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Full Length Research Article

TOXICOPATHOLOGICAL EVALUATION OF *ANNONA MURICATA* LEAF EXTRACT ON SELECTED ORGANS OF ALBINO RATS

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ABSTRACT
Annona muricata (soursop) is a tropical plant widely used in traditional medical practice for the treatment of various ailments, yet comprehensive safety data on its effects on vital organs remain limited. This study investigated the biochemical and histopathological effects of *A. muricata* leaf extracts on the liver, kidneys, testes and ovaries of albino wistar rats. Twenty adult albino rats weighing 180-200g were procured from the animal holding of the Department of Anatomy, University of Benin, and maintained under standard conditions with unrestricted access to feed and water. The rats were divided into four groups with each group consisting of 5 rats. The control Group A received distilled water only, while Group B was administered 250mg/kg of *A. muricata* extract; Group C received 500mg/kg, while Group D was given 1000mg/kg of *A. muricata* extract orally via gavage for one month. Following treatment, the rats were euthanised and blood samples were collected for biochemical analysis while organs were excised for histopathological evaluation. The result revealed no significant changes in haematological parameters, liver function tests, or reproductive hormone levels across all groups ($p > 0.05$). However, significantly elevated sodium and chloride levels were observed in rats administered 1000mg/kg/bw extracts of *A. muricata* when compared to the control ($p < 0.05$). Histopathological evaluation revealed normal histoarchitecture in the control rats, while rats administered with 250mg/kg/bw and 500mg/kg/bw of *A. muricata* exhibited hepatic steatosis with microvacuolar degeneration; however, rats administered with 1000mg/kg/bw of *A. muricata* maintained normal liver histology. All kidney, testis, and ovary sections demonstrated preserved normal architecture across treatment groups. Findings in this study suggest that *A. muricata* leaf extracts exhibit a complex dose-response relationship, with the intermediate doses causing hepatic steatosis while higher doses appear protective. The preservation of the reproductive organ integrity and absence of a significant biochemical toxicity support the traditional use of *A. muricata*, though careful dose optimisation and electrolyte monitoring are recommended for therapeutic applications.

Keywords: Ethnobotanicals, kidney, liver, medicinal plants, reproductive organs.

INTRODUCTION

Annona muricata (soursop) is a tropical plant native to Central and South America, Southeast Asia and Africa. Studies have documented that the leaves of *A. muricata* contain diverse phytochemicals like alkaloids, flavonoids, tannins, and phenolic compounds, which contribute to its antioxidant, anti-inflammatory, and anticancer activities (Rasyid *et al*., 2023; Lekjing *et al*., 2024). Studies have documented the effectiveness of *A. muricata* leaves in traditional medicine for various ailments, including pain relief and anti-parasitic effects, indicating its potential as a therapeutic agent (Rasyid *et al*., 2023; Ibegbulem *et al*., 2023). There is growing interest in exploring the effects of *A. muricata* on reproductive health, largely due to its high antioxidant content which confers protection against oxidative stress, a major contributor to reproductive disorders that impair sperm production and disrupt hormonal balance. Studies suggest that plant extracts such as soursop may alleviate reproductive toxicity caused by environmental pollutants and metabolic disturbances (Ibegbulem *et al*., 2023; Monica and Handayani, 2023). Understanding the mechanism through which *A. muricata* leaves influence reproductive health could provide novel insights into potential interventions for reproductive dysfunction. Recent studies have transitioned towards evaluating the impact of various herbal preparations on various ailments, including the reproductive systems using murine models (Akinpelu *et al*., 2018; Ibegbulem *et al*., 2023). The relevance of using albino rats stems from their physiological similarities to humans when evaluating reproductive toxicology (Nonso *et al*., 2024). Investigating the effects of *A. muricata* leaves on organs and reproductive parameters in this model can pave the way for future clinical applications and therapeutic explorations. Despite the increasing interest in the pharmacological effects of *A. muricata*, particularly its leaves, there remains a distinct lack of comprehensive research specifically investigating their effects on the reproductive system. Several studies have noted the bioactive properties of *A. muricata* leaves, yet empirical data addressing their direct impact on reproductive organ health and function remain limited (Ibegbulem *et al*., 2023; Monica and Handayani, 2023). Also, limited research exists that evaluates how *A. muricata* can counteract such pathological states, particularly in terms of direct organ health impacts and reproductive hormone balance. Furthermore, existing literature seldom highlights the dose-response relationships, the biochemical pathways through which these extracts exert their effects, or histopathological changes in reproductive organs following treatment with *A. muricata* leaf extracts, showcasing a significant knowledge gap (Ehiremen *et al*., 2024; Sunday and Ilesanmi, 2023). Therefore, this study investigates the biochemical and histopathological effects of *A. muricata* leaf extracts on selected organs of albino rats, aiming to elucidate its potential systemic and reproductive impacts. Specifically, the study seeks to determine the effect of the extracts on the histomorphology of the liver, kidneys, testes and ovaries; assess its influence on liver and renal function indices; and investigate its impact on reproductive hormone levels, further providing scientific evidence that enhances understanding of the biological effects and safety profile of *A. muricata* leaf extract,

thus supporting its rational use in herbal medicine.

MATERIALS AND METHODS

Collection of the *Annona muricata* leaves

Fresh *A. muricata* leaves were obtained from Oluku market, Benin City, Edo state, Nigeria. The leaves were authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City. Prof H. A Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin and assigned a Voucher Number: UBH-A591. Following that, a sample was placed in the departmental herbarium.

Extraction of *Annona muricata* Leaves

The extraction was conducted at the Pharmacology department of the University of Benin, Benin City, Nigeria. The leaves were washed in distilled water to remove dirt or contaminants and subsequently airdried in a shaded area to prevent degradation of phytochemicals. Following drying, the leaves were pulverised into fine powder using a laboratory grinder. Fifty grams of the powdered leaves were soaked in 500 mL of ethanol for 48 hours at room temperature. The mixture was continuously stirred with a magnetic stirrer to facilitate optimal extraction, after which filtration was ensured using a fine mesh cloth to separate the liquid extract from the solid remnants. The filtrate was concentrated using a rotary evaporator to yield a viscous extract, which was stored in a cool, dark place until use (Abubakar and Haque, 2020).

