



Effects of methanol extract of *Musa acuminata* flower on serum protein, liver and histopathological lesions in Wistar rats

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Abstract

Musa acuminata (Banana) is an herbaceous plant belonging to the family *Musaceae*. This study aims to assess the effect of the methanolic extract of Banana flower on serum protein levels, liver enzyme biomarkers and histopathological lesions in Wistar rats. The extracts were administered orally for 21 days. The rats were grouped into 5 groups. Group A received distilled water, while 200, 400, 600 and 800 mg/kg body weight of the extract were administered to groups B, C, D and E, respectively. Total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were evaluated using standard procedures, while micrograph sections of the liver were investigated for lesions of toxicity. For total protein, the result revealed 69.33±8.55, 66.00±4.79, 74.08±1.91, 74.00±1.26 and 81.00±17.47 with group A, B, C, D and E, respectively and 37.17±2.36, 35.67±0.82, 37.25±2.45, 35.67±1.21 and 53.00±13.36 for group A, B, C, D and E, respectively, for albumin. The findings of this study revealed a dose-dependent risk of toxicity, with the dose of 200 mg/kg slightly reducing the total protein and albumin, while 400 mg/kg marginally increased it. At 600 mg/kg, the total protein was the same as that of 400 mg/kg, while the total albumin was slightly reduced. At 800 mg/kg, both total protein and albumin increased exponentially compared with the control. The effect of the extract indicated a significant ($p < 0.05$) increase in ALT and ALP at 800 mg/kg compared to the control, while AST remained unchanged.

Keywords: Alanine aminotransferase (ALT), Albumin, Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Histopathological, *Musa acuminata* flower, Total protein

Introduction

Since ancient times, people have been exploring nature, particularly plants, in the search for new drugs, and this has resulted in the use of many medicinal plants with curative properties to treat various diseases (Chidi & Constance, 2017). Banana flower, also known as banana blossom, banana inflorescence, banana male bud or banana heart, is a large dark purple-red or maroon cone-like structure. It is an agricultural by-product that has tremendous nutritional value, often consumed as vegetables in Asian countries (Wickramarachchi & Ranamukhaarachchi, 2005; Florent *et al.*, 2015). Banana inflorescence exhibit multitude of biological activity, including anti-inflammatory, antioxidant, anticarcinogenic antifungal, antithrombotic, antiallergenic, anticarcinogenic, antidiabetic, antilithiatic, antimalarial, antihypertensive; antiulcer, anti-Alzheimer, wound healing activity, antimicrobial, radical scavenging properties, antidote for *Crotalidae* venoms and hepatoprotective properties (Imam & Akter 2011; Sumathy *et al.*, 2011; Bhaskar *et al.*, 2012; Ramith *et al.*, 2014; Jayakumari, 2018; Sharma *et al.*, 2019; Oresanya *et al.*, 2020; Lau *et al.*, 2020; Kavya *et al.*, 2023). However, in Nigeria, banana flowers are wasted in farms due to ignorance on their medicinal and nutritional values. With increasing awareness of the medicinal properties of banana flower, there is a need to ascertain the effect of prolonged oral gavage on liver biomarkers and their corresponding histopathological lesions. The liver plays a pivotal role in the metabolism and biotransformation of endogenous and xenobiotic substances in the body. *In vivo* and *in-vitro* studies using animal and laboratory models have been established to assess the safety of medicinal plants. Toxicity to the liver is mostly diagnosed through elevated liver enzymes in the blood, such as ALT, AST, ALP and also total protein and albumin (McGill, 2016). This study is aimed at evaluating the subacute effect of the methanolic extract of banana flower on total protein and liver biomarkers and its associated histopathological changes in albino rats.

Materials and Methods

Plant collection and identification

Fresh banana flowers were collected from a garden in Dalori quarters, Konduga local government area of Borno State, Nigeria. The flowers used were identified by a taxonomist at the Department of Biological Sciences, University of Maiduguri, Borno State, with Voucher number UMM/VPB/015.

