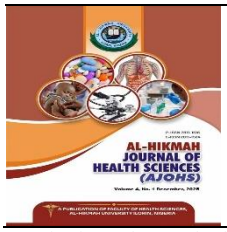
P-ISSN: 2811-1516
E-ISSN: 2811-1524



AL-HIKMAH JOURNAL OF HEALTH SCIENCES (AJOHS)

Official Publication of the Faculty of Basic Medical Sciences

Al-Hikmah University, Ilorin, Nigeria

https://alhikmahuniversity.edu.ng/AJOHS/index.php/journal

Original Article

Sub-Chronic Cardiac Effects of Adimenu Herbal Mixture: Evaluation of CK-MB and Troponin I in Rabbits

Akeem Olayinka Busari[1*], Peter Anuoluwa Folayan[2], Sheriff Afolabi Ayanda[2], Temiloluwa Juliet Olajoye[2], Rukayat Bukola Lawal[2], Abosede Christinah Adebiyi[2], Yetunde Fehintola Oluwasanmi[2], Shefiat Bashir[3], Sulaiman Adebayo Nassar[1], Mufutau Mosunmade Azeez[4], Muhammad Bello Ahmad[5], Idris Yahaya Mohammed[6] and Franklin Folasele Akinola[1]

[1]Department of Medical Laboratory Science, Ladoke Akintola University of Technology, Ogbomoso, Nigeria
[2]Department of Medical Laboratory Science, Kwara State University, Malete, Nigeria.
[3]Department of Medical Laboratory Science, Al-Hikmah University, Ilorin, Nigeria.
[4]Department of Medical Laboratory Science, Ajayi Crowther University, Oyo, Nigeria.
[5]Department of Medical Laboratory Science, Bayero University, Kano, Nigeria.
[6]Department of Chemical Pathology, Bayero University, Kano, Nigeria

ARTICLE INFO

Article History
Received: 26th October, 2025
Accepted: 15th November, 2025
Available online: 10th December, 2025

Keywords
Adimenu Herbal Mixture
Creatine kinase-MB
Troponin I
Cardiac biomarkers
Rabbits

Corresponding Author

Akeem Olayinka Busari
Department of Medical Laboratory Science, Ladoke Akintola University of Technology, Ogbomoso, Nigeria
Phone Number: +2348077609212
Email: busakeem@yahoo.com

ABSTRACT

Background: Adimenu, a locally produced herbal mixture in Nigeria, is widely used to manage several conditions, including hypertension; however, its cardiac safety remains unevaluated. Thus, this study investigated the effects of Adimenu on cardiac biomarkers such as creatine kinase-MB (CK-MB) and cardiac troponin I (cTnI) in rabbits.

Method: Twenty healthy male rabbits were randomly assigned to four groups comprising control, 25% Adimenu, 50% Adimenu, and 100% Adimenu (n = 5 each). Treatments were administered orally for 14 days. During the study, three rabbits were lost, leaving 17 animals that completed the treatment and reached the terminal stage. Body weights and serum CK-MB and cTnI levels were measured, and data were analyzed using one-way ANOVA with post hoc comparisons. Statistical significance was set at $p < 0.05$.

Results: Three animals were lost during the study (two from the 25% group and one from the 100% group), leaving 17 animals that completed treatment. The rabbits given increasing Adimenu concentrations exhibited progressive weight gain, with the 100% group showing the greatest increase ($F = 7.773$, $p = 0.003$). CK-MB levels varied across groups but did not differ significantly ($F = 0.937$, $p = 0.451$), indicating no consistent effect on this marker. In contrast, cTnI levels were significantly elevated in the 25% ($p = 0.005$) and 50% ($p = 0.003$) groups compared to controls; however, not in the 100% group ($p = 0.968$), suggesting a concentration-dependent response with a peak effect at intermediate doses.