Concentration Preparation

The concentrated soursop leaf extract was reconstituted in a 1% acetic acid solution to achieve a final concentration of 1% (w/v). The solution was mixed thoroughly and adjusted to a pH of 4.0 using hydrochloric acid.

Animals

Twenty-four (24) albino rats weighing between 180g and 200g were purchased from the Animal House of the Department of Anatomy, University of Benin, Benin City. The animals were kept in plastic cages with wire gauges for proper ventilation and allowed to acclimatise for two weeks before commencing the experiment. The rats were fed with grower's mash pellets (Standard feed Nigeria Plc) and water *ad libitum* under standard conditions of temperature and relative humidity of 26°C and 46% respectively. The housing facilities were kept sterile, aerated and well-maintained regularly.

Acute Toxicity Test

The lethal dose (LD50) of *A. muricata* extract was established using Locke's method to determine the appropriate dose to be administered. The rats were divided into five (5) groups of two rats in each group. Varying concentrations of *A.muricata* at 1000mg/kg, 1600mg/kg and 1900mg/kg, 2,900mg/kg, and 5,000mg/kg/b.w were orally administered via gavage. The rats were placed under observation for any signs of adverse effects, including lethargy, change in locomotive activity, abnormal behaviour, and death for 48 hours.

Experimental Design

A total of 24 adult rats were randomly assigned to four experimental groups (n = 6 per group), with three males and three females in each group:

1. Group A is the control group that received standard rat pellets and distilled water *ad libitum* for 1 month.
2. Group B had male and female rats that were administered 250mg/kg of *A.muricata* extract for 1 month.
3. Group C had male and female rats that were administered 500mg/kg of *A.muricata* extract for 1 month.
4. Group D had male and female rats that were administered 1000mg/kg of *A. muricata* extract for 1 month.

All animals were administered orally via oral gavage.

Ethical Considerations

The protocol for this study was approved by the Ministry of Agriculture and Food Security, Animal Ethics Committee (MAFSAEC), Benin City, Edo State, with reference number MAFSAEC: 025-07/28/0040. The rats were handled following the Guidelines for the Care and Use of Laboratory Animals.

Specimen collection

Following the administration period, the animals were euthanised using cervical dislocation, and blood specimen was obtained using a cardiac puncture and dispensed into lithium heparin containers and plain bottles. The organs were harvested and immediately fixed in 10% neutral buffered formalin for 24 h (Solomon & Akinbo, 2023; Moronkeji *et al*., 2024).

Biochemical Analyses

Blood samples were collected and analysed for serum levels of albumin, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, and urea. Additionally, reproductive hormones, including testosterone, progesterone, were quantified using enzyme-linked immunosorbent assay (ELISA) kits. For the liver function tests, commercially available ELISA kits procured from Thermo Fisher Scientific, Waltham, MA, USA, were utilised, while renal function tests (creatinine and urea) were assayed using the Renalcheck kidney marker ELISA kit, NephroScience Diagnostics, USA (Dita, 2021). The colourimetric results were analysed using a microplate reader (Axiom Microplate reader Urit-660) at 450nm, ensuring calibration with standards included in the kit (Dita, 2021; Kasama *et al*., 2021). Testosterone, Progesterone were evaluated using the kit by EndoCheck Multi-Hormone ELISA Kit available from suppliers R and D Systems (Minneapolis, MN, USA). The reagents used were equilibrated to 25°C before commencing (Gao *et al*., 2020). The specific substrate solution was added and left for 15 minutes away from sunlight. Stop solutions were added to stop the reaction, and optical density was measured after colour development using an ELISA plate reader at 450nm (Thermo Fisher's Multiskan GO) (Lee *et al*., 2023).

Histopathological investigation

The excised tissues fixed in 10%NBF were processed in an automatic tissue processor machine (Shandon 2000, Leica, Frankfurt, Germany) following the protocol of Moronkeji and Akinbo (2024). Briefly, tissues were dehydrated in different grades of alcohol, cleared in toluene and impregnated in molten paraffin wax for specified periods in the processor machine. Processed tissues were embedded in fresh molten paraffin wax and allowed to set. Embedded paraffin tissue blocks were trimmed at 10µ, sectioned at 3µ, and dried on a hot plate for 15 min. Sections were stained in Cole's haematoxylin for 10 min, rinsed in water, and differentiated in 1% acid alcohol. Sections were counterstained in 1% aqueous

eosin for 3 min, dehydrated in various ascending grades of alcohol, cleared in xylene, and mounted in Canada balsam. Sections were microscopically examined using x10 and x40 objective lenses.

Statistical Analysis

The data collected were analysed using the Statistical Package for Social Sciences (SPSS) software. Results were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was employed to compare mean values among groups, with Tukey's post-hoc test applied for multiple comparisons. A significant level of $p<0.05$ was considered statistically significant.

RESULTS

The effects of *A. muricata* leaf extract on white blood cell parameters across the control and treatment groups are shown in Table 1. Although increases were observed in total white blood cell count, lymphocyte, and granulocyte values among the treated groups, these changes were not statistically significant ($p > 0.05$).

Table 1: ANOVA comparison of WBC analytes across the control and treatment groups

	Group	Mean ± SD	F	p-value
WBC	Control	8.2 ± 3	1.017	0.419
	B	9.4 ± 2.7		
	C	8.4 ± 0.7		
	D	11.3 ± 3.8		
LYM	Control	7.2 ± 2.5	1.103	0.386
	B	8.3 ± 2.1		
	C	7.2 ± 0.8		
	D	9.9 ± 3.5		
LYM (%)	Control	88 ± 2.9	0.844	0.496
	B	89.2 ± 3.5		
	C	85.7 ± 3.1		
	D	87.2 ± 3.1		
MID	Control	0.8 ± 0.4	0.502	0.688
	B	0.8 ± 0.5		
	C	0.9 ± 0.2		
	D	1.1 ± 0.4		
MID (%)	Control	8.5 ± 2.4	0.884	0.477
	B	8 ± 2.8		
	C	10.4 ± 1.7		
	D	9.5 ± 2		
GRA	Control	0.3 ± 0.2	0.324	0.808
	B	0.3 ± 0.2		
	C	0.3 ± 0.1		
	D	0.4 ± 0.1		
GRA (%)	Control	3.5 ± 0.7	0.718	0.56
	B	2.8 ± 0.7		
	C	3.9 ± 1.4		
	D	3.3 ± 1.3		

Keys: WBC, white blood cell; LYM, lymphocyte; LYM (%), percentage of lymphocytes; MID, mid-range leukocytes (monocytes, eosinophils, and basophils); MID (%), percentage of mid-range leukocytes; GRA, granulocyte count; GRA (%), percentage of granulocytes.