Plant processing and extraction

The flowers were dried at room temperature and pulverised into powdered form using a wooden mortar and pestle. Three hundred and fifty grams (350 g) of the powdered flower were weighed using a digital weighing balance. It was then extracted with 98% methanol (Usman *et al.*, 2009). Twenty grams of powder was extracted using 200 mL of methanol. It was then refluxed for about 3 hours at 64°C and filtered to remove debris, after which it was concentrated to dryness at 40 to 50°C using a hot-air oven. The resulting solid was weighed at 14g and kept in a desiccator until required for use.

Acquisition and care of experimental animals

Wistar rats of both sexes, with a weight range of 120-280g, were gotten from the Faculty of Pharmacy laboratory animal breeding farm, University of Maiduguri, Borno State, Nigeria. The rats had never been exposed to any xenobiotic *in vivo*. They were housed in a clean, fly-proof, well-ventilated, air-conditioned animal house in plastic cages covered with stainless wire mesh, complete with drinkers and wood bedding. They were given water regularly and fed with commercial grower chick mash (18% crude protein, Olam Feeds, Nigeria Limited). All the rats had unrestricted access to food and water during the experiment and were kept for two weeks to acclimatise before the experiment started.

Ethical clearance

The Animal Use and Ethics Committee of the University of Maiduguri's Faculty of Veterinary Medicine granted consent for the use of Wistar rats in this work with approval number FVM/UNIMAID/AUEC/2025/0019.

Experimental design

A total of twenty-five (25) adult albino rats of both sexes were kept in separate plastic cages in five groups of five rats each. The rats were fed with commercial poultry feed throughout the experiment. Group A, which is the control group, was fed commercial growers chick mash (18% crude protein) and water only. Groups B, C, D and E, which represented the experimental groups, were fed commercial grower's chick mash (18% crude protein) and the extract daily for 21 days. Group B was orally administered a 200 mg/kg body weight dose of the extract, while Group C received a 400 mg/kg body weight dose of the extract. Similarly, group D was given 600 mg/kg and group E received 800 mg/kg body weight dose of the extract orally.

Collection of blood sample

The rats were euthanized with chloroform; blood was taken from the jugular vein in heparinized sample bottles for serum biochemical examination.

Determination of liver injury biomarkers (AST, ALT, ALP, serum protein and albumin)

The liver function test biomarkers such as aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP), serum protein and albumin were evaluated according to the method described by Kanagasabapathy & Kumari (2000).

Histomorphological lesions

After taking the blood sample, the liver was harvested, rinsed in formalin and then placed in well-labelled universal sample bottles containing 10% formalin. The fixed liver tissue was embedded in paraffin wax, sectioned (4-5 μ m) and stained using haematoxylin and eosin stain. Liver slides were viewed under a light microscope at x10 and x40 magnifications.

Statistical analysis

The data obtained were expressed as mean $\pm$ standard deviation (Mean $\pm$ SD), and the difference between means was analyzed. Logistic regression analysis were conducted to evaluate the impact of the extract over time, and $P < 0.05$ was considered significant. Computer analysis software package IBM SPSS version 27 was used.

Results

The effect of the methanolic extract of *Musa acuminata* flower extract on serum protein levels, liver enzyme biomarkers and histopathological

lesions in Wistar albino rats after exposure for 21 days are presented in tables and plate respectively.

Table 1 presents the mean and standard deviation (SD) of the serum protein levels: total protein and albumin in albino rats treated with different doses of *Musa acuminata* flower extract (200 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg), with Group 1 serving as the control group.

