Trop J Phytochem Pharm Sci, September 2026; 5(7): 683 - 688 **ISSN 2955-1226 (Print)**
ISSN 2955-123(Electronic)

Tropical Journal of Phytochemistry & Pharmaceutical Sciences

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

Original Research Article

Acute and 28-Day Repeated-Dose Oral Toxicity of Aqueous Leaf Extract of *Phyllanthus amarus* In Wistar Albino Rats: Mortality, Clinical Signs and Haematological Indices

Abubakar B. Usman* and Hamisu Abdullahi

Department of Immunology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

ABSTRACT

Phyllanthus amarus is widely used in traditional medicine, but safety information for aqueous leaf preparations remains incomplete. This study evaluated acute and 28-day repeated-dose oral toxicity of an aqueous leaf extract in Wistar rats, with emphasis on mortality, clinical signs and haematological indices. In the acute limit-dose study, nine rats were allocated to control, 2000 mg/kg and 5000 mg/kg groups (n = 3) and observed for 14 days. In the repeated-dose study, 25 rats received distilled water or 250, 500, 750 or 1000 mg/kg/day of extract for 28 days. Full blood count was assessed using an automated haematology analyser, and data were analysed by one-way analysis of variance with Tukey post hoc testing. No mortality or overt toxicity occurred in either study. No deaths at 5000 mg/kg indicated an apparent oral median lethal dose above 5000 mg/kg within this design. In the acute study, selected white- and red-cell indices differed between the 2000 mg/kg group and control, with lower values at 5000 mg/kg than at 2000 mg/kg for some indices. During repeated dosing, white-cell and platelet indices did not differ significantly from control. Overall differences were observed for red blood cell count ($p = 0.003$), haemoglobin ($p = 0.005$) and haematocrit ($p = 0.017$), but no treated group differed significantly from control in post hoc comparisons; these differences occurred only among treated groups. The extract showed a low acute and short-term haematological toxicity signal under the study conditions, although organ-specific, chronic and human safety cannot be inferred.

Keywords: Phyllanthus amarus, Acute oral toxicity, Repeated-dose toxicity, Haematological indices, Median lethal dose, Wistar rats

Received 01 October 2026
Revised 26 October 2026
Accepted 27 October 2026
Published online: 27 November 2026

Copyright: © 2026 Usman and Abdullahi. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Phyllanthus amarus Schumach. and Thonn. (Phyllanthaceae) is a medicinal herb used in several traditional health systems and investigated for hepatoprotective, antioxidant, anti-inflammatory, antiviral and metabolic effects.[1,2] These activities have been associated with lignans, flavonoids, hydrolysable tannins and other phenolic constituents, although composition varies with plant source and extraction method.[2,3]

Therapeutic or ethnomedicinal use does not establish safety. Herbal preparations may contain pharmacologically active constituents that produce dose-related or organ-specific toxicity, and their safety therefore requires systematic experimental evaluation.[4] Acute toxicity studies provide information on immediate clinical effects and lethal-dose range, whereas repeated-dose studies help identify effects that may emerge after sustained exposure.

Previous animal studies of *P. amarus* extracts have generally reported low acute toxicity, including survival at oral doses up to 5000 mg/kg, but the available literature varies in extract type, study design and endpoints assessed.[5,6,7] Haematological data are particularly relevant because changes in leukocyte, erythrocyte and platelet indices may provide early evidence of systemic toxicity or altered haematopoiesis.[8,9,10]

*Corresponding author. mail: b.usman305@gmail.com
Tel: +234 803 078 7257

Citation: Usman AB and Abdullahi H. Acute And 28-Day Repeated-Dose Oral Toxicity of Aqueous Leaf Extract of *Phyllanthus Amarus* In Wistar Albino Rats: Mortality, Clinical Signs and Haematological Indices. Trop J Phytochem Pharm Sci, 2026, 5(7); 683 - 688
http://www.doi.org/10.26538/tjpps/v5i7.2

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

The present study, therefore, evaluated the acute and 28-day repeated-dose oral toxicity of an aqueous leaf extract of *P. amarus* in Wistar rats using mortality, clinical observations and full blood count indices as primary endpoints.