The effects of *A. muricata* leaf extract on platelet count across all groups are displayed in Table 2. Comparative analysis of platelet count values across the treatment and control groups was not significantly different ($p>0.05$). Also, the mean platelet volume, platelet distribution width, plateletcrit and platelet large cell ratio were statistically insignificant ($P>0.05$).

Table 2. ANOVA comparison of platelet values across the control and treatment groups

	Group	Mean ± SD	F	p-value
PLT	Control	484.8 ± 79.1	0.2	0.895
	B	461.8 ± 153.6		
	C	490.8 ± 71.7		
	D	529.5 ± 168.8		
MPV	Control	7.5 ± 0.1	0.866	0.485
	B	7.9 ± 0.4		
	C	7.7 ± 0.6		
	D	7.6 ± 0.2		
PDW	Control	9.3 ± 0.4	0.428	0.736
	B	9.8 ± 2		
	C	9.1 ± 1.1		
	D	8.9 ± 0.9		
PCT	Control	0.4 ± 0.1	0.082	0.969
	B	0.4 ± 0.1		
	C	0.4 ± 0.1		
	D	0.4 ± 0.1		
P-LCR	Control	2.9 ± 2.4	0.506	0.685
	B	6.5 ± 7.5		
	C	5 ± 6.5		
	D	2.4 ± 2.8		

Keys: PLT, platelet count; MPV, mean platelet volume; PDW, platelet distribution width; PCT, plateletcrit; P-LCR, platelet large cell ratio.

Table 3 reveals the haematocrit, haemoglobin, and red cell indices across the groups. Haemoglobin concentration, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red blood cell count, mean corpuscular volume, hematocrit, red cell distribution width–standard deviation and red cell distribution width–coefficient of variation were statistically insignificant across the treatment groups compared with the control ($p > 0.05$).

Table 3. ANOVA comparison of HCT and Red cell indices across the control and treatment groups

	Group	Mean ± SD	F	p-value
HGB	Control	14.1 ± 1.2	0.107	0.954
	B	14.2 ± 1.3		
	C	14.2 ± 0.7		
	D	13.8 ± 0.7		
MCH	Control	18.7 ± 0.7	1.431	0.282
	B	18.8 ± 0.6		
	C	19.2 ± 1.1		
	D	18.1 ± 0.5		
MCHC	Control	35.5 ± 0.5	0.705	0.567

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	B	34.7 ± 1.7		
	C	35.5 ± 0.8		
	D	35.8 ± 1.2		
RBC	Control	7.5 ± 0.4	0.271	0.845
	B	7.5 ± 0.5		
	C	7.4 ± 0.4		
	D	7.6 ± 0.3		
MCV	Control	52.9 ± 1.9	1.712	0.217
	B	54.4 ± 2.9		
	C	54.3 ± 3.9		
	D	50.6 ± 1.6		
HCT	Control	39.8 ± 3.5	0.429	0.736
	B	40.8 ± 2.6		
	C	39.9 ± 2.2		
	D	38.6 ± 2.4		
RDW-SD	Control	33.1 ± 2.2	0.709	0.565
	B	34.2 ± 5.2		
	C	33.1 ± 2.8		
	D	31 ± 1.2		
RDW-CV	Control	15.6 ± 0.8	0.235	0.87
	B	15.7 ± 1.7		
	C	15.2 ± 0.5		
	D	15.2 ± 0.6		

Keys: HGB, haemoglobin concentration; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RBC, red blood cell count; MCV, mean corpuscular volume; HCT, hematocrit; RDW-SD, red cell distribution width–standard deviation; RDW-CV, red cell distribution width–coefficient of variation.

The effects of *A. muricata* leaf extract on renal and hepatic function parameters across all groups are displayed in Table 4. Although a mild, non-significant elevation in urea levels was observed in the treated rats compared with the unexposed control, creatinine values remained statistically insignificant ($p > 0.05$). Similarly, AST, ALT, and ALP levels were not significantly increased in the treatment groups relative to the control ($p > 0.05$). Total and conjugated bilirubin levels were also not significantly different among the groups ($p > 0.05$).

Table 4. ANOVA comparison of the liver and kidney parameters across the groups

	Group	Mean ± SD	F	Sig.
Liver	Control	5.9 ± 0.7	0.125	0.943
	B	6.2 ± 0.7		
	C	5.9 ± 0.7		
	D	6 ± 1.2		
Urea	Control	33 ± 4.7	0.607	0.623
	B	31.5 ± 7.6		
	C	46.3 ± 39		
	D	51.5 ± 30.9		
Creatinine	Control	1 ± 0.1	0.911	0.465
	B	1.2 ± 0.2		
	C	1.1 ± 0.4		
	D	1.2 ± 0.2		
AST	Control	81.3 ± 18.1	0.633	0.608
	B	82.8 ± 15.5		
	C	112.5 ± 78		
	D	118 ± 52.8		
ALT	Control	51.5 ± 7.1	0.683	0.579
	B	49 ± 9.8		
	C	68.5 ± 45.9		
	D	71 ± 27.6		
ALP	Control	49.5 ± 14.9	0.691	0.575
	B	51.3 ± 12.5		
	C	75.5 ± 63.2		
	D	83.8 ± 50.1		
TB	Control	0.3 ± 0.1	0.571	0.644
	B	0.3 ± 0.1		
	C	0.3 ± 0.1		
	D	0.2 ± 0.1		
CB	Control	0.1 ± 0	0	1
	B	0.1 ± 0		
	C	0.1 ± 0		
	D	0.1 ± 0		

Keys: Liver, liver tissue; Urea, plasma urea concentration; Creatinine, plasma creatinine concentration; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TB, total bilirubin; CB, conjugated bilirubin

The effects of *A. muricata* leaf extract on the kidney weight and electrolyte values across all groups are shown in Table 5. Findings revealed no significant changes in the kidney weight and electrolyte values, including sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-), across the treatment groups compared with the control ($p > 0.05$).