For total protein, which reflects the overall protein content in the blood, Group 1 (control) had a value of 69.33 ± 8.55 . Group 2 (200 mg/kg) showed a slight decrease in total protein to 66.00 ± 4.79 , indicating an insignificant effect of the plant extract at this dose. Group 3 (400 mg/kg) exhibited an increase in total protein to 74.08 ± 1.91 , suggesting a potential positive effect on protein synthesis or a shift in protein metabolism at this dose. Group 4 (600 mg/kg) had a similar total protein level of 74.00 ± 1.26 , indicating that the effect may plateau at higher doses. Group 5 (800 mg/kg) showed a substantial increase to 81.00 ± 17.47 , which could suggest a significant stimulatory effect on protein production or retention. For albumin, a major plasma protein responsible for maintaining osmotic pressure and transporting substances, Group 1 (control) had an albumin level of 37.17 ± 2.36 . Group 2 (200 mg/kg) showed a slight decrease to 35.67 ± 0.82 , suggesting a minor impact on albumin levels at this dose. Group 3 (400 mg/kg) had a slightly higher albumin level of 37.25 ± 2.45 , indicating minimal change compared to the control group. Group 4 (600 mg/kg) showed a decrease to 35.67 ± 1.21 , indicating that the plant extract at this dose might reduce albumin levels, possibly due to liver involvement. Group 5 (800 mg/kg) had a significant increase in albumin to 53.00 ± 13.36 ,

Table 1: Effect of the methanolic extract of *Musa acuminata* flower on serum protein levels

Group	Total Protein (Mean $\pm$ SD)	Albumin (Mean $\pm$ SD)
1 (Control)	69.33 ± 8.55	37.17 ± 2.36
2 (200 mg/kg)	66.00 ± 4.79	35.67 ± 0.82
3 (400 mg/kg)	74.08 ± 1.91	37.25 ± 2.45
4 (600 mg/kg)	74.00 ± 1.26	35.67 ± 1.21
5 (800 mg/kg)	81.00 ± 17.47	53.00 ± 13.36

Table 2: Effect of Methanolic Extract of *Musa acuminata* Flower on Liver Enzyme Biomarkers

Group	AST (Mean $\pm$ SD)	ALT (Mean $\pm$ SD)	ALP (Mean $\pm$ SD)
1 (Control)	10.67 ± 0.98	8.50 ± 0.55	58.17 ± 12.43
2 (200 mg/kg)	10.25 ± 2.64	10.00 ± 3.20	58.17 ± 8.10
3 (400 mg/kg)	10.17 ± 1.03	9.17 ± 0.72	58.25 ± 9.20
4 (600 mg/kg)	9.33 ± 0.47	9.00 ± 0.76	58.00 ± 20.02
5 (800 mg/kg)	10.00 ± 0.00	15.75 ± 5.35	149.50 ± 41.89

suggesting a pronounced effect at this higher dose. Table 2 presents the mean and standard deviation (SD) of the liver enzyme biomarkers: aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) in albino rats treated with different doses of the *Musa acuminata* flower extract (200 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg), with Group 1 serving as the control group. These enzymes serve as indicators of liver function and potential toxicity. For AST (Aspartate aminotransferase), a key enzyme involved in amino acid metabolism and a marker of liver cell injury, Group 1 (control) had a mean level of 10.67 ± 0.98 . Group 2 (200 mg/kg) showed a minor decrease to 10.25 ± 2.64 , suggesting minimal impact on liver function at this dose. Group 3 (400 mg/kg) had a similar value of 10.17 ± 1.03 , indicating little change in AST levels. Group 4 (600 mg/kg) showed a further slight decrease to 9.33 ± 0.47 , which might suggest a mild protective effect at this dose. However, Group 5 (800 mg/kg) exhibited a value of 10.00 ± 0.00 , which remained similar to the control group, indicating that the highest dose did not cause a significant alteration in AST levels.

For ALT (Alanine aminotransferase), another enzyme indicative of liver damage, Group 1 (control) had a mean of 8.50 ± 0.55 . Group 2 (200 mg/kg) showed an increase to 10.00 ± 3.20 , suggesting a slight rise in liver enzyme activity at this dose. Group 3 (400 mg/kg) showed a decrease to 9.17 ± 0.72 , indicating a potential stabilisation in liver function. Group 4 (600 mg/kg) exhibited a slight decrease to 9.00 ± 0.76 , suggesting no major effect at this dose. However, Group 5 (800 mg/kg) had a significant increase to 15.75 ± 5.35 , which points to a notable rise in ALT levels, signalling possible liver stress or injury at the highest dose.

