Assessment of the effect of Vitex doniana aqueous leaf extract on pancreatic and cardiac tissues of healthy adult Wistar rats

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Short Report

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Abstract

Background:Vitex doniana Sweet (Black plum) is used in African traditional medicine for diabetes and hypertension, but systematic histopathological safety data on the pancreas and heart are lacking.

Purpose:To evaluate the histological safety profile of Vitex doniana aqueous leaf extract on the pancreas and heart of healthy adult Wistar rats.

Methods: Eighteen adult male Wistar rats were divided into two parallel screening studies (n=3/group). Groups received oral distilled water (control), 1500 mg/kg extract (low dose), or 3000 mg/kg extract (high dose) once daily for 10 days. Body weights were recorded. Pancreatic and cardiac tissues were processed for routine haematoxylin and eosin light microscopy. Data were analysed using two-way repeated measures analysis of variance.

Results: Analysis of variance showed a significant main effect of time ($p < 0.001$) but no significant group–time interaction ($p = 0.117$), indicating normal growth across all groups. Pancreatic histology was completely preserved in all groups. In contrast, myocardial sections from treated groups displayed dose-dependent interstitial oedema without necrosis, fibrosis or inflammation.

Conclusions: In this preliminary screening, Vitex doniana aqueous leaf extract showed an excellent pancreatic safety profile but induced subclinical myocardial interstitial oedema in healthy rats. These findings warrant confirmation in larger cohorts and disease-relevant models before extrapolation to human use.

Introduction

The global burden of metabolic and cardiovascular diseases continues to rise, particularly in low- and middle-income countries (International Diabetes Federation 2021; World Health Organization 2021). This has catalysed a resurgence of interest in medicinal plants as sources of affordable complementary treatments (Ekor 2014). *Vitex doniana* Sweet (Black plum), native to tropical Africa, holds significant ethnobotanical value (Dogara *et al*. 2025). Its leaves, bark and fruits are traditionally employed for managing diabetes mellitus and hypertension (Gbolade 2010; Ogbeche *et al*. 2001). This traditional use directly aligns with the present safety investigation of the aqueous leaf extract in organs (pancreas and heart) relevant to those conditions.

Scientific studies have validated some traditional claims, demonstrating hypoglycaemic, antioxidant, and antihypertensive activities (Ladeji *et al*. 1996; Oche *et al*. 2014; Sulaiman *et al*. 2021). The phytochemical composition includes flavonoids, tannins, saponins, and phenolic acids (Barry *et al*. 2022; Odoom *et al*. 2023). Previous toxicological evaluations have reported a favourable safety profile for *Vitex doniana* extracts (Ajiboye 2015; Onwukwe *et al*. 2020). However, a critical gap exists between documented biochemical effects and a thorough understanding of tissue-level safety. Histopathological examination remains the cornerstone for visualising the direct architectural impact of bioactive substances on organ

integrity (Bancroft and Gamble 2008). Cardiovascular safety of herbal medicines has been a recurring concern (Ernst 2003; Villaescusa *et al*. 2023), warranting careful investigation.

Therefore, this preliminary screening study comprised two parallel investigations designed to provide a comparative histological assessment of *Vitex doniana* aqueous leaf extract on the pancreas and heart of healthy Wistar rats. The use of healthy animals is justified for initial safety screening because any adverse effect observed in a healthy system would be a signal for caution; however, findings must be confirmed in disease models that mimic the traditional use context.

Materials and methods

Plant material and authentication

Fresh leaves of *Vitex doniana* Sweet (family Lamiaceae) were collected from Limawa Raju area, Dutse, Jigawa State, Nigeria, on 15th July 2025. Local name: Dinya (Hausa). English name: Black plum. GPS coordinates: 11°44'59.6"N 9°22'06.4"E. Botanical authentication was performed at the Department of Plant Science, Federal University Dutse, where a voucher specimen was deposited (Voucher No. FUD/PS/2025/VD01). Plant identification was confirmed using the World Flora Online (WFO ID: wfo-0000333061, accessed 16 July 2025). Collection complied with the Nigerian National Biodiversity Regulations and the Convention on Biological Diversity (CBD).