Table 5. ANOVA comparison of kidney weight and electrolyte values across the groups

	Group	Mean ± SD	F	Sig.
Kidney	Control	0.6 ± 0.1	1	0.426
	B	0.5 ± 0.1		
	C	0.5 ± 0.1		
	D	0.5 ± 0.1		
Na^{2+}	Control	137.3 ± 1.5	4.117	0.032
	B	139.8 ± 1.7		
	C	139.3 ± 1.5		
	D	143 ± 3.8		
K^+	Control	3.9 ± 0.6	0.977	0.436

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	B	4.7 ± 0.9		
	C	4.8 ± 1.5		
	D	4 ± 0.3		
Cl⁻	Control	103.5 ± 0.6	5.53	0.013
	B	105.5 ± 1.7		
	C	105 ± 1.8		
	D	107.3 ± 0.5		
HCO_3^-	Control	20.8 ± 3.4	2.368	0.122
	B	17.5 ± 1.9		
	C	17.8 ± 3.3		
	D	22 ± 2.7		

Keys: Kidney, kidney tissue; Na^+, sodium ion; K^+, potassium ion; Cl^-, chloride ion; HCO_3^-, bicarbonate ion.

Figure 1 showed no significant differences in body, testicular, or ovarian weights among the groups. Hormonal evaluation of testosterone and progesterone levels also revealed no statistically significant alterations across the treatment groups relative to the control ($p > 0.05$) (Figure 2).

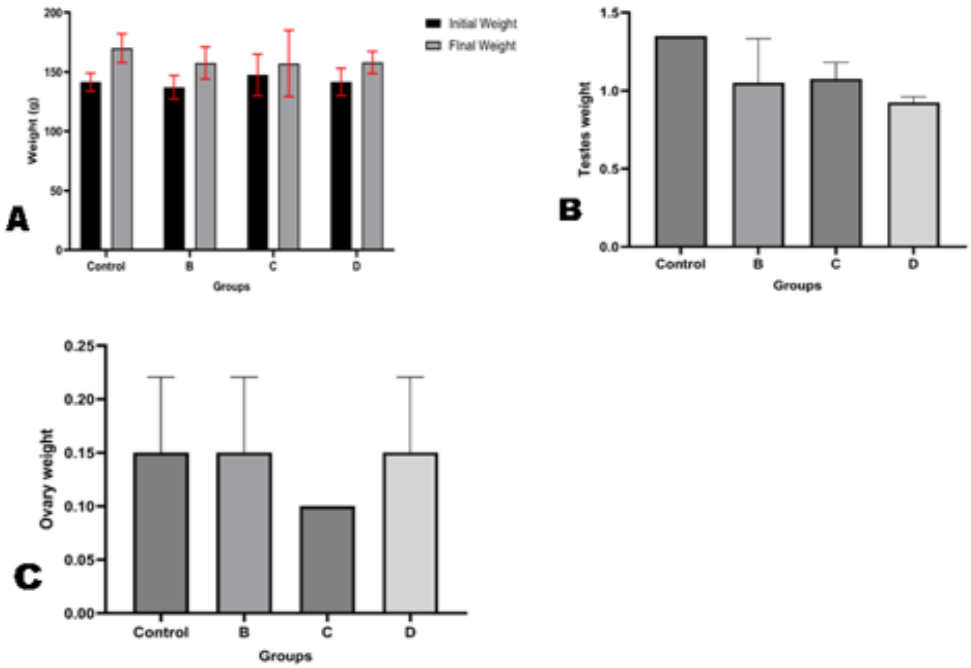

Figure 1. The effect of *Annona muricata* leaves extract on the relative body and reproductive organ (testes & ovary) weight across the groups. a. body weight. b. testes weight. c. ovary weight. There was no statistically significant change ($p > 0.05$) in the body weight, testes and ovary weight of the rats after administration of *Annona muricata* when compared to the control ($p > 0.05$).

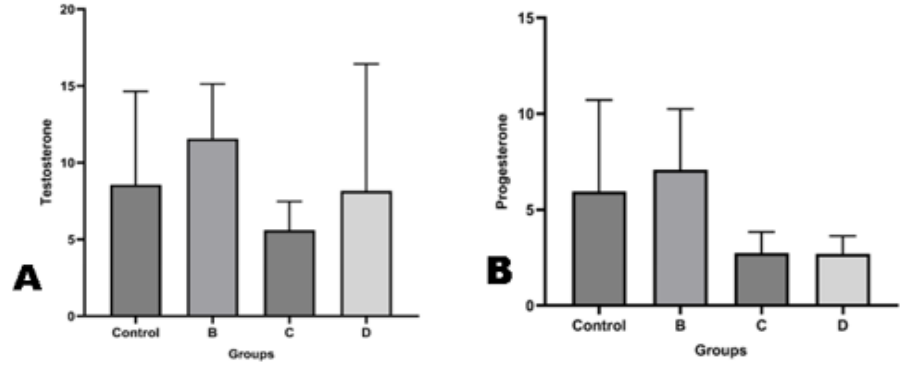

Figure 2. The effect of *Annona muricata* leaf extract on the testosterone and progesterone levels across all the groups. There was no significant difference ($p > 0.05$) in the testosterone and progesterone levels across the *Annona muricata*-treated rats when compared to the control ($p > 0.05$).

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The representative photomicrographs of liver sections from the control and treatment groups are presented in Plate 1. The liver of the control rats (Group A) appeared histologically normal, showing well-preserved hepatocytes with eosinophilic cytoplasm and centrally placed normochromic nuclei. In rats administered with 250mg/kg/bw of *A. muricata* (Group B), liver sections revealed hepatocytes containing microvacuoles consistent with ballooning degeneration, accompanied by mononuclear cell infiltration and sinusoidal Kupffer cell activation. Rats administration with *A. muricata* at 500mg/kg/bw (Group C) exhibited features indicative of steatosis, characterised by ballooning degeneration and prominent Kupffer cell activation within the sinusoids. In contrast, liver sections from *A.muricata*-treated rats at 1000mg/kg/bw (Group D) appeared normal, showing hepatocytes with eosinophilic cytoplasm and centrally located normochromic nuclei with indistinct nucleoli. The sinusoidal spaces were normal, and the overall hepatic architecture was preserved, consistent with normal histological features.