Conclusion: Adimenu produced a dose-related increase in body weight and significantly raised cardiac troponin I at intermediate doses (25% and 50%), while CK-MB remained unaffected. These findings suggest possible cardiotoxic stress at moderate concentrations and an unpredictable response at the highest dose, underscoring the need for dosage regulation and further safety evaluation.

Please cite this article as Busari, A.O., Folayan, P.A., Ayanda, S.A., Olajoye, T.J., Lawal, R.B., Adebiyi, A.C., Oluwasanmi, Y.F., Bashir, S., Nassar, S.A., Azeez, M.M., Ahmad, M.B., Mohammed, I.Y. & Akinola, F.F. (2025). Sub-Chronic Cardiac Effects of Adimenu Herbal Mixture: Evaluation of CK-MB and Troponin I in Rabbits. *Al-Hikmah Journal of Health Sciences, 4*(1), 52-58.

P-ISSN: 2811-1516
E-ISSN: 2811-1524

Introduction

The World Health Organization (WHO) reported that 88% of people used Traditional and Complementary Medicine (T&CM) in 2019. Nigeria is one of the countries with officially established T&CM policies, laws, regulations, programs, and offices; however, the number of countries that use T&CM is likely growing (WHO, 2019). For centuries, the local population has preferred traditional healing methods, such as using plant parts to create contemporary pharmaceutical formulations (herbal medicine), over modern medications (Qadir & Raja, 2021).

Adimenu, a dark brown commercial medicine, a Yoruba term meaning "hold it in the mouth," refers to how it is administered: a locally made herbal mixture from Nigeria is held in the mouth for two to three minutes before being poured. Although the production of the product (Adimenu) was found to be in Oyo and Osun States, it has since expanded to other geopolitical zones in Nigeria, such as the North East in Gombe State and the North Central in Kwara State. The product label for this herbal mixture claims to cure hypertension, severe body pains, stomach disorders, stroke, etc. (Abdullateef & Abdullahi, 2023). According to the Herbal Mixture Container, the ingredients that make up the herbal mixture Adimenu are: *Nicotiana tobaccum Linn* (1%), *Zingiber officinale Roscoe* (7%), *Xylopia aethiopica* Dunal (6%), *Tetrapleura tetraptera Schum*. & *Thonn* (6%), and Water (80%) (Abdullateef & Abdullahi, 2023).

The widespread and growing use of herbal medicine with scientifically unproven safety and efficacy has created public health challenges in terms of safety, efficacy, quality, and rational use (Msomi & Simelane, 2019). Recent research has shown that people have mixed feelings about using herbal mixtures to treat diseases; while many people believe herbal mixtures are effective for curing ailments, others are skeptical, and some are completely opposed to their use, which is thought to pose a threat to human health (Tanya & Ayush, 2020).

The factor contributing to the unfavorable disposition toward its usage is that there is typically no report to verify the herbal preparation's toxicity status and adverse effects. Therefore, it is imperative to evaluate the impact on cardiac biomarkers, a measure of the herbal mixtures' anti-hypertensive properties.

Materials and Methods

Experimental Animals

Twenty healthy male rabbits (≥ 2 months old) with similar baseline weights were obtained from the Kwara State University animal facility. Animals were acclimatized for 7 days under controlled conditions (12-h light/dark, 21–31°C, 45–55% humidity). They were randomly allocated to four groups (control, 25%, 50%, and 100% Adimenu; n=5 each). This experimental study used healthy male rabbits of approximately the same weight; female albino rats were excluded to avoid interference from reproductive hormones on the biochemical parameters measured during the experiment. Twenty healthy male rabbits at least two months old were obtained from Kwara State University's animal house and randomly assigned to four groups: three test groups that received varying concentrations of the Adimenu herbal mixture (25%, 50%, and Absolute) and a control group that was housed in a well-constructed cage and allowed to adapt for 7 days. The animals were housed in a well-ventilated cage made of wood and wire mesh under controlled environmental conditions of 12 12-hour of the day/light per day, temperature between 21 and 31 degrees Celsius, and relative humidity between 45% and 55%. All animals were treated humanely in accordance with the National Society of Medical Research's principle of laboratory animal care (National Institutes of Health Publications no. 80-23, revised 1978) and approved by the Kwara State University Centre for Research and Development (CR&D) with the reference number KWASU/CR&D/REA/2024/0064.

