International Journal of Biotechnology and Microbiology
www.biotechnologyjournals.com
Online ISSN: 2664-7680
Received: 05-01-2026, Accepted: 04-02-2026, Published: 19-02-2026
Volume 8, Issue 1, 2026, Page No. 30-37

Antibacterial and immunomodulatory effects of ethanolic extract of *Cnidoscolus chayamansa* (Hospital too far) leaves

Imarhobobhor L[1], Okorhi Damisa F B[2], Enakireru D B[2]
[1] Department of Microbiology, Faculty of Science, Edo state University Uzaire, Edo State, Nigeria
[2] Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, P.M.B, Delta State, Nigeria

Abstract
The growing prevalence of multidrug-resistant microorganisms and immune-related disorders has intensified the search for plant-derived therapeutic agents. *Cnidoscolus chayamansa*, commonly known as "Hospital Too Far," is traditionally used for managing various ailments, yet its antibacterial and immunomodulatory properties remain poorly documented. This study evaluated the ethanolic extract of *C. chayamansa* (CCEE) against multidrug-resistant bacteria and in Wistar rats. Phytochemical screening revealed alkaloids, terpenoids, steroids, tannins, saponins, anthraquinones, cardiac glycosides, and cyanogenic glycosides. Hematological parameters were assessed using a Susmex machine, while immunological activity was measured through delayed-type hypersensitivity (DTH) and hemagglutination assays. CCEE produced no significant changes in most hematological indices, though mean corpuscular volume increased slightly at 50 mg/kg. DTH assays showed mild stimulation of cellular immunity, with footpad swelling at 50 mg/kg (0.65±1.12) and 200 mg/kg (0.70±1.52), compared to the negative control (0.39±0.66), but markedly lower than the positive control (7.15±0.21). Humoral immunity was suppressed, as antibody titers decreased at both doses relative to controls. Antibacterial testing demonstrated activity only against *Escherichia coli* and *Enterococcus faecalis*, with inhibition zones of 10-14 mm. The minimum inhibitory and bactericidal concentrations for sensitive isolates were 50 mg/ml. Acute and sub-acute toxicity studies revealed no lethality up to 5,000 mg/kg, confirming safety at lower doses. However, histological analysis showed degenerative changes in liver, spleen, kidney, heart, and bone marrow at higher doses (100-200 mg/kg).Overall, CCEE exhibited limited antibacterial activity, suppressed both cellular and humoral immunity, and was safe at lower doses, though higher concentrations caused organ alterations.

Keywords: Antibacterial, immunomodulatory, ethanolic extract, *cnidoscolus chayamansa*, wistar rats, toxicity, phytochemicals

Introduction
The prevalent development of multidrug-resistant microbes and malfunctioning of the immune system is a significant health concern to the population. (WHO, 2020; Murray *et al*., 2022) Alternative therapeutic agents, thus, have been the use of medicinal plants which are rich in phytochemical content and which are relatively safe. (Abubakar *et al*., 2021) [2] The world health organization defines medicinal plants as plant that had substances that were useful in therapeutic purposes or as precursors of drug production. (WHO, 2019; WHO, 2023)
Cnidoscolus chayamansa is an evergreen shrub commonly applied in traditional medicine in treating infections and inflammatory diseases. Its bioactive constituents have been proposed in previous studies to play a role in antibacterial and immunomodulatory action. The present study was thus aimed at the assessment of the antibacterial and immunomodulatory property of the ethanolic and aqueous leaf extracts of *C. chayamansa* on the basis of the experiment *in vitro* and *in vivo* models.

Materials and Method
The sample of Fresh *Cnidoscolus chayamansa* leaves was taken in a garden in Akure, Nigeria and confirmed by the University of Benin experts. The extracts were prepared by washing and drying the leaves over a few months before being ground into powder after which they were subjected to ethanol and water using a Soxhlet extractor. It was then concentrated, dried and stored to be used in subsequent experiments (Abubakar *et al*., 2021; Zahid *et al*., 2025) [2, 37].

Collection and maintenance of test organisms
Five bacteria isolates from clinical samples were obtained from the Department of Microbiology, University of Benin, Benin City Edo State: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Enterococcus faecalis*. The pure isolates were chosen based on their clinical and pharmacological importance. They were maintained in Nutrient agar slant at the temperature of 37 ^{0}C and preserved in the refrigerator at 4 ^{0}C for further use. (Abdulraheem, I. A. 2022; Princewill *et al*., 2025) [1, 30]

Supporting Materials
Media preparation
Normal microbiology cultures such as Mueller Hinton agar and nutrient broth were prepared. There were other chemicals such as cyclophosphamide (immune suppressant), levamisole (immune stimulant) and various solvents. (Leaf, S. P 2021; Sitati, C. N. W 2025) [24, 33].

Sheep Red Blood Cells
Sheep blood used in this research was collected in a local slaughterhouse, treated to eliminate undesired elements and a suspension made. This was employed to test the rats with immune responses. (Iwetan *et al*., 2022, 2025) [21, 22]

Phytochemical Screening
Both ethanolic and aqueous extracts were checked for natural compounds such as alkaloids, flavonoids, tannins, saponins, steroids, and glycosides all of which are known to have biological activity. (Fachriyaha *et al*., 2020; Adeleye and Risenga 2022) [3]

Experimental Animals
The study was done on albino rats. They were kept in clean and well ventilated conditions and were fed common diets and given some time to acclimatize before the experiments commenced. All the processes were based on international animal care principles. (Prepared by the Animal Facilities Standards Committee of the Animal Care Panel, 2021) [29]

Hematology and Toxicity
The rats were split into groups whereby some were only administered with water and others with various doses of the plant extract (50, 100 or 200 mg/kg) during the three weeks. They were monitored on daily changes in health and body weights were recorded. The blood samples were examined in terms of the red and white count, the hemoglobin, platelets, and other indicators. Liver, kidney, and spleen were examined using a microscope to determine whether it had been damaged. Body weights were also compared to the organ weights to determine changes.