Materials and Methods

Plant material, authentication and extraction

Fresh leaves of *P. amarus* were collected from the Kwalkwalawa area, Dundaye village, Wamakko Local Government Area, Sokoto State, Nigeria, during the rainy season (August-September 2019). The plant was authenticated at the Herbarium of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, and a voucher specimen was deposited under voucher number UDUH/ANS/0202. Air-dried leaves were powdered, and 100 g of material was macerated in 1000 mL of distilled water overnight with gentle agitation. The mixture was filtered through Whatman No. 1 filter paper, concentrated *in vacuo* at 50 ± 5°C and lyophilised to obtain the crude aqueous extract.

Experimental animals and ethical approval

Thirty-four Wistar rats of either sex (9-11 weeks old; 150 ± 20 g) were obtained from the Department of Veterinary Pharmacology, Ahmadu Bello University, Zaria, Nigeria. Rats of both sexes were randomly allocated to the experimental groups; the number of male and female rats in each group was not recorded, and data from both sexes were therefore pooled and not analysed separately. Animals were acclimatised for two weeks in the animal house of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, under standard husbandry conditions (ambient temperature 30 ± 5°C, relative humidity 55 ± 5% and 12-hour light/dark cycle), with free access to standard pelletised feed and water. Ethical approval was

© 2026 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

obtained from the Usmanu Danfodiyo University Faculty of Veterinary Medicine Animal Research Ethics Committee (UDUS/FAREC/AUP-R07/2019), and procedures were conducted in accordance with institutional animal-welfare requirements.

Acute oral toxicity study
A fixed-group limit-dose acute oral toxicity design was used. The limit doses (2000 and 5000 mg/kg body weight) and the 14-day observation period were adopted from Organisation for Economic Co-operation and Development (OECD) Test Guideline 425.[11] However, the design did not follow the sequential up-and-down procedure of that guideline, in which single animals are dosed in sequence and each subsequent dose is selected according to the outcome in the preceding animal, without a concurrent control group. Instead, fixed groups of three rats, including a concurrent vehicle control, were used. Nine rats were allocated to three groups of three. Group I received distilled water, whereas Groups II and III received a single oral dose of extract at 2000 and 5000 mg/kg body weight, respectively. Within each extract group, rats were dosed one at a time at 24-hour intervals (Group II on days 1-3 and Group III on days 4-6). Animals were observed closely during the first hours after dosing and thereafter daily for 14 days for mortality and overt clinical signs, including changes in fur or mucous membranes, behaviour, tremors, salivation and diarrhoea. On day 15, animals were anaesthetised and blood was collected into anticoagulated tubes for haematological assessment.

Twenty-eight-day repeated-dose oral toxicity study
Twenty-five rats were randomly allocated to five groups of five. Group I received distilled water, while Groups II-V received 250, 500, 750 and 1000 mg/kg body weight of aqueous extract, respectively, by oral gavage once daily for 28 consecutive days. Animals were monitored daily for mortality and overt clinical signs. On day 29, animals were anaesthetised and blood was collected by cardiac puncture into ethylenediaminetetraacetic acid (EDTA)-anticoagulated tubes.

Haematological analysis
Full blood count was determined on EDTA-anticoagulated whole blood using an automated impedance-based haematology analyser (Genesis HA6000, Perlong Medical Equipment, China). Measured indices included total white blood cell count and differential indices, red blood cell count, haemoglobin, haematocrit and red-cell indices, as well as platelet count and platelet indices.

Statistical analysis
Data were analysed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA) and are presented as mean ± standard error of the mean (SEM). Group differences were evaluated using one-way analysis of variance followed by Tukey's honestly significant difference (HSD) post hoc test. Statistical significance was set at $p \leq 0.05$.