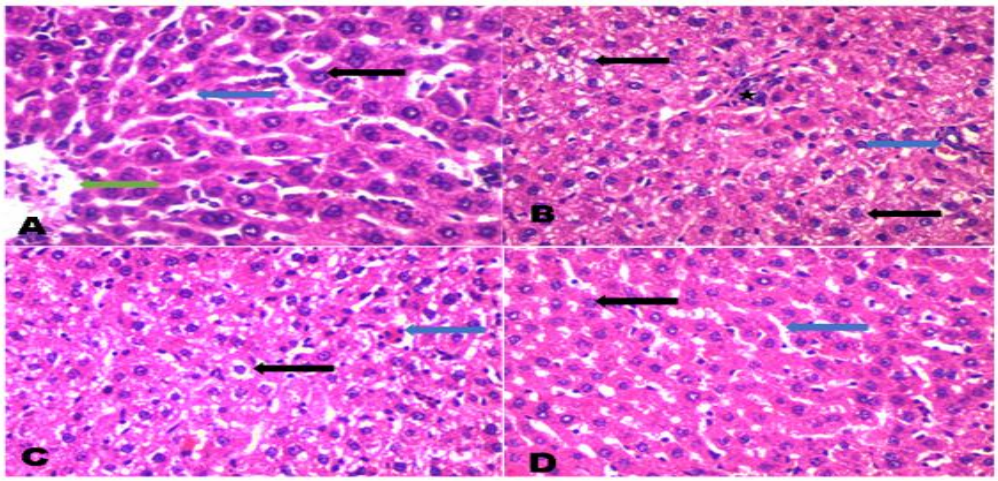

Plate 1: Representative photomicrographs of liver sections from control and treatment groups. **(A)** Control group showing normal hepatocytes (black arrow) with eosinophilic cytoplasm and centrally placed normochromic nuclei, with normal appearing sinusoids (blue arrow) and central vein (green arrow). **(B)** Group B showing hepatocytes with microvacuolar ballooning degeneration, mononuclear infiltration (★), and sinusoidal Kupffer cell activation (blue arrow), indicative of steatosis. **(C)** Group C showing similar features of steatosis with ballooning degeneration and mild Kupffer cell activation (blue arrow). **(D)** Group D showing normal hepatocytes with eosinophilic cytoplasm and centrally placed nuclei, consistent with normal hepatic architecture.

The representative photomicrographs of kidney sections from the control and treatment groups is shown in shown in Plate 2. The kidneys of the control rats appeared normal, characterised by intact glomeruli with normal mesangium, blood vessels, and epithelial lining. The renal tubules were oval-shaped and lined by cuboidal epithelial cells, with some tubules containing pale eosinophilic material within their lumina. Similarly, kidney sections from the treatment groups showed normal glomerular architecture with well-preserved mesangial cells, blood vessels, and epithelial lining. The renal tubules appeared normal, lined by cuboidal epithelial cells, and exhibited no evidence of degeneration, necrosis, or inflammatory infiltration. Overall, the histological features across the treatment groups were consistent with normal renal morphology.

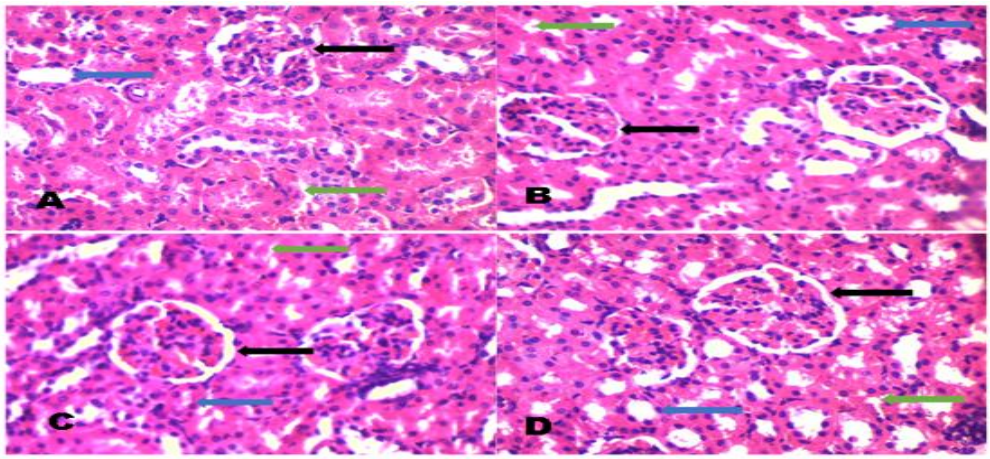

Plate 2. Representative photomicrographs of kidney sections from control and treatment groups.

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Key:

- **(A)** Section of the kidney showing normal glomeruli (thick arrow) with intact mesangium, blood vessels, and epithelial lining. The tubules (thin arrow) are oval-shaped, lined by cuboidal epithelium, and contain pale eosinophilic material. Features consistent with a normal kidney.
- **(B)** Kidney section showing well-preserved glomeruli (thick arrow) with normal mesangium, blood vessels, and epithelium. Tubules (thin arrow) are oval-shaped and lined by cuboidal cells with pale eosinophilic contents. Features in keeping with normal renal structure.
- **(C)** Section of the kidney showing normal glomeruli (thick arrow) and mesangium with intact vasculature and epithelial lining. The renal tubules (thin arrow) are oval-shaped and lined by cuboidal epithelium containing pale eosinophilic material. Features consistent with normal kidney histology.
- **(D)** Kidney section showing normal glomeruli (thick arrow) with well-preserved mesangium, blood vessels, and epithelium. Tubules (thin arrow) appear oval-shaped, lined by cuboidal cells, and contain pale eosinophilic material. Features consistent with normal renal morphology.