Immunomodulatory Studies
The effect of the plant extract on the immune system was seen using another group of rats. The extract was administered to some groups and to others together with cyclophosphamide (to suppress immunity) and levamisole (to stimulate immunity). (Barakat, *et al*., 2023) Immune responses were measured in two ways:

- **Delayed-type hypersensitivity:** Rats were injected with sheep red blood cells and then they were tested to ascertain the amount to which their paw swelled an indicator of immune response. (Ahmad, *et al*., 2023) [12]
- **Humoral response:** Antibodies against the sheep red blood cells were tested in a hemagglutination test using the blood samples. (Athanasiou *et al*., 2021)

Antibacterial Testing
Agar well diffusion was done on the extracts basically by dropping extracts in wells on a bacterial plate and determining the clear zones where the bacteria did not grow. The outcomes were contrasted with the conventional antibiotics such as gentamycin and ciprofloxacin. To find the amount of extract required to prevent or kill bacteria, (Akinduti, *et al*., 2022) [6] determined the minimum inhibitory and bactericidal concentrations.

Acute Toxicity
Safety was tested by administering increasing doses of the extract to rats beginning with very small doses up to very high doses (5,000mg/kg). The 24-hour observation was made on them in case of any illness or abnormal behavior. At any dose, no toxic effects were observed. (Frederickson, *et al*., 2022)

Data Analysis
Statistical analysis was done with SPSS software to analyze all the results. The significance of differences between groups was taken to be significant below probability value of 0.05. (Fiandini, *et al*., 2024)

1. Phytochemical Composition
Phytochemical screening showed that the ethanolic extract of *Cnidoscolus chayamansa* had alkaloids, tannins, terpenoids, steroids, saponins, anthraquinones, cardiac glycosides and cyanogenic glycosides whereas the aqueous extract had only steroid, saponins, anthraquinones, cardiac glycosides and cyanogenic glycosides. Both extracts did not contain flavonoids. (Ajiboye *et al*., 2021; García-Ramos *et al*., 2024) [5, 19].

2. Physical and Behavioral Observations
Throughout 21 days of treatment, rats in the 50mg/kg group were relatively normal in feeding and movement behavior, rats in 100 and 200mg/kg groups were slower in activity and less fed. One of the rats in the 200 mg/kg group had dirty fur and bloody nostrils prior to being sacrificed. (OECD, 2021; Turner *et al*., 2021) [34]

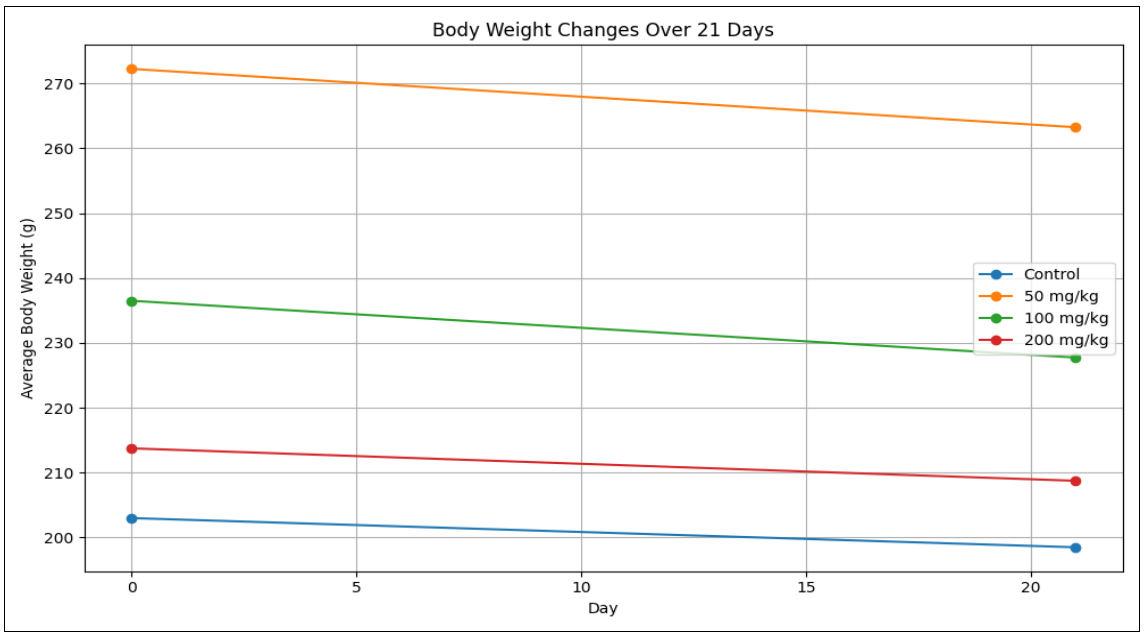


Fig 2: Shows average body weight changes across groups

3. Immunological Responses

Delayed-Type Hypersensitivity (DTH)

Extract-treated groups showed mild footpad swelling (0.65-0.70 mm), far lower than the positive control (Levamisole, 7.15 mm). When combined with cyclophosphamide, swelling increased compared to cyclophosphamide alone.