Results and Discussion

Acute mortality, clinical observations and apparent lethal-dose range
No mortality occurred in the control, 2000 mg/kg or 5000 mg/kg groups during the 14-day observation period (Table 1). No treatment-related changes in fur, mucous membranes, behaviour, tremors, salivation or diarrhoea were recorded. Within the present design, survival at 5000 mg/kg is consistent with an apparent oral median lethal dose above 5000 mg/kg.
No mortality occurred in the control, 2000 mg/kg or 5000 mg/kg groups during the 14-day observation period (Table 1). No treatment-related changes in fur, mucous membranes, behaviour, tremors, salivation or diarrhoea were recorded. Within the present design, survival at 5000 mg/kg is consistent with an apparent oral median lethal dose above 5000 mg/kg.

Table 1: Acute oral toxicity observations in Wistar rats

Group	N	Dose (mg/kg body weight)	Clinical/behavioural changes (24 h/72 h/14 d)	Mortality
I (control)	3	Distilled water	None/None/None	0/3
II	3	2000	None/None/None	0/3
III	3	5000	None/None/None	0/3

N = number of animals per group.

The absence of mortality at 5000 mg/kg is broadly consistent with previous reports using methanolic or ethanolic *P. amarus* preparations, in which no deaths were observed at similar high oral doses.[5,6,7] Similarly, no mortality or overt signs of toxicity were reported after high single oral doses (up to 3000 mg/kg) of aqueous and methanol leaf extracts of *Senna alata* in mice.[12] However, the acute experiment used only three animals per group and a fixed-group design, which did not follow the sequential up-and-down procedure of OECD Test Guideline 425. It was therefore not designed to provide a precise dose-response estimate for lethality or non-lethal haematological effects. The sex distribution within treatment groups was also not recorded, precluding sex-specific analysis. Accordingly, the result is best interpreted as evidence of low acute lethality under the conditions tested rather than proof of complete safety or a definitive GHS hazard classification.[15]

Acute Haematological findings
Fourteen days after single-dose administration, selected white blood cell and red blood cell indices were significantly higher in the 2000 mg/kg group than in control ($p < 0.05$), while some indices were significantly lower at 5000 mg/kg than at 2000 mg/kg ($p < 0.05$) (Figures 1-3). Platelet indices did not show a consistent significant pattern across the acute groups.

This non-monotonic pattern does not provide evidence of a simple dose-dependent haematotoxic response. Comparable acute studies of *P. amarus* have generally reported preservation of haematological parameters across a wide dose range, although differences in solvent, plant material, animal strain and analytical platform limit direct comparison.[5,7] Furthermore, because the acute assessment was limited to clinical observations and haematological indices, it cannot exclude biochemical, organ-specific, reproductive, genotoxic or other systemic toxic effects

Twenty-eight-day repeated-dose findings
No mortality or overt clinical signs of toxicity were observed during 28 days of daily administration at 250-1000 mg/kg body weight. White blood cell and platelet indices did not differ significantly between treated groups and control ($p > 0.05$) (Figures 5 and 6). Overall analysis of variance identified differences among groups for red blood cell count ($p = 0.003$), haemoglobin ($p = 0.005$) and haematocrit ($p = 0.017$). Post hoc analysis showed that these differences occurred between treated groups; no treated group differed significantly from control for these three indices (Table 2 and Figure 4). Thus, the significant overall analysis of variance for these three indices reflects differences among treated groups, mainly those involving the 1000 mg/kg group, and does not constitute evidence of extract-related toxicity relative to the vehicle control.