Extract preparation

The aqueous extract was prepared by cold maceration. Leaves were washed, air-dried at room temperature and mechanically pulverised. 100 g of powdered leaves was soaked in 2.5 L of distilled water for 72 h with intermittent agitation. The mixture was filtered through muslin cloth and then Whatman No. 1 filter paper. The filtrate was concentrated using a water bath at 40 °C. The percentage yield was 12.32% (w/w). The extract was stored at 4 °C until use. Preliminary phytochemical screening revealed the presence of flavonoids, tannins, saponins and phenolic compounds, consistent with previous reports (Agbafor and Nwachukwu 2011; Odoom *et al*. 2023; Kamal *et al*. 2022).

Dose selection

Doses (1500 and 3000 mg/kg) were selected following the organization for economic co-operation and development OECD 420 fixed-dose procedure (OECD 2002) and exceed the estimated human equivalent dose derived from traditional use (approximately 500–1000 mg/kg for a 60 kg adult). The higher dose was chosen to challenge the system for potential toxicity signals.

Ethical approval and animal welfare

Ethical approval was obtained from the Animal Research Ethics Committee of the Faculty of Basic Medical Sciences, Federal University Dutse (Approval No. FBMS/REC25/017). The study was conducted in accordance with the ARRIVE guidelines, the NIH Guide for the Care and Use of Laboratory Animals,

and OECD principles. Only male rats were used to avoid hormonal variability associated with the estrous cycle. This sex limitation is acknowledged as a limitation.

Experimental design

For each study (pancreatic and cardiac), nine adult male Wistar rats weighing 129–193 g were used. Animals were housed under standard conditions (12-h light/dark cycle, 22 ± 2 °C) with free access to standard rodent chow and water, acclimatised for two weeks. Sample size n = 3 per group is consistent with toxicological screening for qualitative histopathological detection (Nakakaawa *et al*. 2023).

Group A (Control): Received 1.3 mL/kg of distilled water orally.
Group B (Low dose): Received extract at 1.3 mL/kg (~1500 mg/kg).
Group C (High dose): Received extract at 2.6 mL/kg (~3000 mg/kg).

Treatments were administered once daily via oral gavage for 10 consecutive days. Body weights were recorded weekly.

Histological processing

Twenty-four hours after the final administration, animals were humanely sacrificed. The pancreas and heart were excised, rinsed in normal saline, and fixed in 10% neutral buffered formalin for 48 h. Tissues were processed through graded ethanol, cleared in xylene, and embedded in paraffin. Sections of 5 µm thickness were cut and stained with haematoxylin and eosin. Stained slides were examined under a light microscope by two independent observers blinded to the treatment groups across a minimum of five randomly selected high-power fields per tissue section.

Data analysis

Body weight data were expressed as mean ± standard deviation. Descriptive statistics and line graphs were generated using Microsoft Excel. A two-way repeated measures analysis of variance was performed using statistical package for the social sciences (SPSS) version 20.0 to evaluate the main effects of treatment, time, and their interaction. Because the primary endpoint was qualitative histopathological screening and the group × time interaction was non-significant ($p = 0.117$), no post-hoc pairwise tests were conducted. Statistical significance was set at $p \leq 0.05$.

Results

General observations and body weight

All animals survived the 10-day treatment period without overt signs of toxicity. Two-way repeated measures analysis of variance revealed a significant main effect of time ($F(2,12) = 25.75$, $p < 0.001$) and a significant main effect of group ($F(2,6) = 33.46$, $p < 0.001$), but the group × time interaction was not significant ($F(4,12) = 2.31$, $p = 0.117$), indicating that weight changes over time did not differ between

treatment groups. Body weight data are therefore not shown in a table but are available from the corresponding author.