Sample Size Determination

This computation technique is based on the law of diminishing returns. It is referred to as the "resource equation" method and is employed when estimating standard deviation or effect size is impossible due to the lack of previous findings, when multiple endpoints are being measured, or when intricate statistical procedures are being used for analysis (Charan *&* Kantharia, 2013). According to this method, a value “E” was measured, which is the degree of freedom of analysis of variance (ANOVA). The value of E should lie between 10 and 20. The resource equation method was applied to determine sample adequacy. With 20 animals allocated to 4 groups (5/group), $E=N-G=20-4=16$

This lies within the recommended 10–20 range, confirming the adequacy of the sample size.

Herbal Mixture Preparation and Administration

The Adimenu herbal mixture was purchased from a local store in Ilorin Metropolis. Following the preparation of ingredients, distilled water was used as a diluting medium to produce the herbal mixture in different concentrations (Abdullateef & Abdullahi, 2023). The formula RV/O was used to reconstitute the various concentrations of the Admineu herbal mixture, where R = required concentration, V = required volume, and O = original concentration (100%).

The herbal mixture was administered according to the Organization for Economic Cooperation and

P-ISSN: 2811-1516
E-ISSN: 2811-1524

Development (OECD) Guideline 407 for the sub-acute oral toxicity test with slight modifications (OECD, 2008).

Experimental Protocol
Following the International guidelines for this toxicological study (OECD, 2008), the animals were treated for fourteen (14) days and observed for any signs of toxicity and/or mortality.

Weighing of Animals
At the end of the experiment, the laboratory animals were weighed on a Marsden V-22 veterinary scale, which has a bright LCD and easy-to-read weight readings.

Table 1. Herbal mixture preparation and administration

Concentration	Proportion of diluent	Proportion of Adimenu herbal mixture
25%	75% of Distilled water	25% Adimenu herbal mixture
50%	50% of Distilled water	50% Adimenu herbal mixture
100%	Nil	100% Adimenu herbal mixture

Table 2. Experimental protocol

Group	Treatment	Total number of animals	Inference
1	Feed + Water *ad libitum*	5	Control
2	Feed + 25% Adimenu + Water *ad libitum*	5	Low dose
3	Feed + 50% Adimenu + Water *ad libitum*	5	Medium dose
4	Feed + 100% Adimenu + Water *ad libitum*	5	High dose

The animals were fasted for 12 hours at the end of the experiment, and they were then anesthetized with diethyl ether to induce anesthesia. About 3 ml of blood will be collected using the heparinized capillary tube from the medial canthus of the eye under the nictitating membrane. The blood was dispensed into a plain bottle; after clotting occurred, it was centrifuged at 3000 revolutions per minute (rpm) for 5 minutes, and the supernatant was collected into plain tubes for serum analysis of the cardiac biomarker.

Creatine Kinase-MB Estimation
The level of CK-MB was estimated using the Enzyme-linked immunoassay (ELISA) method described by Stein and Bohner (1985).

Principle
The reagent contains a polyclonal antibody (specific to the CK-M monomer), which so completely inhibits CK-MM activity and one-half of CK-MB activity. Only the activity of the non-inhibited B monomer subunit, representing half of the CK-MB activity, is measured. The method assumes that CKBB activity in the specimen is essentially zero.

Troponin I Estimation
The level of troponin I was estimated in rabbits treated with Adimenu and the control group using the chemiluminescence immunoassay (CLIA) method.