© 2026 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)

Table 2: Significant Tukey HSD pairwise comparisons for red-cell indices in the 28-day repeated-dose study

Parameter	Comparison	Mean difference	p value
RBC count	Group II vs Group IV	1.67 × 10^{12}/L	0.025
RBC count	Group IV vs Group V	-2.23 × 10^{12}/L	0.002
Haemoglobin	Group III vs Group V	-2.18 g/dL	0.041
Haemoglobin	Group IV vs Group V	-3.06 g/dL	0.003
Haematocrit	Group IV vs Group V	-11.04 percentage points	0.009

Group I = distilled-water control; Groups II-V = 250, 500, 750 and 1000 mg/kg body weight/day, respectively. No treated-versus-control comparison for RBC count, haemoglobin or haematocrit was statistically significant.

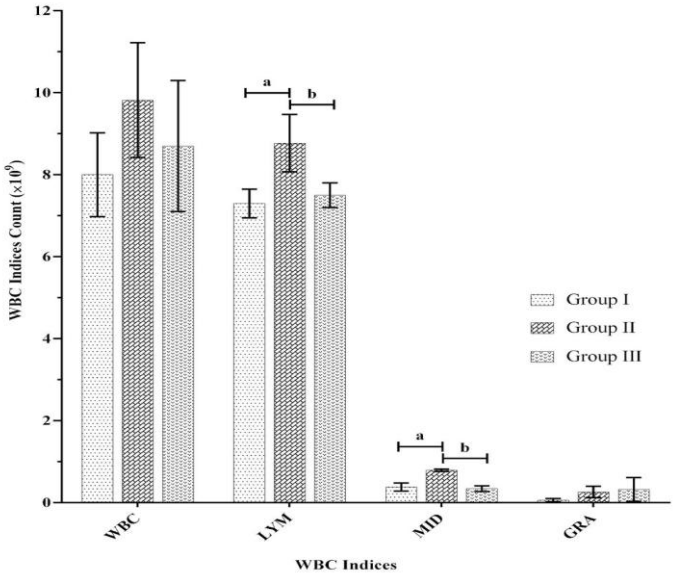

Figure 1: Acute-study white blood cell indices. Values are mean ± SEM. Group I = control; Group II = 2000 mg/kg; Group III = 5000 mg/kg. WBC = white blood cells; LYM = lymphocytes; MID = mid-sized cells; GRA = granulocytes. a, $p < 0.05$ for Group II versus control; b, $p < 0.05$ for Group III versus Group II.

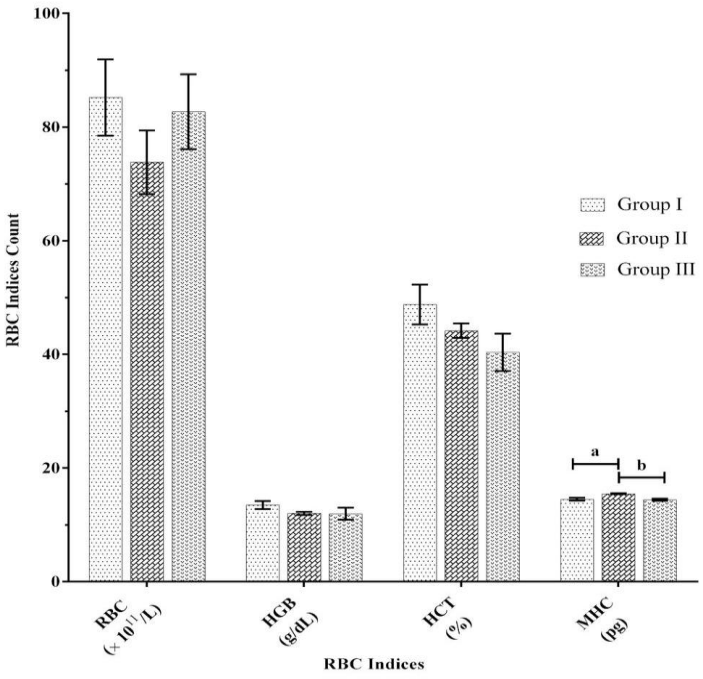

Figure 2: Acute-study red blood cell indices. Values are mean ± SEM. Group I = control; Group II = 2000 mg/kg; Group III = 5000 mg/kg. RBC = red blood cells; HGB = haemoglobin; HCT = haematocrit. MHC = mean corpuscular haemoglobin (pg). a, $p < 0.05$ versus control; b, $p < 0.05$ for Group III versus Group II.