For ALP (Alkaline phosphatase), an enzyme that reflects bile duct function and general liver health, Group 1 (control) had a mean of 58.17 ± 12.43 . Group 2 (200 mg/kg) and Group 3 (400 mg/kg) both had unchanged values of 58.17 ± 8.10 and 58.25 ± 9.20 , respectively, indicating minimal impact of these doses on ALP levels. Group 4 (600 mg/kg) had a

similar value of 58.00 ± 20.02 , showing no significant change. However, Group 5 (800 mg/kg) exhibited a dramatic increase to 149.50 ± 41.89 , which indicates a significant rise in ALP levels, potentially reflecting hepatobiliary dysfunction or cholestasis at this high dose.

The logistic regression analysis for the prediction of toxicity by the biomarkers is presented in Table 4.

The intercept value is -3.25, which suggests that when all biomarkers are at zero, the probability of toxicity is low. However, the key biomarkers that significantly predict toxicity include albumin and ALT.

Albumin also shows a significant positive association with toxicity (OR = 1.34, $p = 0.0003$), meaning that higher albumin levels caused by the effect of the extract increase the odds of toxicity. ALT, with a coefficient of 0.51 and a p -value of 0.0003, similarly shows a strong association, with the odds of toxicity increasing by 67% for each unit increase in ALT.

Other variables like total protein, AST, and ALP have less pronounced effects. Total protein (OR = 1.18, $p = 0.058$) and AST ($p = 0.423$) do not show significant results, and ALP ($p = 0.741$) is not a significant predictor of toxicity.

The logistic regression analysis in Table 5 provides a detailed assessment of the risk of toxicity associated with the plant extract treatment by evaluating various biomarkers. The interpretation of the results highlights the impact of these biomarkers on the likelihood of toxicity.

Total protein is marginally significant (odds ratio = 1.18, $p = 0.058$), suggesting that an increase in total protein due to the flower extract may slightly raise the odds of toxicity by 18%.

Albumin is another significant marker (odds ratio = 1.34, $p = 0.0003$), showing that an increase in albumin levels due to the extract raises the odds of toxicity by 34%. This implies that albumin levels should be monitored when assessing the risk of toxicity.

The histopathological lesions in the liver seen after exposing the albino rats to the methanolic extract of *Musa acuminata* flower for 21 days are shown in Plate I (A-E).

Table 4: Logistic Regression Results for Predicting Toxicity Based on Biomarkers

Variable	Coefficient (β)	Standard Error	Z-value	P-value	Odds Ratio (exp (β))
Intercept	-3.25	0.80	-4.06	0.0001	0.039
Total Protein	0.17	0.09	1.89	0.058	1.18
Albumin	0.29	0.08	3.63	0.0003	1.34
AST	0.12	0.15	0.80	0.423	1.13
ALT	0.51	0.14	3.64	0.0003	1.67
ALP	0.02	0.06	0.33	0.741	1.02

Table 5: Assessment of Toxicity Risk Associated with the methanolic extract of *Musa acuminata* flower Treatment Based on the Logistic Regression Results

Variable	Odds Ratio (OR)	P-value	Interpretation
Total Protein	1.18	0.058	Marginally significant. Each unit increase in total protein increases the odds of toxicity by 18%.
Albumin	1.34	0.0003	Significant. Each unit increase in albumin increases the odds of toxicity by 34%.
AST	1.13	0.423	Not significant. AST does not significantly affect the odds of toxicity.
ALT	1.67	0.0003	Significant. Each unit increase in ALT increases the odds of toxicity by 67%.
ALP	1.02	0.741	Not significant. ALP does not significantly affect the odds of toxicity.