Principle
The CLIA kit uses the Sandwich-CLIA principle. The micro-CLIA plate provided has been pre-coated with an antibody specific to cTn-I. Standards or samples are added to the micro-CLIA plate wells and combined with the specific antibody. Then, a biotinylated detection antibody specific for cTn-I and Avidin Horseradish Peroxidase (HRP) conjugates are added successively to each microplate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain cTn-I, biotinylated detection antibody, and Avidin-HRP conjugate will appear fluorescent. The relative light unit (RLU) value is measured by the chemiluminescence immunoassay analyzer. The RLU value is positively associated with the concentration of cTn-I present in the sample.

Data Analysis
The data analysis was conducted using one-way Analysis of variance (ANOVA) and post hoc multiple comparisons for the data collected. A Pearson correlation (r) coefficient was employed to explore relationships between the quantitative variables; a p-value less than 0.05 ($p<0.05$) was considered significant.

Results
Three animals were lost during the study (two from the 25% group and one from the 100% group), resulting in final group sizes of n=3 and n=4 for the 25% and 100% groups, respectively.
Body weight, CK-MB, and troponin levels were assessed across the four experimental groups. Adimenu administration produced a dose-dependent increase in body weight, with the 100% group showing the highest mean (1493.5 ± 227.21 g) compared with the control (717.60 ± 196.81 g). This effect was statistically significant ($R^2 = 0.642$, $F = 7.773$, $p = 0.003$).

P-ISSN: 2811-1516
E-ISSN: 2811-1524

Table 3. Comparison of the mean ± SEM of the weight and cardiac biomarkers (CK-MB and troponin) of the albino rabbits

Parameters Mean ± SEM	Control (n = 5)	25% Adimenu (n = 3)	50% Adimenu (n = 5)	100% Adimenu (n = 4)	R^2	F	P-Value
Weight (g)	717.60±196.81	936.33±229.32	1058.8±296.78	1493.5±227.21	0.642[a]	7.773	0.003*
CK-MB (U/L)	47.91 ± 3.74	44.21±2.07	60.45± 26.30	55.15± 8.17	0.178[b]	0.937	0.451
Troponin (pg/ml)	116.11± 57.95	189.52± 3.39	184.67 ± 10.14	138.61± 23.78	0.513[c]	4.566	0.021*

Mean ± SEM = Mean ± Standard error of the Mean, n = number of rabbits in a group. CK-MB = Creatine Kinase-Muscle-Brain subunit

For CK-MB, mean values ranged from 44.21 ± 2.07 U/L (25% Adimenu) to 60.45 ± 26.30 U/L (50% Adimenu), with the control group at 47.91 ± 3.74 U/L. Variability was high, and the model was not significant (R^2 = 0.178, F = 0.937, p = 0.451), indicating no consistent effect of Adimenu on CK-MB activity. Troponin levels increased markedly at 25% (189.52 ± 3.39 pg/ml) and 50% (184.67 ± 10.14 pg/ml) compared with control (116.11 ± 57.95 pg/ml), before declining at 100% (138.61 ± 23.78 pg/ml). This pattern was statistically significant (R^2 = 0.513, F = 4.566, p = 0.021), suggesting that moderate concentrations of Adimenu (25–50%) consistently elevate troponin, while the highest dose produces a less stable effect.