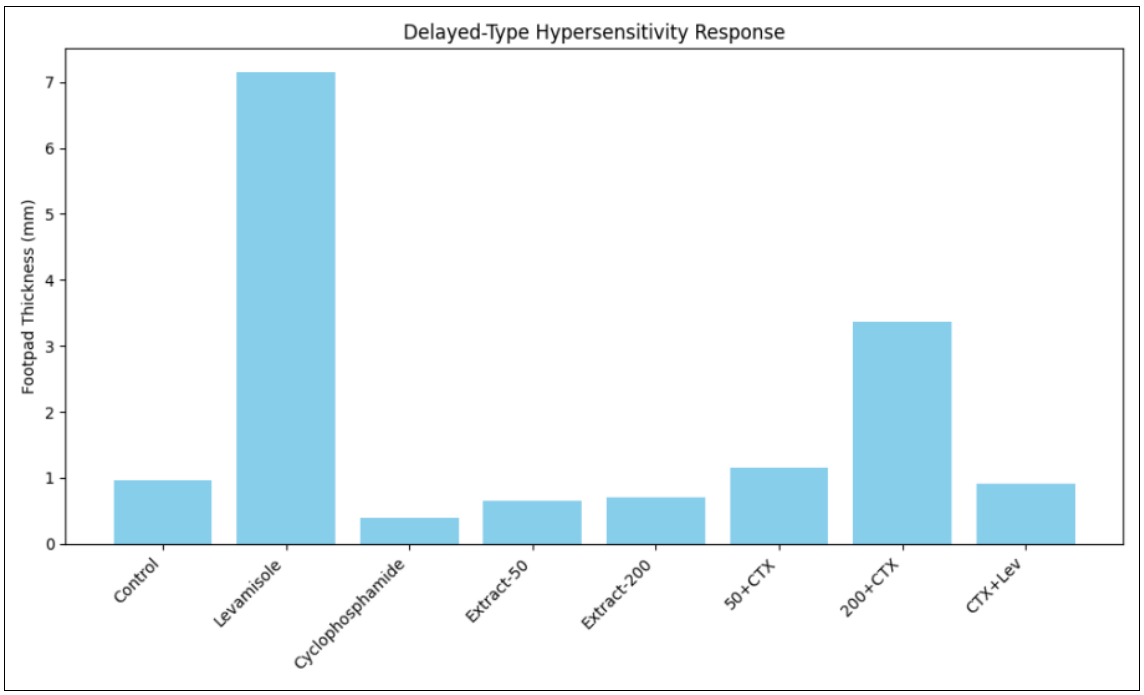


Fig 3: presents DTH footpad thickness across groups

Hemagglutination Assay

Antibody titers were reduced in extract-treated groups (0.21-0.23) compared to controls (0.27-0.28). Extract + cyclophosphamide further suppressed titers, while cyclophosphamide + levamisole increased titers (0.33).

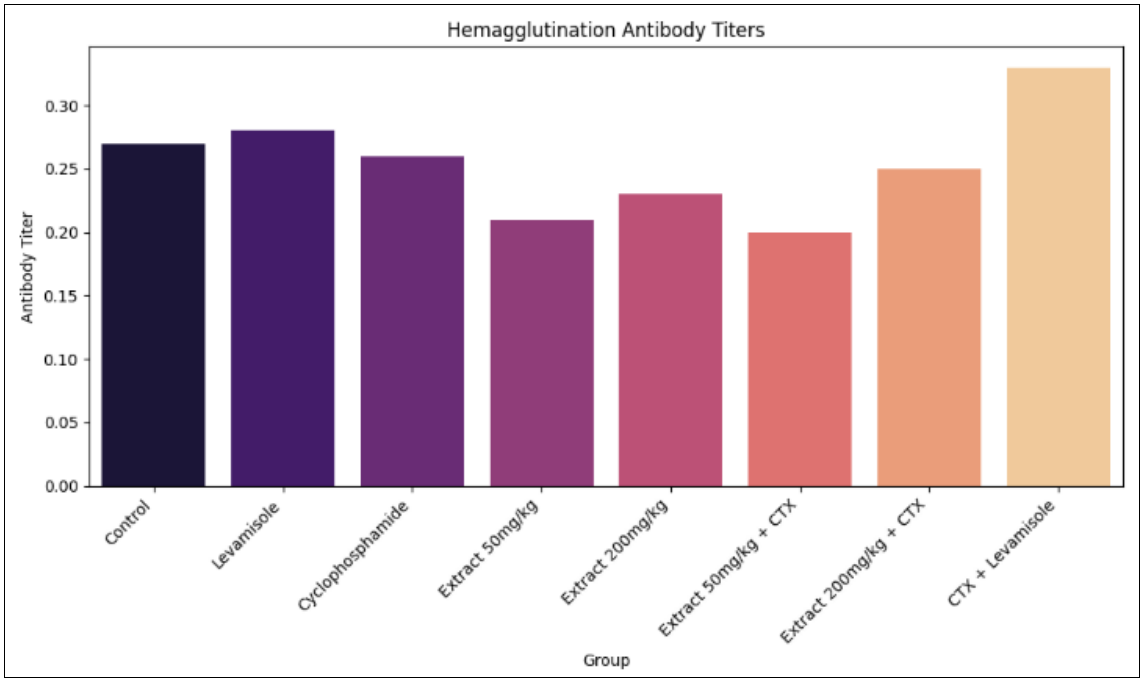


Fig 4: Shows antibody titers across groups

4. Hematological Parameters

There was a significant reduction of WBC and MCH values in the treated groups as compared to the controls. At 50 mg/kg MCV and MCH were slightly increased. The values of RBC, HGB, HCT, PLT, and PCT decreased in all the treated groups. At higher doses, there was an increase in relative organ weights of liver and spleen.