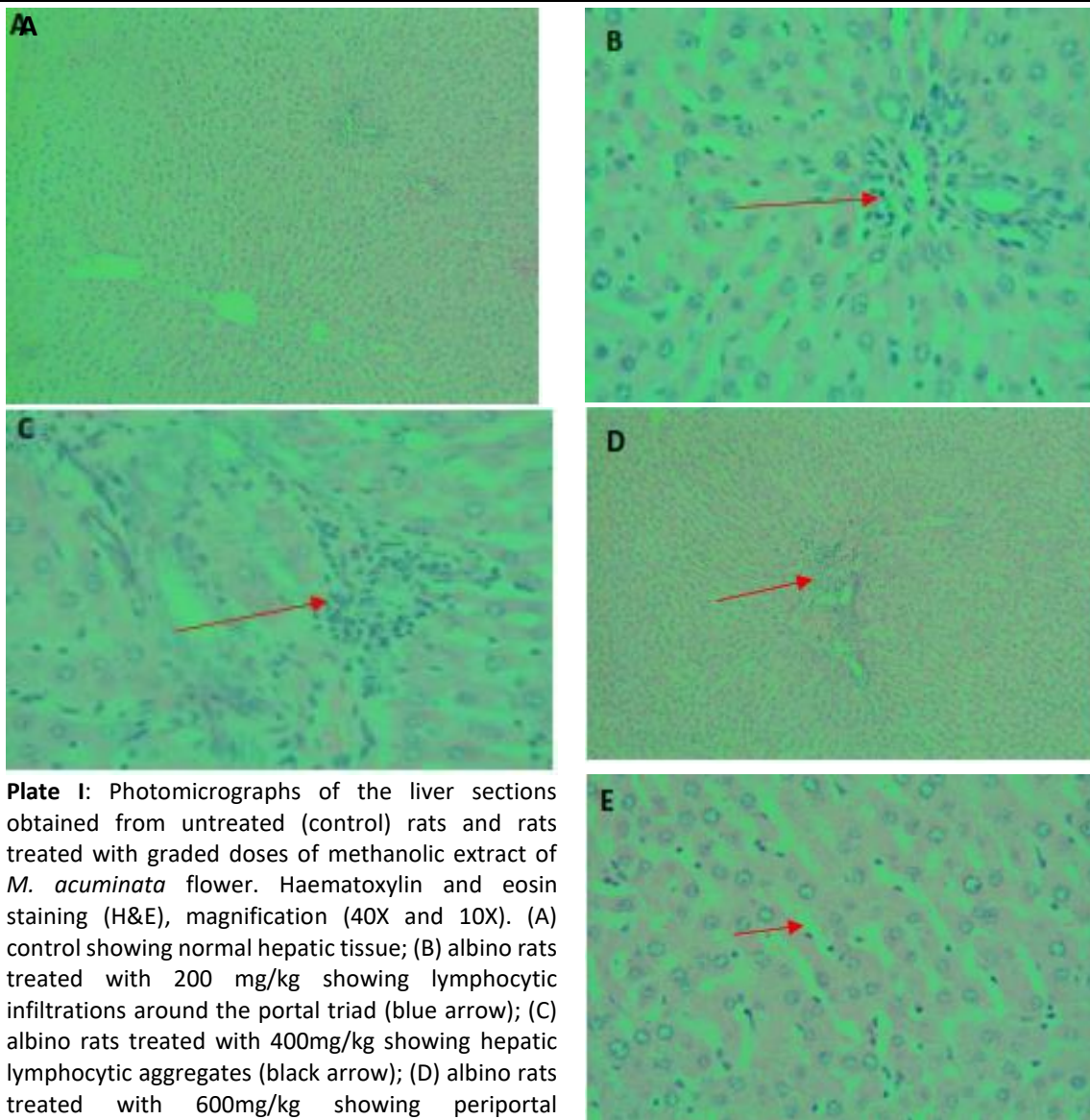


Plate I: Photomicrographs of the liver sections obtained from untreated (control) rats and rats treated with graded doses of methanolic extract of *M. acuminata* flower. Haematoxylin and eosin staining (H&E), magnification (40X and 10X). (A) control showing normal hepatic tissue; (B) albino rats treated with 200 mg/kg showing lymphocytic infiltrations around the portal triad (blue arrow); (C) albino rats treated with 400mg/kg showing hepatic lymphocytic aggregates (black arrow); (D) albino rats treated with 600mg/kg showing periportal lymphocytic aggregates (black arrow);