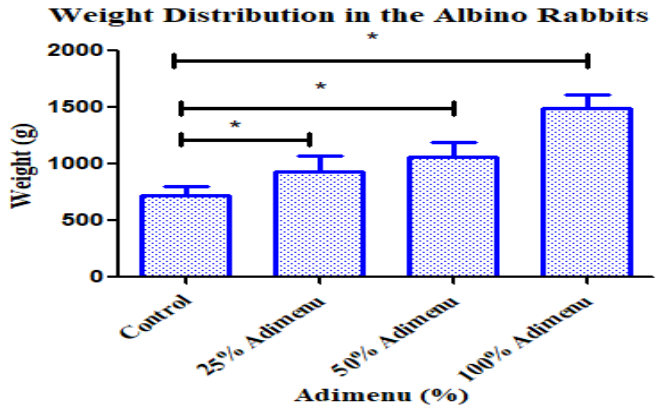


Fig. 1. Comparison of rabbits' body weights across the group
** = significant at p < 0.05*

Fig. 1 shows a comparison of the group's rabbits' body weights. Post hoc analysis revealed significant differences in body weight across treatment groups. Rabbits treated with 100% Adimenu exhibited the greatest weight gain, with values significantly higher than the control (p = 0.002) and 25% Adimenu groups (p = 0.044). In addition, the 25%, 50% and 100% groups showed significantly greater body weights compared with the control group (p < 0.05). These findings demonstrate a dose-dependent increase in body weight, with the most pronounced effect observed at the highest concentration (100% Adimenu).

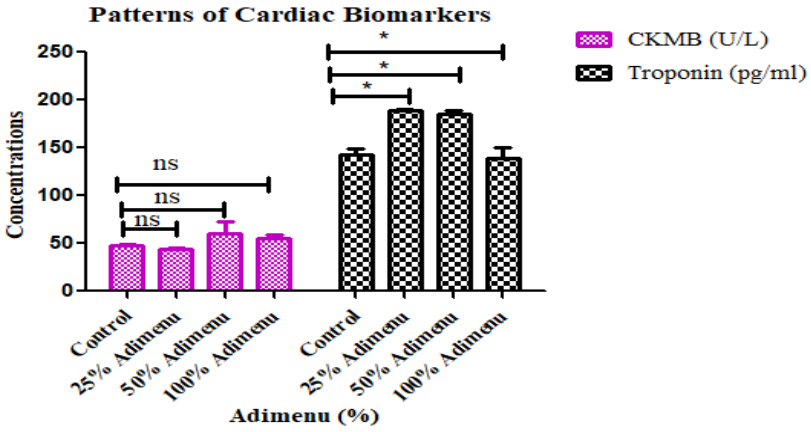


Fig. 2. Comparison of CK-MB and troponin I across the group
** = significant at p < 0.05, ns = not significant*

P-ISSN: 2811-1516
E-ISSN: 2811-1524

Fig. 2 compares the CK-MB and troponin across the group. The post hoc comparisons of CK-MB revealed no statistically significant differences among groups ($p > 0.05$), indicating that Adimenu administration did not significantly alter CK-MB activity in a consistent pattern.

In contrast, troponin levels showed a distinct pattern. Both the 25% and 50% Adimenu groups exhibited significantly higher troponin concentrations compared with the control group ($p < 0.05$), with values remaining consistently elevated between these two doses. However, at 100% Adimenu troponin levels declined and were no longer significantly different from the control. This pattern suggests that moderate doses of Adimenu (25-50%) result in a reproducible rise in troponin, whereas the highest dose produces a less stable effect, indicating a cardioprotective effect.

In Table 4, the Pearson's correlation analysis demonstrated a strong and statistically significant positive association between Adimenu concentration and body weight ($r = 0.774$, $p < 0.001$), indicating that increasing doses of Adimenu were consistently associated with greater weight gain. In contrast, no significant correlations were observed between Adimenu concentration and CK-MB ($r = 0.289$, $p = 0.261$) or troponin ($r = 0.036$, $p = 0.890$), suggesting that variations in these cardiac biomarkers were not directly explained by Adimenu dosage. These findings indicate a dose-dependent relationship between Adimenu administration and body weight, whereas the effects on cardiac biomarkers appear variable and not directly linked to dose concentration.