The representative photomicrographs of testicular histoarchitecture across the control and treatment groups is shown in shown in Plate 3. The testicular sections of the control rats revealed oval-shaped seminiferous tubules containing Sertoli cells and sperm cells at various stages of maturation. The tubules were surrounded by a thin basement membrane, with interstitial spaces containing Leydig cells, consistent with normal testicular morphology. Similarly, the testicular sections from all treatment groups appeared histologically normal, showing well-organised seminiferous tubules with Sertoli and germ cells at different maturation stages. The interstitium contained normal Leydig cells, and no observable pathological lesions were detected, indicating preserved testicular integrity across the groups

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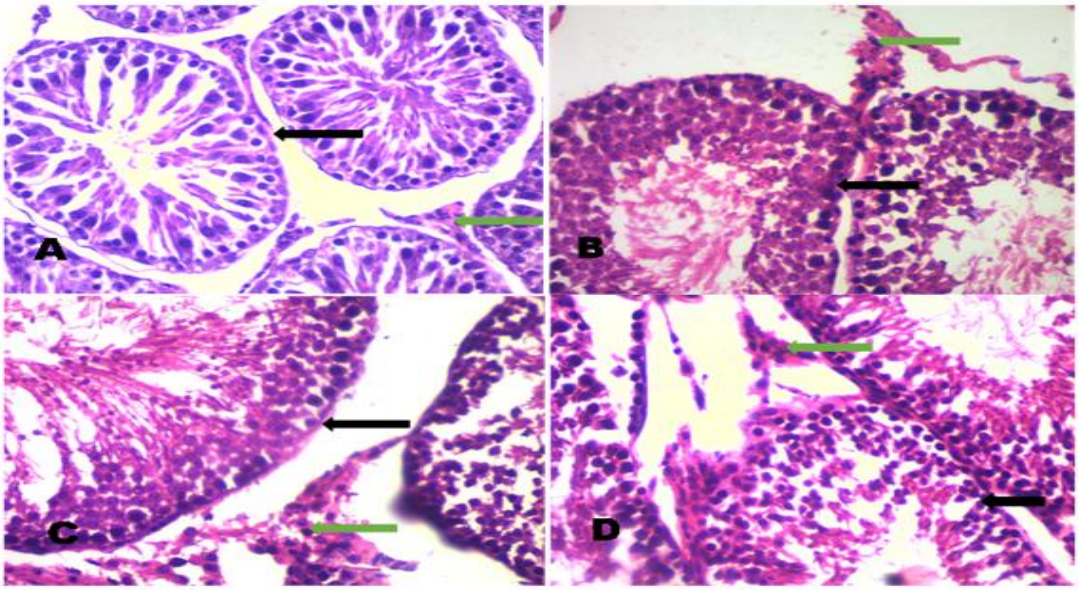

Plate 3. Representative photomicrographs of testicular sections from control and treatment groups.
(A) Section of the testis showing oval-shaped seminiferous tubules (thick arrow) containing Sertoli cells and sperm cells at various stages of maturation. The tubules are enclosed by a thin membrane, with Leydig cells (thin arrow) present in the interstitium. Features are consistent with normal testicular architecture.
(B) Testicular section showing well-organised seminiferous tubules (thick arrow) containing Sertoli and sperm cells at different maturation stages. The tubules are surrounded by a thin membrane, with Leydig cells (thin arrow) visible in the interstitium.
(C) Section of the testis showing seminiferous tubules (thick arrow) with normal cellular organisation, including Sertoli and sperm cells at varying stages of development. A thin membrane surrounds the tubules, and Leydig cells (thin arrow) are evident in the interstitium.
(D) Testicular section showing normal seminiferous tubules (thick arrow) containing Sertoli cells and sperm cells at multiple stages of maturation. The tubules are bounded by a thin membrane, and Leydig cells (thin arrow) are present within the interstitium.

The representative ovarian histoarchitecture across all groups is displayed in Plate 4. The ovary of the unexposed control rats appeared normal and devoid of pathological lesions, characterised by follicles containing oocytes surrounded by theca and granulosa cells, as well as follicles at different stages of maturation within a well-organised ovarian stroma. Likewise, ovarian sections from the treatment groups showed similar histological features, with follicles containing oocytes encased by theca and granulosa cell layers and multiple follicles at various maturation stages. Overall, the ovarian morphology across all groups remained normal, consistent with preserved ovarian structure and function.

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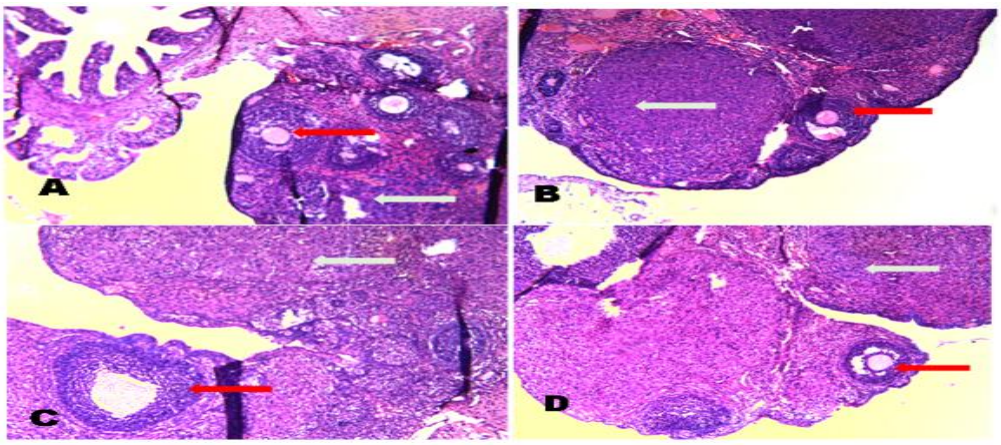

Plate 4. Representative photomicrographs of ovarian sections from control and treatment groups
Key:
A. Ovarian section showing a follicle with oocyte (thin arrow) surrounded by theca and granulosa cells; other follicles at varying maturation stages are present in the stroma (thick arrow). Features are in keeping with normal ovarian tissue
B. Ovarian section with a follicle containing an oocyte (thin arrow) and surrounding theca and granulosa cells; multiple maturing follicles are observed in the stroma (thick arrow). Features are in keeping with normal ovarian tissue**.**
C. Ovarian section showing a follicle with oocyte (thin arrow) and supporting cells; additional follicles at different maturation stages are present in the stroma (thick arrow). Features are in keeping with normal ovarian tissue.
D. Ovarian section displaying a follicle with oocyte (thin arrow) and surrounding theca and granulosa cells; other follicles at various maturation stages are seen in the stroma (thick arrow). Features are in keeping with normal ovarian tissue.