© 2026 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)

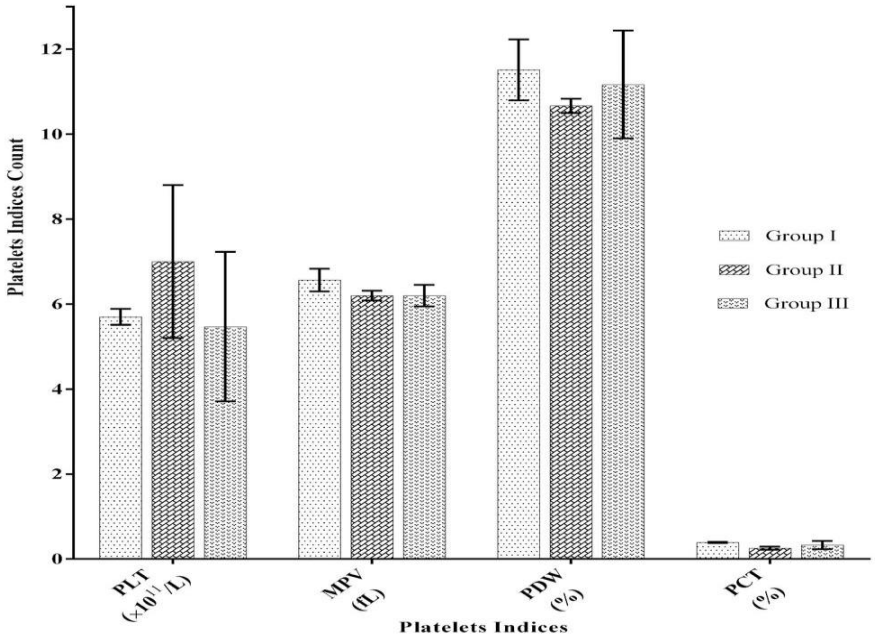

Figure 3: Acute-study platelet indices. Values are mean ± SEM. Group I = control; Group II = 2000 mg/kg; Group III = 5000 mg/kg. PLT = platelet count; MPV = mean platelet volume; PDW = platelet distribution width; PCT = plateletcrit.