Table 4. Pearson Correlation Analysis Between Adimenu Administration, Weight, CKMB, and Troponin

Parameters	Statistics	Adimenu (%)	Weight (Kg)	CKMB (U/L)	Troponin (pg/ml)
Adimenu (%)	r	1.000	0.774	0.289	0.036
	p-value		0.000*	0.261	0.890
Weight (Kg)	r	0.774	1.000	0.273	-0.094
	p-value	0.000*		0.289	0.719
CKMB (U/L)	r	0.289	0.273	1.000	0.145
	p-value	0.261	0.289		0.579
Troponin (pg/ml)	r	0.036	-0.094	0.145	1.000
	p-value	0.890	0.719	0.579	

*r = Pearson Correlation Coefficient, P-value = statistically significant value at set ≤ 0.05, * = significant at p < 0.05*

Discussion

The widespread and increasing use of Adimenu herbal medicine, despite its unproven safety, efficacy, and rational basis, prompted this investigation into its effects on cardiac biomarkers. Given its traditional claims as a remedy for ailments such as hypertension, our study sought to experimentally investigate these assertions.

Adimenu concentration was found to cause a dose-dependent increase in body weight, with an R^2 value of 0.642, accounting for 64% of weight variability (Tables 3-4; Fig. 1). This strong predictive relationship suggests that Adimenu may stimulate appetite and enhance food intake, leading to progressive weight gain at higher doses. Mechanistically, this effect could reflect alterations in metabolic regulation, possibly through stimulation of orexigenic neuropeptides or disruption of lipid metabolism, resulting in elevated cholesterol and triglyceride levels that further compound cardiovascular risk. The significant weight gain observed aligns with the findings of Sulaiman et al. (2020), who reported that *Nicotiana tabacum* extract induced a dose-dependent rise in serum triglycerides in rats, thereby predisposing them to cardiometabolic disturbances. Such metabolic alterations may place additional hemodynamic stress on the heart, increasing the risk of hypertrophy and cardiac dysfunction.

In contrast, CK-MB levels demonstrated only minor, non-significant variations across the treatment groups (Tables 3–4; Fig. 2). CK-MB, a selective marker of myocardial injury, is frequently elevated in acute myocardial infarction (AMI). However, the low R^2 value (0.178) in our study indicates that Adimenu concentration accounted for only a small proportion of CK-MB variability, suggesting limited predictive value. The marked intra-group variability, particularly in the 50% group, highlights the inconsistent nature of Adimenu's effect on this biomarker. These findings agree with Sultani et al. (2020), who emphasized that the effects of herbal mixtures are often heterogeneous due to differences in composition and dosage, as well as individual variability in metabolic handling of phytochemicals. This variability likely explains the inconsistent CK-MB responses seen in our study. However, our results diverge from those of Ibrahim et al. (2017), who reported nicotine-induced significant elevations in CK-MB activity as a marker of cardiac injury. One possible explanation is that CK-MB, although useful for detecting acute injury, may be less sensitive to subtle or chronic myocardial stress compared to newer biomarkers such as troponin.