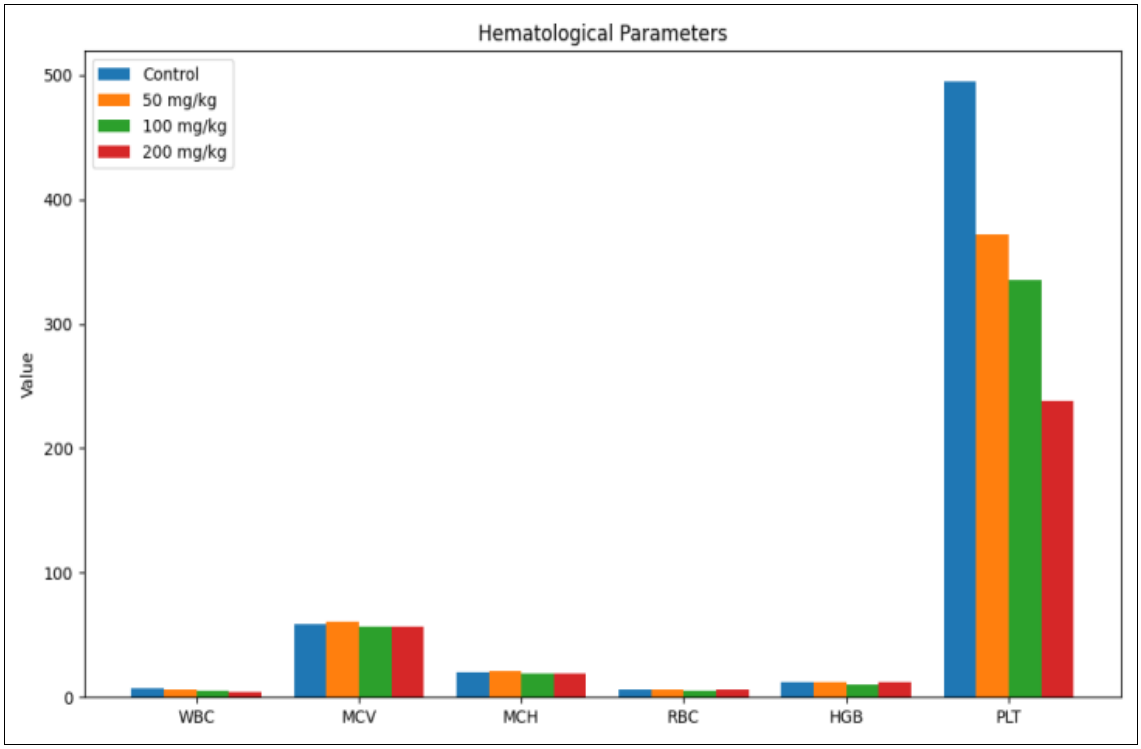


Fig 5: Summarizes hematological parameters across groups

5. Histopathology

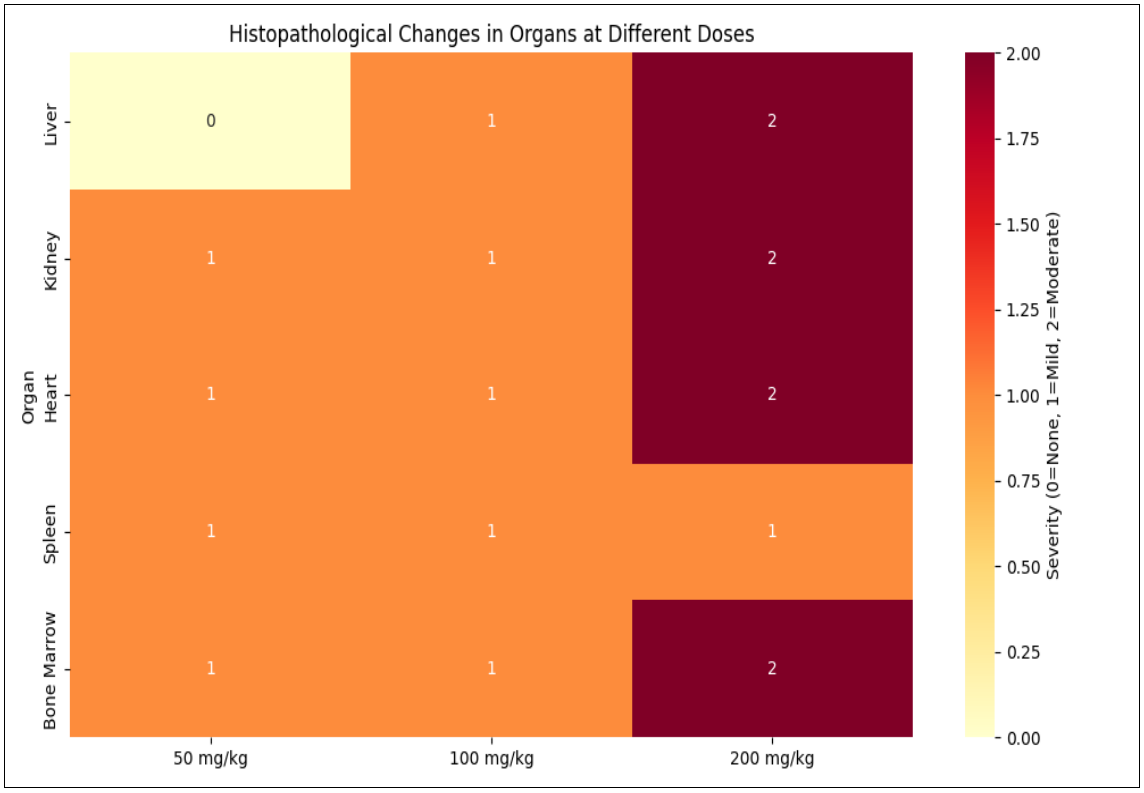
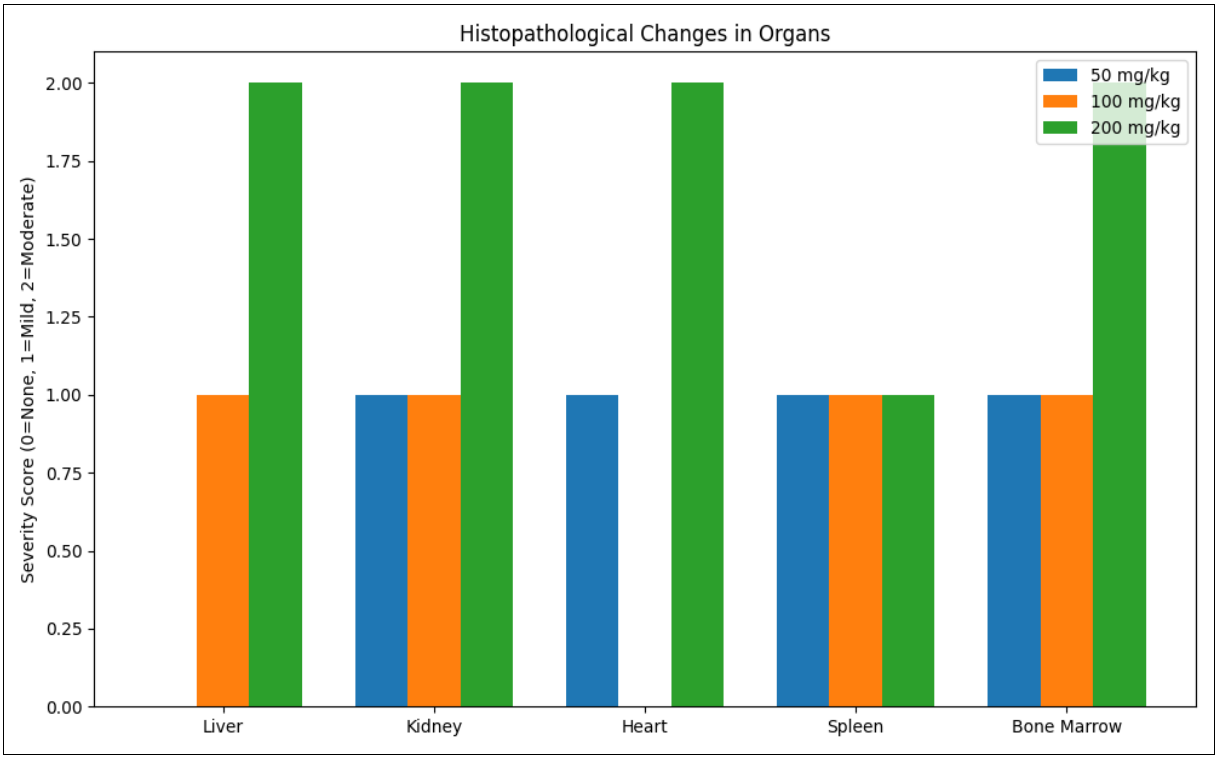