Pancreatic histology

Pancreatic histology was indistinguishable between control and treated groups. The architectural integrity of exocrine acini and endocrine Islets of Langerhans was completely preserved in all animals, with no pathological alterations such as vacuolation, inflammation, necrosis, or islet degeneration (Table 1, Fig. 1).

Table 1 Summary of pancreatic histological findings (n=3/group)

Group	Acinar integrity	Islet morphology	Pathology
Control	Normal	Normal, discrete	Absent
Low dose	Normal	Normal, discrete	Absent
High dose	Normal	Normal, discrete	Absent

Cardiac histology

Control sections exhibited normal, compact myocardial architecture with tightly adjoined cardiac muscle fibres. In low-dose sections, interstitial oedema was observed, characterised by clear fluid-filled spaces separating cardiomyocyte bundles. High-dose sections showed variable findings ranging from normal to mild interstitial oedema. No evidence of cardiomyocyte necrosis, fibrosis, or active inflammation was observed in any treated group (Table 2, Fig. 2).

Table 2 Summary of cardiac histological findings (n=3/group)

Group	Myocardial architecture	Interstitial oedema	Other pathology
Control	Normal, compact	Absent	Absent
Low dose	Mildly disorganized	Present (+)	Absent
High dose	Variable (normal to mild disorganisation)	Mild to moderate (++ at most)	Absent

Discussion

This preliminary screening reveals a dichotomy in tissue-level response to *Vitex doniana* aqueous leaf extract. While the pancreas demonstrated complete histological resilience, the heart exhibited interstitial oedema without necrosis or inflammation.

The absence of pancreatic damage aligns with the plant's traditional use in diabetes management (Ogbeche *et al*. 2001; Oche *et al*. 2014) and is supported by previous toxicological profiles (Dogara *et al*. 2025; Onwukwe *et al*. 2020). The pancreatic safety observed here is consistent with a sub-acute toxicity study in female rats that reported no major organ damage (Forcados *et al*. 2021).

In contrast, the induction of myocardial interstitial oedema represents a potential safety signal. Oedema can impair diastolic filling and disrupt electrical coupling. The mechanism may be haemodynamic, possibly via arteriolar vasodilation increasing capillary hydrostatic pressure, analogous to peripheral oedema seen with calcium channel blockers (Makani *et al*. 2011; Messerli 2002). Similar vasodilatory effects have been reported for the seeds of *Vitex doniana* (Ogbeche *et al*. 2001). This creates a paradox: a plant used for heart ailments may induce a subclinical structural alteration in a healthy baseline model. However, the clinical relevance of this finding in diseased states (e.g., hypertensive or diabetic hearts) remains unknown.

Limitations

Sample size (n=3 per group) is suitable for preliminary screening but insufficient for definitive conclusions. Acute exposure (10 days) only; no functional cardiovascular assessments, serum biomarkers, or female animals were included. The traditional use is for disease states (diabetes, hypertension); findings may differ in disease models.

Conclusion

In this preliminary screening, *Vitex doniana* leaf extract exhibited an excellent safety profile in the pancreas but induced dose-dependent myocardial interstitial oedema in healthy Wistar rats without evidence of cardiomyocyte injury. While its use for metabolic indications (diabetes) appears structurally safe in healthy animals, cardiovascular applications require further evaluation in disease-relevant models and with larger sample sizes. These findings should be confirmed in sub-chronic studies with functional cardiovascular assessments.

Declarations

Compliance with Ethical Standards

Funding

This study was not supported by any funding.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical

standards of the institution at which the studies were conducted (Federal University Dutse, approval No. FBMS/REC25/017).

Informed consent

For this type of study informed consent is not required.

Consent for publication

For this type of study consent for publication is not required.

Data availability

The histopathology images supporting this study are openly available in the Mendeley Data repository at https://data.mendeley.com/datasets/hpmt8fvm6p/1, DOI: 10.17632/hpmt8fvm6p.1.