P-ISSN: 2811-1516
E-ISSN: 2811-1524

Troponin analysis revealed more robust findings. Adimenu treatment significantly influenced troponin levels ($R^2 = 0.513$, $p < 0.05$), with the 25% and 50% groups exhibiting marked and consistent elevations compared with controls. Troponin I, a highly specific indicator of cardiac myocyte injury, is known to rise in response to ischemic or toxic insults. The increase at moderate doses of Adimenu suggests a reproducible cardiotoxic effect, possibly mediated by nicotine-induced oxidative stress, endothelial dysfunction, or enhanced platelet aggregation. Rasmussen and Jin (2021) demonstrated similar findings, reporting nicotine-associated perturbations in cardiovascular biomarkers, while Jain et al. (2021) further highlighted nicotine's ability to promote thrombogenesis and endothelial injury mechanisms that align with the elevated troponin observed here. Mechanistically, these effects are consistent with the known pharmacology of nicotine, a constituent of Adimenu. Nicotine activates sympathetic pathways, elevating catecholamines, heart rate, and blood pressure, thereby increasing myocardial oxygen demand. Concurrently, it promotes oxidative stress, endothelial dysfunction, and platelet activation, which predispose to ischemia and infarction. These mechanisms align with the elevated troponin levels in our study and reinforce the findings of Benowitz & Burbank (2016), who reported that nicotine profoundly disrupts cardiovascular biomarker homeostasis. Interestingly, the 100% Adimenu group showed a decrease in troponin levels, indicating that natural compounds have cardioprotective effects by lowering cardiac biomarkers, including troponin levels, and multiple studies support this protective mechanism across various plant-derived compounds. Gallic acid pretreatment (15 mg/kg), one of Adimenu's components, significantly reduced serum troponin-T levels in rats with isoproterenol-induced myocardial infarction while also improving other cardiac markers and antioxidant status (Priscilla & Prince, 2009). N,α-L-rhamnopyranosyl vincosamide from Moringa oleifera leaves reduced troponin-T levels and other cardiac markers in isoproterenol-induced cardiotoxicity, indicating cardioprotective properties through free radical scavenging (Panda et al., 2013). Syzygium polyanthum ethanol extract reduced troponin T levels in benzene-induced cardiac damage in a dose-dependent manner (Soemijarto *et al*., 2021). Broader evidence has shown that dietary natural bioactive compounds, including polyphenolic compounds and other plant-derived molecules, possess protective effects against cardiovascular diseases through various molecular mechanisms (Sharifi-Rad et al., 2020). Thus, consumption of Adimenu at higher concentrations may have a cardioprotective effect against cardiovascular diseases, with the significant decrease observed in troponin I levels at 100% concentration.

Conclusion

The study observed that Adimenu significantly increases body weight and decreases troponin levels in albino rabbits at the higher dose, but its effect on CK-MB levels is inconsistent. The low troponin I concentrations observed in 100% concentration groups suggest that Adimenu has a cardioprotective effect and thus suggest safety evaluation and dosage regulation.

Public Health Implications

Adimenu is consumed without regulation. Our findings highlight potential cardiac risks, especially at intermediate concentrations, emphasizing the need for consumer protection, standardization, and further toxicological evaluation.

Recommendation

The findings of this study should be considered hypotheses that require further investigation, including larger cohorts, detailed histopathological mechanisms and longer-term dosing of Adimenu.

Limitations and Future Directions

This study's limitations are as follows:

i. Small sample size and animal mortality reduced statistical power.

ii. Absence of histopathological confirmation of cardiac injury.

Consent

It is not applicable.

Ethical Approval

This study was approved by the Kwara State University Centre for Research and Development (CR&D) with the reference number KWASU/CR&D/REA/2024/0064.

Disclaimer (Artificial Intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Acknowledgement

We thank almighty God for the successful completion of this research work.

Competing Interests

Authors have declared that no competing interests exist.

P-ISSN: 2811-1516
E-ISSN: 2811-1524

References

Abdullateef, R. A., & Abdullahi, H. J. (2023). Herbal mixture (Adimenu) toxicological evaluations using the *Allium cepa L.* assay. *EAS J Biotechnol Genet, 5*(4), 98–105.

Benowitz, N. L., & Burbank, A. D. (2016). Cardiovascular toxicity of nicotine: Implications for electronic cigarette use. *Trends in Cardiovascular Medicine, 26*(6), 515–523. https://doi.org/10.1016/j.tcm.2016.03.001

Charan, J., & Kantharia, N. (2013). How to calculate sample size in animal studies? *Journal of Pharmacology and Pharmacotherapeutics, 4*(4), 303–306. https://doi.org/10.4103/0976-500X.119726

Ibrahim, Z. S., Soliman, M. M., Mahmoud, S., & Shukry, M. (2017). Cardioprotective effects of curcumin against diabetes and nicotine-combined oxidative stress. *African Journal of Traditional, Complementary and Alternative Medicines, 14*(6), 64–71.