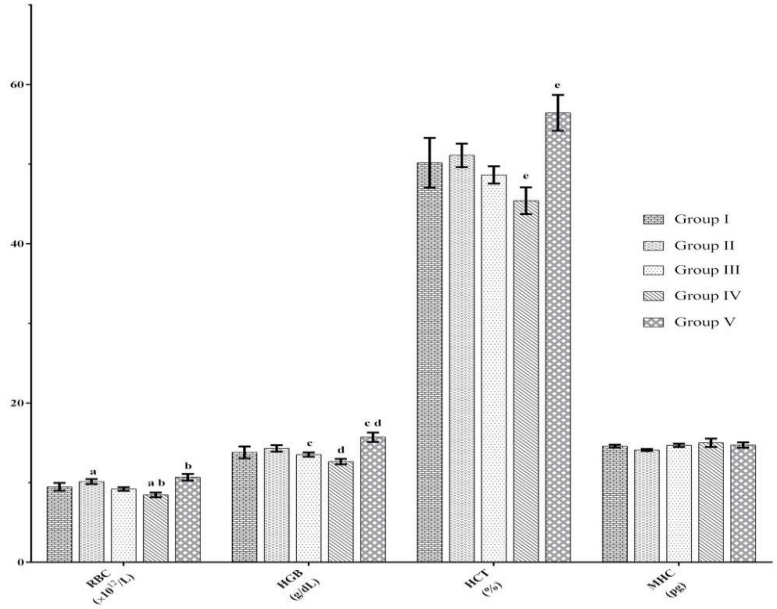

Figure 4: Red blood cell indices after 28 days of repeated oral administration. Values are mean ± SEM. Group I = control; Groups II-V = 250, 500, 750 and 1000 mg/kg/day, respectively. RBC = red blood cells; HGB = haemoglobin; HCT = haematocrit. MHC = mean corpuscular haemoglobin (pg). Bars sharing the same letter (a-e) differ significantly from each other (Tukey HSD, $p < 0.05$; Table 2); no treated group differed significantly from control.

The absence of treated-versus-control differences in red-cell variables, together with stable white-cell and platelet indices and the absence of clinical toxicity, does not indicate a consistent haematological adverse-effect pattern during the 28-day exposure period. The differences among treated groups did not follow a dose-related pattern and, given the small group size (n = 5), are most parsimoniously attributed to biological variability. This is consistent with other 28-day oral studies in Wistar rats, in which red-cell indices were largely unaffected by repeated administration of plant extracts or other test materials.[12,13]

For context, the current OECD repeated-dose 28-day guideline includes broader clinical, biochemical, organ-weight and histopathological endpoints and recommends both male and female animals at each dose level.[14] Because these additional endpoints were not assessed, and exposure was limited to 28 days, the present findings are restricted to mortality, clinical signs and haematological indices. They therefore cannot support conclusions on systemic or organ-specific safety, reproductive or genotoxic effects, chronic toxicity, or human safety.[15]

© 2026 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)

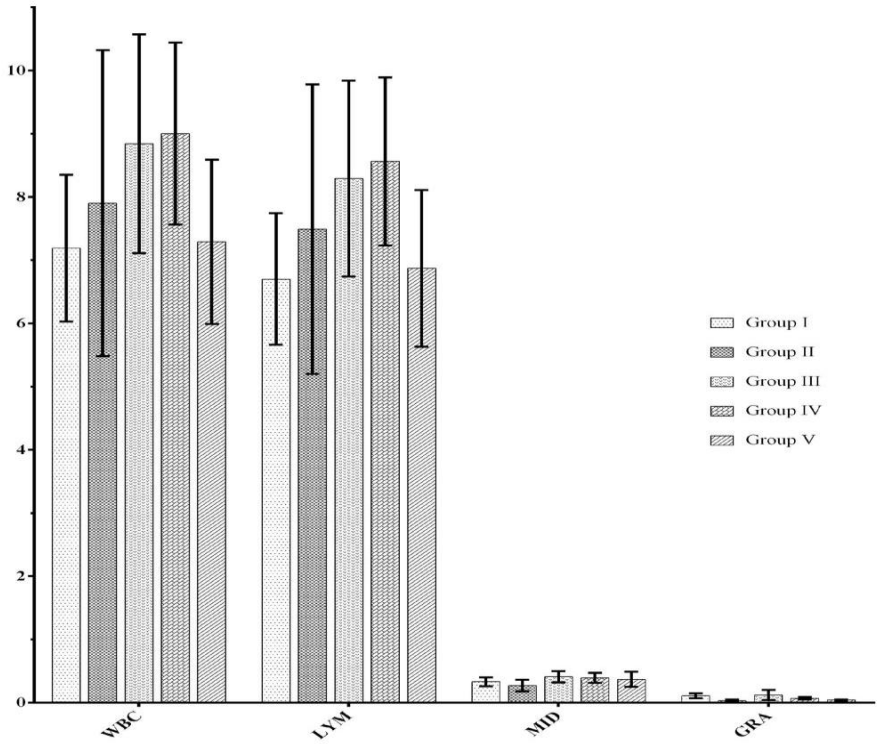

Figure 5: White blood cell indices after 28 days of repeated oral administration. Values are mean ± SEM. Group I = control; Groups II-V = 250, 500, 750 and 1000 mg/kg/day, respectively. WBC = white blood cells; LYM = lymphocytes; MID = mid-sized cells; GRA = granulocytes.

