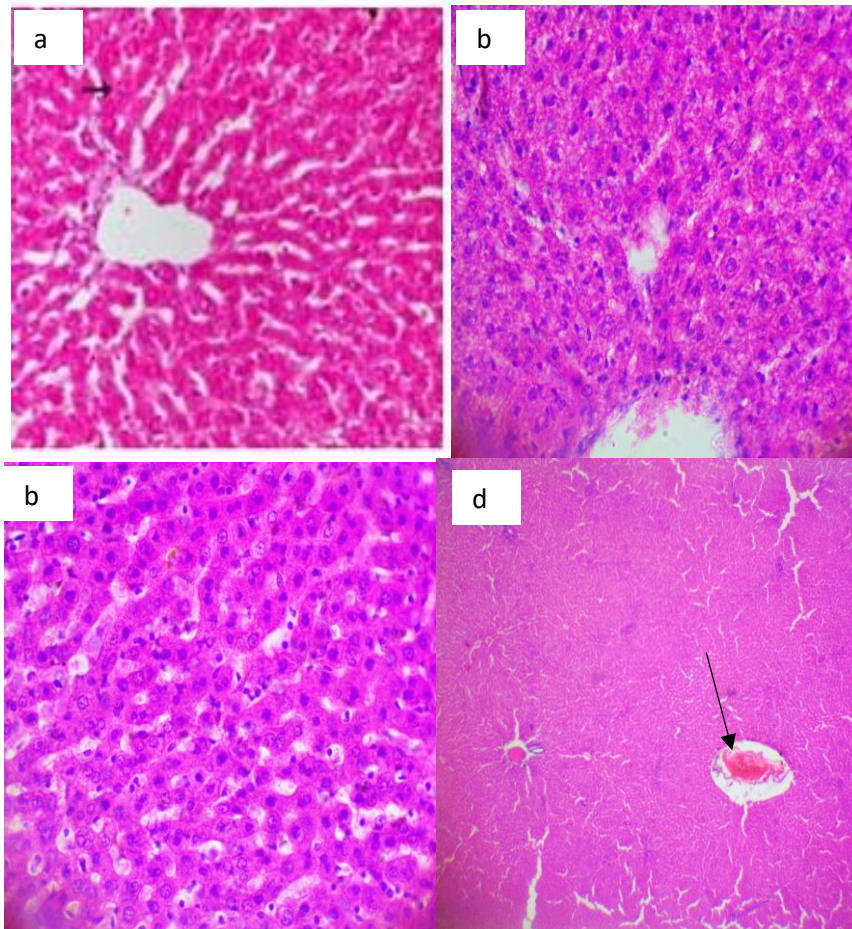


Figure 3: Photomicrograph of sections of liver of Wistar rats administered graded doses of *P. africana* for 28 days. a= vehicle control; b= 250 mg/kg; c= 500 mg/kg d= 1000 mg/kg

Figure 4 (a-d) showed the histology of the kidney of rats administered graded doses of methanol extract of *P. africana*. The photomicrographs showed normal architecture of renal corpuscles and tubules.

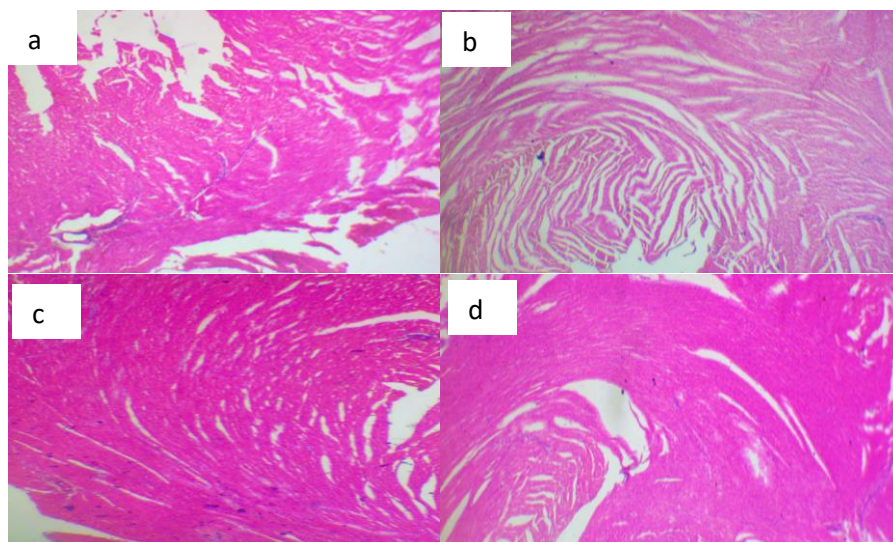


Figure 4: Photomicrograph of sections of kidney of Wistar rats administered graded doses of *P. africana* for 28 days. a= vehicle control; b= 250 mg/kg; c= 500 mg/kg d= 1000 mg/kg

DISCUSSION

The present study was aimed at determining the phytochemical screening and toxicity study of the methanol stem bark extract of *Prosopis africana* in albino Wistar rats. The percentage yield of the dried stem bark of *P. africana* after extraction with methanol was found to be 12.6%, which suggests a good yield for further analysis. Phytochemical screening of the methanolic extract demonstrated the presence of alkaloids, glycosides, saponins, carbohydrate, terpenoids, flavonoids, and tannins, which have been reported to possess various pharmacological activities (Huang *et al.*, 2018).