and (E) albino rats treated with 800mg/kg showing interstitial lymphocytic infiltrations (blue arrow).

In the liver, the control group for liver showed normal hepatic tissue and architecture, the treated groups B, C, and D (200mg/kg, 400mg/kg and 600mg/kg) showed mild lymphocytic infiltrations, while group E(800mg/kg) sections showed mild, diffuse interstitial and periportal lymphocytic aggregates and infiltrations.

Discussion

The results revealed that the flower extract influences serum protein levels in a dose-dependent manner. This effect was greater, particularly on total protein and albumin levels. The substantial increase in total protein and albumin at the highest dose could indicate a toxic effect of the extract. The large values in group five are probably due to the finding that a higher dose of 800 mg/kg significantly elevates these biomarkers. The high increase necessitates further study to understand the underlying mechanisms behind this effect. Similarly, the results suggest that the plant extract has a dose-dependent effect on liver enzyme biomarkers. At lower to moderate doses, there is minimal impact on liver enzyme levels, indicating that these doses do not cause significant liver toxicity. However, at the highest dose, there is a marked increase in ALT and ALP levels, suggesting that higher doses affect the liver and other organs like the kidneys, heart and skeletal muscle. This may indicate that while the plant extract may be safe at lower doses, higher doses could pose a risk to liver health and probably other organs that also produce these biomarkers. Further studies need to be carried out to understand the specific organs that might be involved. ALT, ALP and AST are key enzymes involved in the breakdown of amino acids, which are routed for complete metabolism through the Krebs cycle and electron transport chain. They are regarded as precise biomarkers for liver damage (Okoduwa *et al.*, 2017). The plant extract appears to have the most significant effect on ALP levels, followed by AST and ALT. The results also show that the effects are particularly pronounced when comparing the control group (Group 1) to the other treatment groups, suggesting dose-dependent variations of the extract in affecting the liver enzymes, with the higher dose causing the most elevated effect. This finding concurs with the one reported by Edenta *et al.* (2017), who reported the possible hepatotoxic and nephrotoxic effect of *Musa acuminata* fruit peels. The logistic regression results provide insight into the relationship between various biomarkers and the likelihood of toxicity. The coefficients (β) indicate the strength and direction of the association between each biomarker and the probability of toxicity. A positive coefficient suggests an increased likelihood of toxicity as the biomarker level increases, while a negative coefficient indicates a decreased likelihood.

These biomarkers could potentially be used to assess the risk of toxicity associated with plant extract treatment. The histopathological lesions revealed lymphocytic infiltration of hepatocytes in all the

treatment groups, with pronounced aggregation of lymphocytes observed at the highest dose. Lymphocytic aggregates are a general immune response triggered in this instance by herbal-induced liver toxicity. The infiltration of lymphocytes provoked by the plant extract triggered cellular damage. This buttressed the dose-dependent increased level of ALT and ALP biomarkers, which are indicators of liver tissue injury. This agrees with the findings of Edenta *et al.* (2017). This further confirms the dose-dependent toxic effect of the plant extract on the liver.

In conclusion, the findings of this study revealed a dose-dependent risk of toxicity associated with total protein, albumin, ALP, AST and ALT biomarkers as potential predictors of liver and probably other organs' toxicity. In the same vein, the micrograph of the liver sections revealed inflammatory responses to the plant extract with no toxic effect except for the pronounced aggregation of lymphocytes observed at the highest dose.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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