Fig 6-10: show representative photomicrographs of liver, kidney, heart, spleen, and bone marrow

- **Liver:** Mild fatty changes at 100 mg/kg; inflammatory infiltration and thickened portal vein at 200 mg/kg.
- **Kidney:** Mild glomerular thickening at 100 mg/kg; tubular necrosis at 200 mg/kg.
- **Heart:** Slight thickening of coronary artery at 50 and 200 mg/kg.
- **Spleen:** Mild dilation of central artery at all doses.
- **Bone marrow:** Fat cell infiltration at 50-100 mg/kg; reduced marrow cells at 200 mg/kg.



6. Antibacterial Activity

The ethanolic extract inhibited *E. coli* and *E. faecalis* with zones of inhibition ranging from 10-14 mm. MIC and MBC were both 50 mg/ml. No activity was observed against *S. aureus*, *P. aeruginosa*, or *K. pneumoniae*. The aqueous extract showed no antibacterial activity.

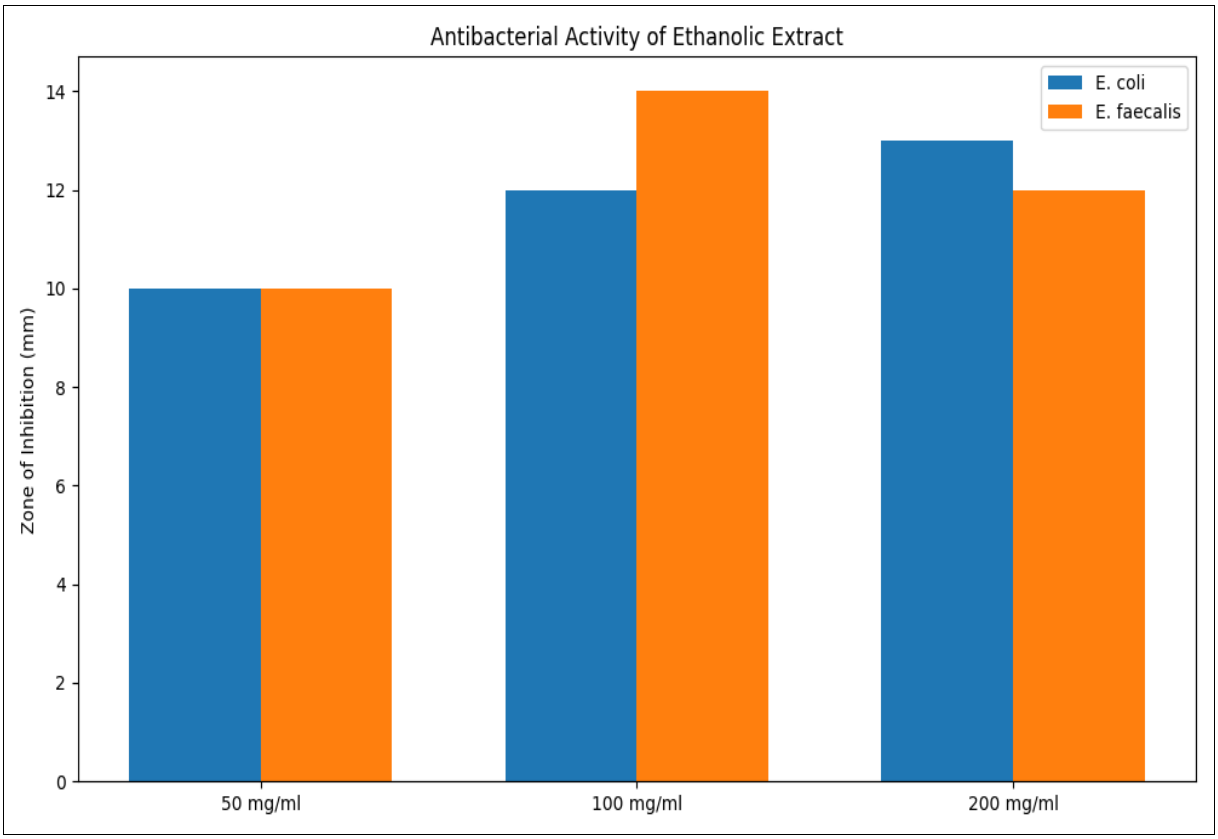
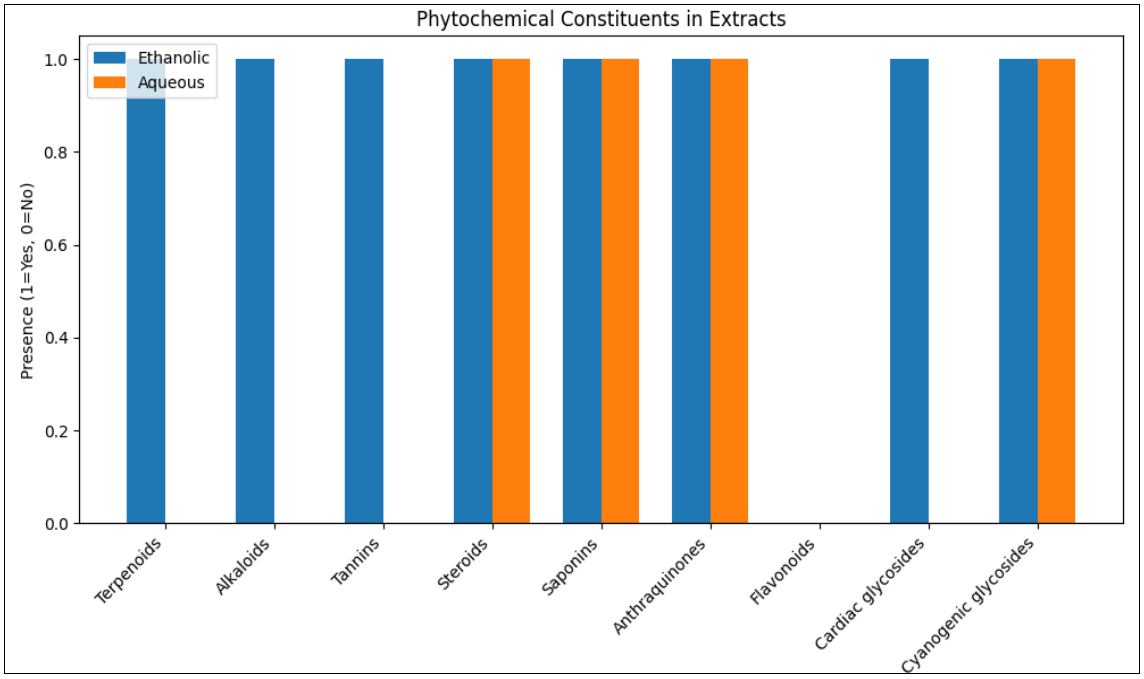


Fig 11: Compares inhibition zones of ethanolic extract against sensitive isolates

7. Toxicity Studies

Acute toxicity studies revealed no lethality up to 5,000 mg/kg (Lorke's method). Sub-acute studies showed histological changes at higher doses (100-200 mg/kg), but minimal alterations at 50 mg/kg.Figure



Discussion

The current research examined the immunomodulation action of *Cnidoscolus chayamansa* leaf extracts on rats and the antimicrobial effects of the leaf extracts on selected multidrug-resistant bacterial strains. The screening of phytochemicals showed that the ethanolic extract possessed a variety of bioactive compounds such as terpenoids, tannins, saponins, steroids, alkaloids, cardiac and cyanogenic glycosides, and anthraquinones but the aqueous counterpart exhibited a low phytochemical profile. This difference in phytochemical composition has been reported before and has been observed to be due to solvent-dependent extraction efficiencies and phytochemical distributions in plant matrices. Ajiboye *et al*., 2021; Salehi *et al*., 2022; García-Ramos *et al*., 2024) [5, 19, 31].