References

1. Agbafor KN, Nwachukwu N (2011) Phytochemical analysis and antioxidant property of leaf extracts of *Vitex doniana* and *Mucuna pruriens*. Biochem Res Int 2011:459839. https://doi.org/10.1155/2011/459839
2. Ajiboye TO (2015) Standardized extract of *Vitex doniana* Sweet stalls protein oxidation, lipid peroxidation and DNA fragmentation in acetaminophen-induced hepatotoxicity. J Ethnopharmacol 164:273–282. https://doi.org/10.1016/j.jep.2015.01.026
3. Bancroft JD, Gamble M (eds) (2008) Theory and Practice of Histological Techniques, 6th edn. Churchill Livingstone Elsevier, London
4. Barry PR, Sanogo R, Diallo D (2022) Toxicological profile, phytochemical analysis and anti-inflammatory properties of leaves of *Vitex doniana* Sweet (Verbenaceae). Heliyon 8(8):e10080. https://doi.org/10.1016/j.heliyon.2022.e10080
5. Dogara AM, Al-Rawi SS, Hama HA (2025) Traditional uses, bioactive compounds and pharmacological uses of *Vitex doniana* Sweet: A Review. Ethnobot Res Appl 30:1–130. https://doi.org/10.32859/era.30.12.1-130
6. Ekor M (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 4:177. https://doi.org/10.3389/fphar.2013.00177
7. Ernst E (2003) Cardiovascular adverse effects of herbal medicines: a systematic review of the recent literature. Can J Cardiol 19(7):818–827 (PMID: 12813616)
8. Forcados GE, James DB, Scoltz W *et al* (2021) In vitro cytotoxicity and in vivo sub-acute toxicity studies on *Vitex doniana* (Verbenaceae) leaves extract in MCF-7 breast cancer cells and female Wistar rats. Comp Clin Pathol 30:387–396. https://doi.org/10.1007/s00580-021-03229-w
9. Gbolade AA (2010) Ethnobotanical study of *Vitex doniana* Sweet (Black plum) in Nigeria. Int J Med Plant Res 2(1):1–6

10. International Diabetes Federation (2021) IDF Diabetes Atlas, 10th edn. International Diabetes Federation, Brussels
11. Kamal N, Mio Asni NS, Rozlan INA *et al* (2022) Traditional medicinal uses, phytochemistry, biological properties, and health applications of *Vitex* sp. Plants 11:1944. https://doi.org/10.3390/plants11151944
12. Ladeji O, Okoye ZSC, Uddoh F (1996) Effects of *Vitex doniana* stem bark extract on blood pressure. Phytother Res 10(3):245–247. https://doi.org/10.1002/(SICI)1099-1573(199605)10:3<245::AID-PTR806>3.0.CO;2-L
13. Makani H, Bangalore S, Romero J *et al* (2011) Peripheral edema associated with calcium channel blockers: incidence and withdrawal rate – a meta-analysis of randomized trials. J Hypertens 29(7):1270–1280. https://doi.org/10.1097/HJH.0b013e3283472643
14. Messerli FH (2002) Vasodilatory edema: a common side effect of antihypertensive therapy. Curr Cardiol Rep 4(6):479–482. https://doi.org/10.1007/s11886-002-0114-7
15. Nakakaawa L, Gbala ID, Cheseto X, Bargul JL, Wesonga JM (2023) Oral acute, sub-acute toxicity and phytochemical profile of *Brassica carinata* A. Braun microgreens ethanolic extract in Wistar rats. J Ethnopharmacol 305:116121. https://doi.org/10.1016/j.jep.2022.116121
16. Oche O, Sani I, Ndako JA (2014) pancreatic islet regeneration and some liver biochemical parameters of leaf extracts of *Vitex doniana* in normal and streptozotocin-induced diabetic albino rats. Asian Pac J Trop Biomed 4(2):124–130. https://doi.org/10.1016/S2221-1691(14)60220-5
17. Odoom JF, Adu F, Aboagye SY (2023) Chemical composition, antioxidant, and antimicrobial activities of the leaf and fruit essential oils of the West African plum, *Vitex doniana*. J Chem 2023:9959296. https://doi.org/10.1155/2023/9959296
18. OECD (2002) Test No. 420: Acute Oral Toxicity – Fixed Dose Procedure. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. https://doi.org/10.1787/9789264070943-en
19. Ogbeche KA, Eniojukan JF, Onyenwenyi OC (2001) Blood pressure lowering effect of the seeds of *Vitex doniana*. J Clin Sci 1(1):22–24
20. Onwukwe OS, Ukwuani JN, Onyemelukwe AO *et al* (2020) Assessment of the sub-acute toxicity profile of *Vitex doniana* (Sweet) leaf fractions on albino rats. Annu Res Rev Biol 35(2):86–95. https://doi.org/10.9734/arrb/2020/v35i230192
21. Sulaiman MA, Abubakar AM, Garba R (2021) Curative effects of aqueous and ethanol stem bark extracts of *Vitex doniana* on doxorubicin-induced cardiotoxicity in rats. Asian J Res Biochem 8(2):44–55. https://doi.org/10.9734/ajrb/2021/v8i230178
22. Villaescusa L, Zaragozá C, Zaragozá F, Tamargo J (2023) Herbal medicines for the treatment of cardiovascular diseases: Benefits and risks – A narrative review. Int J Cardiol 385:44–52. https://doi.org/10.1016/j.ijcard.2023.04.045
23. World Health Organization (2021) Cardiovascular diseases (CVDs). https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). Accessed 25 May 2026