Jain, D., Chaudhary, P., Varshney, N., Bin Razzak, K. S., Verma, D., Khan Zahra, T. R., ... & Calina, D. (2021). Tobacco smoking and liver cancer risk: Potential avenues for carcinogenesis. *Journal of Oncology, 2021*(1), 5905357.

Msomi, N. Z., & Simelane, M. B. (2019). Herbal medicine. In *InTech: Rijeka, Croatia* (pp. 215–227).

National Institutes of Health Publications. (1978). NIH Guide. https://grants.nih.gov/grants/guide/historical/1978_11_27_Vol_07_No_18.pdf

OECD. (2008). *Test No. 407: Repeated dose 28-day oral toxicity study in rodents.* OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing. https://doi.org/10.1787/9789264070684-en

Panda, S., Kar, A., Sharma, P., & Sharma, A. (2013). Cardioprotective potential of N,α-L-rhamnopyranosyl vincosamide, an indole alkaloid, isolated from the leaves of *Moringa oleifera* in isoproterenol-induced cardiotoxic rats: In vivo and in vitro studies. *Bioorganic & Medicinal Chemistry Letters, 23*(4), 959–962. https://doi.org/10.1016/j.bmcl.2012.12.060

Priscilla, D. H., & Prince, P. S. (2009). Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. *Chemico-Biological Interactions, 179*(2–3), 118–124. https://doi.org/10.1016/j.cbi.2008.12.012

Qadir, S. U., & Raja, V. (2021). Herbal medicine: Old practice and modern perspectives. In *Phytomedicine* (pp. 149–180). Academic Press.

Rasmussen, M., & Jin, J. P. (2021). Troponin variants as markers of skeletal muscle health and diseases. *Frontiers in Physiology, 12*, 747214.

Sharifi-Rad, J., Rodrigues, C. F., Sharopov, F., Docea, A. O., Can Karaca, A., Sharifi-Rad, M., ... & Calina, D. (2020). Diet, lifestyle and cardiovascular diseases: Linking pathophysiology to cardioprotective effects of natural bioactive compounds. *International Journal of Environmental Research and Public Health, 17*(7), 2326. https://doi.org/10.3390/ijerph17072326

Soemijarto, D. A. T., Lister, I. N. E., & Lie, S. (2021, July). Reduction of tropinin levels in rats through cardioprotective activity of *Syzygium polyanthum* ethanol extract. In *2021 IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce)* (pp. 1–5). IEEE. https://doi.org/10.1109/InHeNce52833.2021.9537281

Stein, W., & Bohner, J. (1985). Influence of autoantibodies to creatine kinase-BB on assays for MB isoenzyme. *Clinical Chemistry, 31*(7), 1189–1192.

Sulaiman, F. A., Nafiu, M. O., Yusuf, B. O., Muritala, H. F., Adeyemi, S. B., Omar, S. A., ... & Otohinoyi, D. A. (2020). The GC-MS fingerprints of *Nicotiana tabacum L.* extract and propensity for renal impairment and modulation of serum triglycerides in Wistar rats. *J. Pharm. Pharmacogn. Res, 8*(3), 191–200.

Sultani, R., Tong, D. C., Peverelle, M., Lee, Y. S., Baradi, A., & Wilson, A. M. (2020). Elevated triglycerides to high-density lipoprotein cholesterol (TG/HDL-C) ratio predicts long-term mortality in high-risk patients. *Heart, Lung and Circulation, 29*(3), 414–421.

Tanya, D., & Ayush, S. (2020). Use of herbal medicine on coronavirus. *Acta Scientific Pharmaceutical Sciences, 4*(4), 61–63.

World Health Organization. (2019). *WHO global report on traditional and complementary medicine 2019.* World Health Organization.