Sub-acute toxicological evaluation revealed that ethanolic extract 100mg/kg and 200mg/kg did indeed lead to reduced feed and water consumption, which may be a property of an appetite-suppressant. Despite a decrease in hematological parameters when compared with the control animals, no statistical significance ($p < 0.05$) could be seen. The general depression of erythrocyte counts and hemoglobin, packed cell volume and indices has the potential to reveal inhibition of haemopoietic system or elevated hemolysis, which have been previously attributed to some phytoconstituents like alkaloid and tannins. (OECD, 2021)

Footpad thickness in reaction to antigenic challenge was increased in all groups immunologically but rats in the extract treatment had attenuated responses compared to negative and positive controls. This finding points to the possibility of cell-mediated immunity suppression, which may occur through an inhibitory action on the T-lymphocyte activation or the cytokine signalling. Also, the decrease in circulating antibody titres indicate an impairment of humoral immune responsiveness, and this might come about by interfering with the antigen processing pathway or activation pathway of lymphocytes. These trends of immunosuppression are consistent with the literature that certain phytochemicals, such as saponins and cyanogenic glycosides, have the ability to alter the activity of immune cells. (Mahmoud, *et al* 2021) [25]

The antimicrobial assay also did not find any inhibitory effects of the aqueous extract against *Escherichia coli* and *Enterococcus* faecalis but only the ethanolic extract showed measurable antibacterial action. The ethanolic extract has got antibacterial potential which can be attributed to tannins which are well known to have antimicrobial property against Gram negative and Gram positive bacteria. The results are consistent with some of these studies which show selective antibacterial activity of extracts of *Cnidoscolus* species, although activity across disorders and isolates has been found to vary. (Saraiva, *et al* 2012, De Oliveira-Júnior, *et al* 2018, Do Nascimento, *et al* 2025) [14, 15, 32].