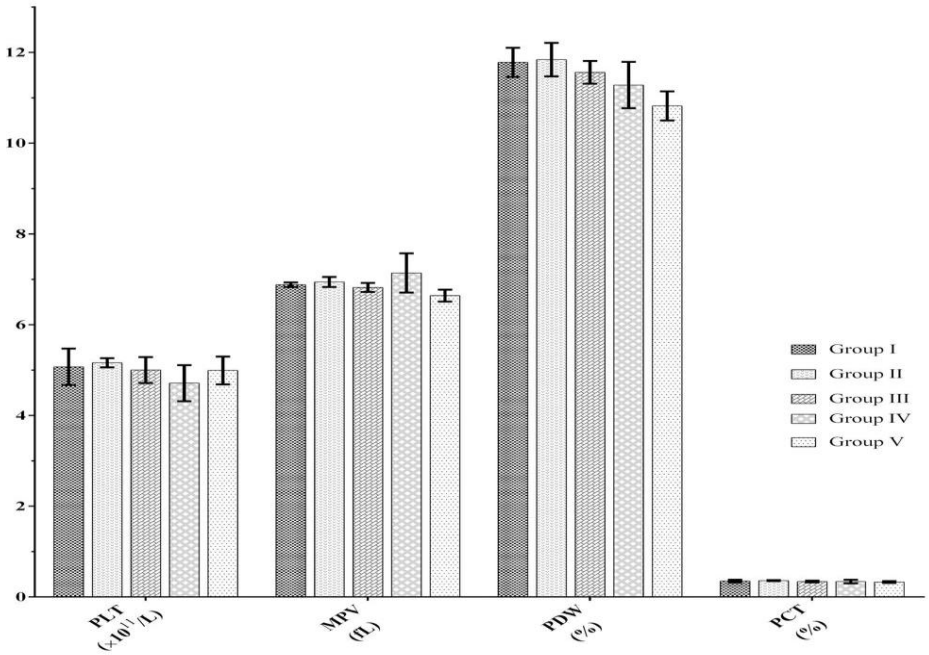

Figure 6: Platelet indices after 28 days of repeated oral administration. Values are mean ± SEM. Group I = control; Groups II-V = 250, 500, 750 and 1000 mg/kg/day, respectively. PLT = platelet count; MPV = mean platelet volume; PDW = platelet distribution width; PCT = plateletcrit.

Conclusion

Under the conditions of this study, aqueous *Phyllanthus amarus* leaf extract produced no mortality or overt clinical toxicity after a single oral dose up to 5000 mg/kg or during 28 days of daily administration up to 1000 mg/kg. The haematological findings did not show a consistent dose-related adverse pattern, and no repeated-dose treatment group differed significantly from control in red blood cell count, haemoglobin or haematocrit after post hoc testing. These findings indicate a low acute and short-term haematological toxicity signal in Wistar rats, but they do not establish organ-specific, chronic

© 2026 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

or human safety. Longer-duration studies with broader systemic-toxicity endpoints and chemical standardization of the extract are warranted.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

Acknowledgement

The authors acknowledge the support of the technical staff of the Department of Immunology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