DISCUSSION

Medicinal plants play an integral role in the management of several ailments and diseases and are increasingly gaining global attention due to their therapeutic potential and perceived lower toxicity compared to conventional drugs (Moronkeji *et al*. 2024; Adeniyi *et al*., 2024). The bioactive constituents of several medicinal plants offer promising alternatives or adjuncts in disease prevention and treatment, thus supporting the growing interest in phytotherapy as a safer and more sustainable approach to health management (Abubakar *et al*., 2025; Moronkeji *et al*., 2025). *A.muricata* is widely used in traditional medicine to treat various conditions, including infections, cancer and diabetes, and this ethnomedicinal importance has prompted scientific studies into its therapeutic potential across different cultures (Chan *et al*., 2023; Ehiremen *et al*., 2024). In this study, the haematological, biochemical and histopathological effects of *A. muricata* leaf extract were evaluated on the liver, kidneys, testes, and ovaries at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg over one month, revealing both therapeutic benefits and dose-dependent adverse effects. Findings revealed that *A. muricata* did not significantly alter the full blood count (FBC) across all treatment groups compared to the control ($p > 0.05$). This finding agrees with the report of Adegboyega *et al*. (2021), who observed that ethanolic extracts of *A. muricata* leaves preserved haematological stability in sodium arsenite-induced toxicity models. The maintenance of white blood cell counts, lymphocyte proportions, haemoglobin concentrations, and platelet levels indicates that the extract does not exert notable haematotoxic effects at the tested doses, thereby supporting its haematological safety for potential therapeutic use. Nonetheless, these observations differ from some previous studies that reported possible immunomodulatory influences of *A. muricata* leaf extracts. Gavamukulya *et al*. (2019) reported alterations in certain haematological parameters following *A. muricata* administration, although their study used different extraction methods and dosing regimens. These differences may be due to variations in extraction techniques, phytochemical composition, and treatment duration, emphasising the need for standardised extraction and dosing protocols to ensure consistent and reproducible evaluation of the plant's therapeutic efficacy and safety.

The biochemical studies showed no statistically significant difference in the AST, ALT, ALP, total bilirubin, and albumin levels across treatment groups when compared to the control. This finding is particularly noteworthy given the critical role of the liver in drug metabolism and detoxification. The maintenance of normal enzyme levels suggests that *Annona muricata* extract does not induce acute hepatotoxicity at the administered doses, consistent with reports by Usunobun (2014) and Usunobun and Okolie (2016), who demonstrated hepatoprotective properties of the extract against dimethylnitrosamine-induced liver damage. However, the histopathological examination revealed a concerning pattern of steatosis (fatty degeneration) in groups B and C (250mg/kg and 500mg/kg), characterised by hepatocytes containing microvacuoles and ballooning degeneration. Interestingly, group D rats (1000 mg/kg) maintained normal hepatocytic morphology. This paradoxical finding suggests a non-linear dose-response relationship, potentially indicating that while higher doses may activate protective mechanisms or adaptive responses, intermediate doses might induce metabolic stress leading to lipid accumulation. The steatotic changes observed contradict previous studies by Oladele *et al*. (2020) and Fakunle *et al*. (2024), who reported hepatoprotective effects of *A. muricata* extracts. This discrepancy may be explained by differences in extraction methods, treatment duration, or the presence of specific phytochemical compounds that vary with preparation techniques. The development of steatosis without corresponding elevation in liver enzymes suggests early-stage metabolic disruption that may not yet manifest in biochemical markers, emphasising the

importance of histopathological evaluation in toxicity studies. The study revealed significant effects on electrolyte balance, particularly sodium and chloride levels, with group D (1000mg/kg) showing significantly elevated concentrations compared to controls ($p < 0.05$). These changes suggest potential effects on renal electrolyte handling mechanisms, possibly involving alterations in sodium-potassium ATPase activity or changes in glomerular filtration and tubular reabsorption processes. Despite these biochemical changes, histopathological examination of the kidney appeared normal across all treatment groups. The preservation of renal morphology while observing electrolyte disturbances indicates functional rather than structural alterations, which may represent early adaptive responses to the bioactive compounds in the extract. This finding partially supports the nephroprotective effects reported by Ehiremen *et al*. (2024), who demonstrated *A.muricata*'s ability to mitigate cadmium-induced renal damage, though their study focused on protective rather than direct effects. The maintenance of normal creatinine and urea levels, despite electrolyte changes, suggests preserved overall renal function. However, the electrolyte imbalances warrant careful monitoring in clinical applications, particularly in populations with compromised renal function or those taking medications affecting electrolyte balance. One of the most significant findings of this study was the absence of adverse effects on reproductive organs and hormones. Both testes and ovaries maintained normal histological architecture across all treatment groups, with no evidence of structural distortion, cellular degeneration, or inflammatory changes. The seminiferous tubules showed normal spermatogenesis stages, intact Sertoli cells, and well-preserved Leydig cells in the interstitium. Similarly, ovarian sections demonstrated normal follicular development stages, healthy theca and granulosa cells, and appropriate stromal organisation. These histological findings were corroborated by stable reproductive hormone levels, with no significant changes in testosterone or progesterone concentrations across treatment groups. This preservation of reproductive function contrasts with some concerns raised about the potential reproductive toxicity of certain plant extracts and aligns with traditional uses of *A. muricata* that do not report reproductive adverse effects. The results support findings by Ibegbulem *et al*. (2023), who reported beneficial effects of *A. muricata* on prostate health and hormone balance. Furthermore, the study's findings are consistent with the reports of Ehiremen *et al*. (2024), who demonstrated the protective effects of the extract on testicular histomorphology in cadmium-induced toxicity models. The preservation of reproductive function suggests that *A. muricata* leaf extract does not interfere with normal gonadal function and may even provide protective benefits against reproductive toxicity. The varied effects observed in this study likely reflect the complex phytochemical composition of *Annona muricata* leaves, which includes flavonoids, alkaloids, phenolic compounds, and acetogenins. The antioxidant properties attributed to flavonoids and phenolic compounds may explain the protective effects observed in reproductive organs and the maintenance of overall organ function (Pieme *et al*., 2014; Aguilar-Hernández *et al*., 2019). The manifestation of hepatic steatosis at intermediate doses, with preserved hepatic function at higher doses, suggests a non-linear, dose-dependent metabolic response. Acetogenins in *A. muricata* are known to modulate cellular metabolism and may contribute to these biphasic hepatic effects (Rady *et al*., 2018). The electrolyte changes observed at high doses may result from the extract's effects on membrane transport proteins or hormonal systems regulating fluid and electrolyte balance. The flavonoid content, particularly quercetin and kaempferol, has been associated with effects on renal tubular function and may explain the observed sodium and chloride elevation (Astuti *et al*., 2021). The findings in this study support the therapeutic potential of *A. muricata* leaf extracts as evidenced by the preservation of reproductive function and minimal haematological or overt organ toxicity. However, dose-dependent adverse effects- including hepatic steatosis at intermediate doses and electrolyte disturbances at high doses indicate the need for careful dose optimisation and monitoring. Several limitations of this study should be acknowledged. The short treatment duration, limited sample size, absence of reversibility assessment and lack of extraction method comparison. Future research should prioritise long-term safety evaluations, mechanistic investigations, extract standardisation, reversibility studies and clinical trials to establish safe and effective dosing parameters.