Histopathologic assessment showed organ-specific adverse effects of medium and high doses, especially of heart, liver, and spleen, kidney, and bone marrow tissues. Although no evidence of acute toxicity was detected, chronic or repeated exposure at higher levels seems to be potentially dangerous of organ dysfunction, which could be possibly attributed to bioactivity of secondary metabolites such as saponins, hydrocyanides, and terpenoids. (Alamgir.*et al* 2018, Ohiagu, *et al* 2021) [8, 27] The above observations highlight the challenge of understanding the safety of medicinal plants, in which the history of traditional use does not necessarily correspond to lack of toxicity. (Khoobchandani, 2024, Aliu, *et al* 2025) [9, 23]

Collectively, the data indicate that *C. chayamansa* leaf extracts exhibit a multifaceted biological profile characterized by immunomodulatory suppression, selective antibacterial activity, and potential organ toxicity at higher dosage regimens. These results expand upon existing phytochemical and pharmacological knowledge of *C. chayamansa* and parallel reports of its traditional therapeutic applications and limitations in related pharmacological contexts. (Al Kazman, *et al* 2022, Chihomvu, *et al* 2024) [7, 13].

Conclusion

In summary, chronic administration of *Cnidoscolus chayamansa* ethanolic leaf extract alters hematological indices and immune responsiveness in rats, and demonstrates inhibitory activity against selected multidrug-

resistant bacteria. While no acute lethal effects were observed, higher and repeated dosing may predispose to organ-specific toxicity. These findings suggest that although *C. chayamansa* contains bioactive compounds with potential pharmacological utility, caution is warranted regarding its prolonged or high-dose use. Further research is recommended to elucidate the mechanisms underlying its immunomodulatory effects and to clarify the scientific basis for its traditional use in the management of anemia and other ailments, as well as to establish safe therapeutic boundaries.

Declaration of Competing Interest

The authors certify that they have NO affiliation with or involvement in any organization or entity with any financial in the subject matter discussed in this manuscript.

Acknowledgements

I appreciate the authorities and staff of Federal University of Petroleum Resources, Effurun (FUPRE) (Environment Management and Toxicology Department) and Edo State University Uzaire, Edo State (Department of Microbiology, Faculty of Science).

Data availability: Data will be made available on request.

References

1. Abdulraheem IA. *In vitro* Antimicrobial Assay and Phytochemical Screening of the Bioactive Components of *Datura metel* (LINN)(GEGEMU) on Selected Clinical Isolates (Master's thesis, Kwara State University (Nigeria)), 2022.
2. Abubakar MS, Shehu RA, Umar M. Medicinal plants as sources of bioactive compounds for antimicrobial and immunomodulatory activities: A review. Journal of Ethnopharmacology,2021:268:113576. https://doi.org/10.1016/j.jep.2020.113576
3. Adeleye OC, Risenga IM. Screening of phytochemical profile and biological activities in the leaves, stems and roots of South African *Portulacaria afra* using four extraction solvents. Biomedical and Pharmacology Journal,2022:15(3):1561-1572.
4. Ahmad W, Jantan I, Haque MA, Arsyad L. Magnoflorine from *Tinospora crispa* upregulates innate and adaptive immune responses in Balb/c mice. International Immunopharmacology,2022:111:109081.
5. Ajiboye BO, Oyinloye BE, Ogunyinka BI. Phytochemical screening and antioxidant activity of medicinal plants: Influence of extraction solvents. Journal of Food Biochemistry,2021:45(6):e13741. https://doi.org/10.1111/jfbc.13741
6. Akinduti PA, Emoh-Robinson V, Obamoh-Triumphant HF, Obafemi YD, Banjo TT. Antibacterial activities of plant leaf extracts against multi-antibiotic resistant *Staphylococcus aureus* associated with skin and soft tissue infections. BMC complementary medicine and therapies,2022:22(1):47.
7. Al Kazman BS, Harnett JE, Hanrahan JR. Traditional uses, phytochemistry and pharmacological activities of Annonacae. Molecules,2022:27(11):3462.
8. Alamgir ANM. Secondary metabolites: Secondary metabolic products consisting of C and H; C, H, and O; N, S, and P elements; and O/N heterocycles. In Therapeutic use of medicinal plants and their extracts: volume 2: phytochemistry and bioactive compounds (pp. 165-309). Cham: Springer International Publishing, 2018.
9. Aliu TB, Obun FE, Raji H, Badmus K. Safety Evaluation and Concerns of Natural Products in Traditional Medicine. AROC Pharm. Biotechnol,2025:5:9-17.
10. Antimicrobial resistance and plant-derived bioactive compounds: An overview of phytochemicals as alternative therapeutics. Frontiers in Pharmacology,2022:13:805843. https://doi.org/10.3389/fphar.2022.805843
11. Athanasiou LV, Spanou VM, Katsogiannou EG, Katsoulos PD. Hematological Features in Sheep with IgG and IgM Antibodies against *Borrelia burgdorferi* sensu lato. Pathogens,2021:10(2):164.
12. Barakat H, Alkhurayji RI, Aljutaily T. Immune-boosting potentiating properties of *Brassica nigra* hydroalcoholic extract in cyclophosphamide-induced immunosuppression in rats. Foods,2023:12(19):3652.
13. Chihomvu P, Ganesan A, Gibbons S, Woollard K, Hayes MA. Phytochemicals in drug discovery—A confluence of tradition and innovation. International journal of molecular sciences,2024:25(16):8792.
14. De Oliveira-Júnior RG, Ferraz CA, Pontes MC, Cavalcante NB, da Cruz Araujo EC, de Oliveira AP, *et al*. Antibacterial activity of terpenoids isolated from *Cnidoscolus quercifolius* Pohl (Euphorbiaceae), a Brazilian medicinal plant from Caatinga biome. European Journal of Integrative Medicine,2018:24:30-34.
15. Do Nascimento JB, Gonçalves Castro JW, Inácio da Silva M, Viturino JJF, Pereira da Silva M, Donelardy ACC, *et al*. Chemical Profile with Antibacterial and Modulatory Activity of Extracts from the Stem Bark of *Cnidoscolus quercifolius* Pohl. Current Bioactive Compounds,2025:21(2):E130524229907.
16. Fachriyaha E, Kusrinia D, Haryanto IB. Phytochemical test, determination of total phenol, total flavonoids and antioxidant activity of ethanol extract of moringa. J Kim Sains Apl,2020:23(8):290-4.
17. Fiandini M, Nandiyanto ABD, Al Husaeni DF, Al Husaeni DN, Mushiban M. How to calculate statistics for significant difference test using SPSS: Understanding students comprehension on the concept of steam engines as power plant. Indonesian Journal of Science and Technology,2024:9(1):45-108.
18. Frederickson SC, Steinmiller MD, Blaylock TR, Wisnieski ME, Malley JD, Pandolfo LM, *et al*. Comparison of juvenile feed protocols on growth and spawning in zebrafish. Journal of the American Association for Laboratory Animal Science,2021:60(3):298-305.
19. García-Ramos R, López-García L, Hernández-González R. Phytochemical composition and antimicrobial potential of ethanolic and aqueous extracts of *Cnidoscolus chayamansa*. Biotecnia,2024:26:123-135. https://doi.org/10.18633/biotecnia.v26.2233
20. García-Ramos R, López-García L, Hernández-González R. *In vitro* and in silico antioxidant and antimicrobial activity of ethanolic extracts of *Cnidoscolus chayamansa* leaves. Biotecnia,2024:26:123-135. https://doi.org/10.18633/biotecnia.v26.2233