Figures

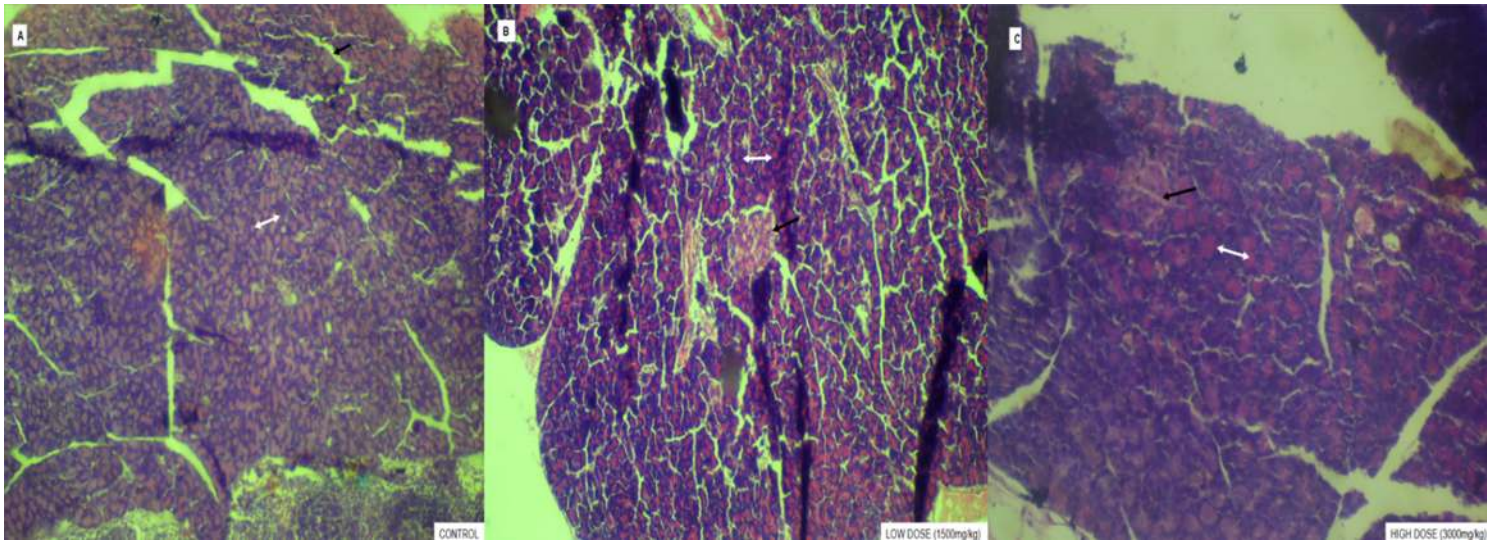

Figure 1

Photomicrographs of pancreatic tissue (haematoxylin and eosin, ×200). (A) Control group – normal acini and Islet of Langerhans. (B) Low dose (1500 mg/kg) – preserved architecture. (C) High dose (3000 mg/kg) – normal histology. Black arrows: Islets of Langerhans; white arrows: exocrine acini.

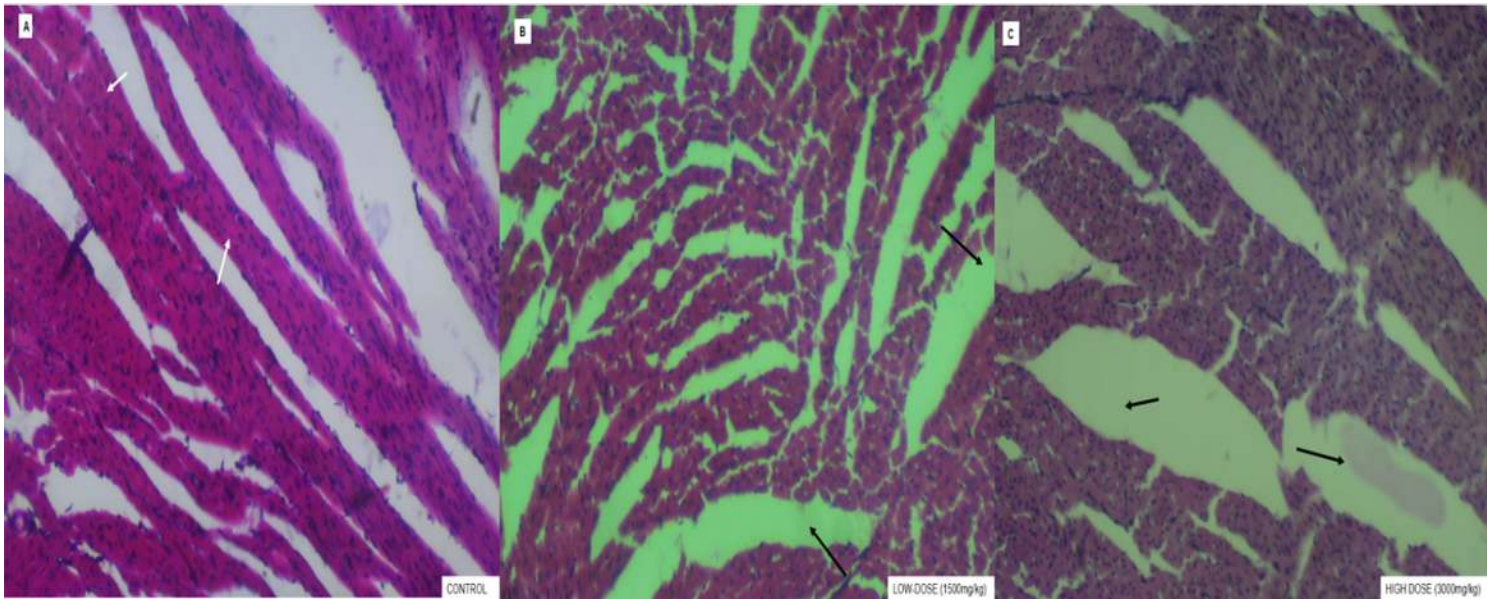

Figure 2

Photomicrographs of myocardial tissue (haematoxylin and eosin, ×400). (A) Control – compact myocardial fibres. (B) Low dose – mild interstitial oedema (black arrows). (C) High dose – mild to moderate fluid-filled spaces separating cardiomyocyte bundles. No necrosis, fibrosis or inflammation visible.

Supplementary Files

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