References

1. Patel JR, Tripathi P, Sharma V, Chauhan NS, Dixit VK. *Phyllanthus amarus*: ethnomedicinal uses, phytochemistry and pharmacology: a review. J Ethnopharmacol. 2011; 138(2): 286-313. Doi: 10.1016/j.jep.2011.09.040
2. Asaduzzaman M, Nahar L, Biswas MS, Podder MK, Rahman MM. *Phyllanthus amarus* as a multifunctional medicinal herb: bioactive compounds, mechanisms, and clinical perspectives. Pharmacol Res Mod Chin Med. 2026; 18: 100752. Doi: 10.1016/j.prmcm.2026.100752
3. Jantan I, Haque MA, Ilangkovan M, Arshad L. An insight into the modulatory effects and mechanisms of action of *Phyllanthus* species and their bioactive metabolites on the immune system. Front Pharmacol. 2019; 10: 878. Doi: 10.3389/fphar.2019.00878
4. Mensah MLK, Komlaga G, Forkuo AD, Firempong C, Anning AK, Dickson RA. Toxicity and safety implications of herbal medicines used in Africa. In: Builders PF (Ed.). Herbal medicine. London: IntechOpen; 2019. Doi: 10.5772/intechopen.72437
5. Kushwaha SK, Dashora A, Dashora N, Patel JR, Kori ML. Acute oral toxicity studies of the standardized methanolic extract of *Phyllanthus amarus* Schum & Thonn. J Pharm Res. 2013; 6(7): 720-724. Doi: 10.1016/j.jopr.2013.04.020
6. Oduola T, Muhammad AA, Aiyelabegan F, Tajudeen M, Okalawon SO. Hepatotoxic assessment of *Phyllanthus amarus* leaf extract in Wistar rats. Eur J Med Plants. 2018; 23(4): 1-11. Doi: 10.9734/EJMP/2018/41238
7. Abu OD, Okolie NP, Ekperusi SE, Marcel LI. Acute toxicity study of ethanol extract of *Phyllanthus amarus* leaves in Wistar albino rats. Biomed J Sci Tech Res. 2024; 57(2): 49010-49013. Doi: 10.26717/BJSTR.2024.57.008970
8. Ebhohon SO, Asoya EV, Iyare HE, Akerele OR, Ezedimbu MC. Effect of aqueous leaf extract of *Justicia carnea* on hematological parameters of male Wistar rats exposed to thioacetamide. Trop J Phytochem Pharm Sci. 2023; 2(2): 55-58. Doi: 10.26538/tjpps/v2i2.2
9. Obidah W, Onoja RI, Johnson J, Eze VF, David A, Suleiman N, Linus VB, Midala SD, Ini M, Ja'afar JN, Zailani HA. Therapeutic and overdose effects of camel urine in male Wistar rats. Trop J Phytochem Pharm Sci. 2025; 4(7): 303-307. Doi: 10.26538/tjpps/v4i7.3
10. Ajani OS, Obiechefu CH, Ake AS. Therapeutic effects of *Cyperus esculentus* on monosodium glutamate-induced haemo-biochemical and steroidogenic anomalies in male Wistar rats. Trop J Phytochem Pharm Sci. 2025; 4(2): 37-44. Doi: 10.26538/tjpps/v4i2.1
11. Organisation for Economic Co-operation and Development (OECD). Test No. 425: Acute oral toxicity: up-and-down procedure. OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing; 2022. Doi: 10.1787/9789264071049-en
12. Baba RT, Salaudeen RA, Aladodo M, Dangana ZA, Muhammad ZK, Sowole HB, Sani HU, Yakubu F. Acute and sub-acute toxicity studies of *Senna alata* on mice and Wistar rats. Trop J Phytochem Pharm Sci. 2026; 5(6): 663-671. Doi: 10.26538/tjpps/v5i6.6
13. Okpokam DE, Okere SO, Anyanwu GO. Effect of bread improver dough conditioner used in commercial bread production on some biochemical, oxidative stress and haematological parameters in male Wistar rats. Trop J Phytochem Pharm Sci. 2025; 4(9): 398-403. Doi: 10.26538/tjpps/v4i9.4
14. Organisation for Economic Co-operation and Development (OECD). Test No. 407: Repeated dose 28-day oral toxicity study in rodents. OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing; 2025. Doi: 10.1787/9789264070684-en
15. United Nations. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). (10th revised ed.). New York and Geneva: United Nations; 2023.

© 2026 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License