In the acute toxicity study, all animals survived phase one of the test, which suggested that the extract has a low toxicity profile. However, the animal administered with 5000 mg/kg of the stem bark extract died in phase two, and the LD₅₀ of the extract was calculated as 3807.88 mg/kg, which suggests that caution should be taken when administering very high doses of the extract. These findings are consistent with previous reports on the toxicity of *P. africana* extract (Obode *et al.*, 2020).

Furthermore, we observed that there were no variations in food/water consumption among the various interventions. The rats administered 1000 mg/kg of the extract showed weight change compared to the control rats, which may suggest an effect on metabolic processes. However, all other treatment groups did not show any weight change compared to the control group. Except for red blood cell and white blood cell counts, all other haematological parameters were not affected by the varying dose of *P. africana* extract. This suggests that the extract may have a selective effect on certain blood cells. This was similar to what was reported by Antai *et al.* (2009).

Additionally, the histology of the liver, kidney, and heart did not show any signs of malformation, which suggests that the extract may not have a toxic effect on these organs.

These findings are consistent with previous reports on the safety of different *P. africana* parts extract (Adelakun *et al.*, 2017; Obode *et al.*, 2020)

In conclusion, the present study provides evidence of the phytochemical constituents of *P. africana* extract, which may have pharmacological activities. The low toxicity profile observed in the acute toxicity study suggests that caution should be taken when administering high doses of the extract. The selective effect on certain blood cells and weight change observed in the rats may suggest an effect on metabolic processes. The absence of malformation in the histology of vital organs suggests that the extract may be safe for consumption. Further studies are required to elucidate the pharmacological activities of the phytochemical constituents of *P. africana* extract and its mechanism of action.

CONCLUSION

Based on the acute toxicity study, the LD₅₀ of methanol stem bark extract of *Prosopis africana* was determined to be 3807.88 mg/kg. The 28-day sub-acute toxicity study revealed that the compound could be tolerated up to a dose of 1000 mg/kg with minimal untowards effects. This study therefore demonstrates the relative tolerability *vis-à-vis* side effects of the extract at 1000 mg/kg. However, since this is a preliminary toxicological study, a more comprehensive toxicity study involving non-rodent animals is required to corroborate our findings to set a safe dose levels for clinical studies.

Declaration of competing interest

The authors declare that they have no known competing financial or personal interest.

REFERENCES

- Adelakun, K. M., Ajagbe, S. O., Ampitan, T. A. and Adeyemi, M. O. (2017). Toxicity effect of African mesquite (*Prosopis africana*) seed on African catfish (*Clarias gariepinus*) juveniles. *Bulletin of Animal Health and Production in Africa*, **65**:201-208.
- Ajiboye, A. A., Agboola, D. A., Fadimu, O. Y. and Afolabi, A. O. (2013). Antibacterial, phytochemical and proximate analysis of *Prosopis africana* (Linn) seed and pod extract. *Futa Journal of Research in Sciences*, **9**:101-109.
- Antai, A. B., Ofem, O. E., Ikpi, D. E., Ukafia, S. and Agiang, E. A. (2009). Phytochemistry and some haematological changes following oral administration of ethanolic root extract of *Gongronema latifolium* in rats. *Nigerian Journal of Physiological Sciences*, **24**(1): 79-83.
- Dorato, M. A. and Buckley, L. A. (2006). Toxicology in the drug discovery and development process. *Current Protocols in Pharmacology*, Chapter 10, <https://doi.org/10.1002/0471141755.ph1003s32>
- Huang, W., He, J., Nisar, M. F., Li, H. and Wan, C. (2018). Phytochemical and Pharmacological Properties of *Chaenomeles speciosa*: An Edible Medicinal Chinese Mugua. *Evidence-based Complementary and Alternative Medicine : eCAM*, 2018:9591845.
- Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, **54**(4): 275-287.
- Obode, O. C., Adebayo, A. H., and Li, C. (2020). Phytochemical and toxicological evaluations of *Prosopis africana* (GUILL. and PERR.) extract on albino wistar rats. *Toxicological research*, **37**(2): 183-195.
- Parasuraman, S. (2011). Toxicological screening. *Journal of Pharmacology & Pharmacotherapeutics*, **2**(2): 74-79.
- Rolls, G. (2011). Microtomy and paraffin section preparation. Leica Biosystem, Wetzlar, Germany, pp. 3-9.

Trease, G. and Evans, W. (2002) Phytochemicals. In: *Pharmacognosy*, 15th Edition, Saunders Publishers, London, 242-393.

Yanda, L., Tatsimo, S. J. N., Tamokou, J. D., Matsute-Takongmo, G., Meffo-Dongmo, S. C., Meli Lannang, A., and Sewald, N. (2022). Antibacterial and Antioxidant Activities of Isolated Compounds from *Prosopis africana* Leaves. *International Journal of Analytical Chemistry*, 2022: 4205823.