21. Iwetan BB, Kweki GR, Onobrudu DA, Ugochukwu U, Andy OO, Ewhre LO, *et al*. Hepatorenal protection of *Justicia carnea* leaf aqueous extract on sheep red blood cell-induced immunotoxicity in mice. Tropical Journal of Pharmaceutical Research, 2025, 24(5).
22. Iwetan BB, Obianime AW, Ewhre LO, Kweki GR. The Antioxidant Modulating Properties of *Justicia carnea* Extract on Sheep Red Blood Cells Immunized Mice. J. Pharmaceut. Res. Int,2022:34(33B):58-74.
23. Khoobchandani M. Unveiling the Complexity of Herbal Medicine: Safety, Toxicity, and Regulatory Challenges. In Medicinal Applications of Phytopharmaceuticals (pp. 269-282). Cham: Springer Nature Switzerland, 2024.
24. Leaf SPOEA. *In vitro* Antibacterial, *in vivo* Immunomodulatory and (Doctoral dissertation, Kenyatta University), 2021.
25. Mahmoud HS, Almallah AA, Gad EL-Hak HN, Aldayel TS, Abdelrazek HM, Khaled HE. The effect of dietary supplementation with *Nigella sativa* (black seeds) mediates immunological function in male Wistar rats. Scientific Reports,2021:11(1):7542.
26. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, *et al*. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. The Lancet,2022:399(10325):629-655. https://doi.org/10.1016/S0140-6736(21)02724-0
27. Ohiagu FO, Chikezie PC, Chikezie CM. Toxicological significance of bioactive compounds of plant origin. Pharmacognosy Communications,2021:11(2):67-77.
28. Organisation for Economic Co-operation and Development (OECD). OECD guideline for the testing of chemicals: Repeated dose 28-day oral toxicity study in rodents (Test No. 407). OECD Publishing, 2021. https://doi.org/10.1787/9789264070684-en
29. Prepared by the Animal Facilities Standards Committee of the Animal Care Panel. Guide for laboratory animal facilities and care. ILAR journal,2021:62(3):345-358.
30. Princewill N, Chinedu NA, Diaku JI, Godwin EC. Antibiotics Evaluation of Bacterial Isolates Associated with Imo State University Microbiology Laboratory and Sub-Offices. World News of Natural Sciences,2025:58:318-328.
31. Salehi B, Ata A, Anil Kumar NV, Sharopov F, Ramírez-Alarcón K, Ruiz-Ortega A, *et al*., 2022.
32. Saraiva AM, Almeida DM, Tavares EA, Caetano MN. Phytochemical screening and antibacterial activity of four *Cnidoscolus* species (Euphorbiaceae) against standard strains and clinical isolates. Journal of medicinal plants Research,2012:6(21):3742-3748.
33. Sitati CNW. Antimicrobial and Immunomodulatory Effects of Aqueous Extracts of Edible Mushrooms *Termitomyces striatus* in Infected BALB/c Mice (Doctoral dissertation, COPAS-JKUAT), 2025.
34. Turner PV, Brabb T, Pekow C, Vasbinder MA. Administration of substances to laboratory animals: Routes of administration and factors to consider. Journal of the American Association for Laboratory Animal Science,2021:60(4):427-435. https://doi.org/10.30802/AALAS-JAALAS-20-000105
35. World Health Organization. Antimicrobial resistance. World Health Organization, 2020. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
36. World Health Organization. WHO global report on traditional and complementary medicine 2019-2023. World Health Organization, 2023.
37. Zahid A, Saeed T, Aihetasham A, Ali MA, Shakir J, Tabassum I, *et al*. Effect of Leaf Extract of *Syzgium cumini* and *Curcuma longa* on Gut Fauna of *Heterotermes indicola*. Agricultural Research Reports,2025:3(1):16-23.