Conclusion

This study comprehensively evaluated the histopathological and biochemical effects of *Annona muricata* leaf extract in albino rats, providing insights into both its therapeutic potential and safety profile. Results showed preserved reproductive organ integrity and normal haematological parameters across all doses, supporting its traditional use as a safe herbal remedy. However, a non-linear dose-response was observed: intermediate doses (250–500 mg/kg) induced hepatic steatosis, while higher doses (1000 mg/kg) maintained normal liver histology. Elevated sodium and chloride at the highest dose suggested functional renal effects without structural damage. These findings highlight the importance of precise dosing, monitoring of electrolytes, and comprehensive safety evaluations for herbal medicines. Overall, the study validates the traditional use of *A. muricata*, reveals previously unreported effects, and underscores the need for evidence-based clinical application and further research to optimise therapeutic use.

REFERENCES

Abubakar A.R, Haque M. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *J Pharm Bioallied Sci*. 12(1):1-10.

Abubakar, A. A., Arawande, J. O., Adeniyi, T. D., Moronkeji, A., Aina, O., & Salau, S. (2025). In Vivo anti-Plasmodium Potential of Aloe barbadensis Anthraquinones and its Combination with Amodiaquine. *Tropical Journal of Natural Product Research*, *9*(10).

Adegboyega, A., Odunola, O., Akinwumi, K., Babalola, O. and Akinwande, A. (2021). Ameliorative effect of ethanol extract of *Annona muricata* leaves in sodium arsenite-induced toxicity in male wistar rats. *International Journal of Biochemistry Research and Review*, 2(4): 8-15.

Adeniyi T.D., Moronkeji A, Akinlami, O.O., Firdaws O, and Mofesola M.A. (2024). Evaluation of the Combined Extract of Rauwolfia Vomitoria and Aframomum Melegueta on the Kidney and Liver of Adult Male Wistar Rats. *Sokoto Journal of Medical Laboratory Science* 9(2): 203–12.

Aguilar-Hernández, G., García-Magaña, M., Vivar-Vera, M., Sáyago-Ayerdi, S., Sánchez-Burgos, J., Morales-Castro, J., and Montalvo-González, E. (2019). Optimization of ultrasound-assisted extraction of phenolic compounds from *Annona muricata* by-products and pulp. *Molecules*, 24(5):

904-908.

Akinpelu, M., Gerald, E. I., Ayodele, B. R., Akpu, A. O. & Chuks, I. M. (2018). Evaluation of herbal cocktail used in the treatment of malaria on liver tissue of adult Wistar rats. *Journal of Medicinal Plants Research*, 12(28), 508-521.

Astuti, Y., Priambodo, W. and Septiyana, D. (2021). Overview of wound assay methods extract ethanol leaves soursop (*Annona muricata*) on widr colon cancer cells. *Bali Medical Journal*, 10(3): 1385-1389.

Chan, W. J. J., Beale, P., McLachlan, A. J., Hanrahan, J. R., & Harnett, J. E. (2024). The safety and tolerability of Annona muricata leaf product in people living with cancer: study protocol. *Advances in Integrative Medicine*, 11(3), 143-148.

Dita, M. (2021). Enzyme linked immunosorbent assay (elisa). *Natural Sciences Engineering and Technology Journal*, 1(2): 29-38.

Ehiremen, S. E., Iyare, G., and Omorodion, N. T. (2024). Restorative effect of *Annona muricata* on histomorphology of testicular tissue of cadmium tumor induced albino rats. *Journal Of Health, Applied Sciences and Management*, 7(2): 66-80.

Fakunle, P., Samuel, E., Kayode, O., and Kehinde, B. (2024). Assessment of protective roles of aqueous seed extract of sour-sop (*Annona muricata*) in cerebellum following cadmium-induced neurotoxicity of adult wistar rats. *Asian Journal of Research in Medical and Pharmaceutical Sciences,* 13(1): 9-20.

Gao, Y., Guo, H., Moumouni, P., Liu, M., Li, J., Efstratiou, A., and Xuan, X. (2020). Development and evaluation of an enzyme-linked immunosorbent assay based on recombinant tgsrs2 for serodiagnosis of toxoplasma gondii infection in cats. *Journal of Veterinary Medical Science*, 82(11): 1662-1665.

Gavamukulya, Y., Maina, E., Meroka, A., Madivoli, E., Shemy, H., Magoma, G., and Wamunyokoli, F. (2019). Liquid chromatography single quadrupole mass spectrometry (lc/sq ms) analysis reveals presence of novel antineoplastic metabolites in ethanolic extracts of fruits and leaves of *Annona muricata*. *Pharmacognosy Journal*, 11(4): 660-668.

Ibegbulem, C., Ujowundu, C., Ukairo, D., and Ihejieto, H. (2023). Effect of *allium cepa* (onion) bulb and *Annona muricata* (soursop) pulp juices on body weight, prostate weight, selected hormones and prostate-specific antigen (psa) in testosterone and oestradiol-induced benign prostatic hyperplasia in albino rats. *Asian Journal of Research in Biochemistry,* 13(3): 94-103.

Kasama, T., Sun, M., Kaji, N., Akiyama, S., Yuzawa, Y., Tokeshi, M., and Baba, Y. (2021). Microchip immunoassays for monitoring renal function: rapid, low-cost, and highly sensitive quantification of urinary biomarkers of diabetic nephropathy. *Micromachines*, 12(11): 1353.

Lee, D., Asmare, N., and Sarioglu, A. (2023). Paper-based multi-well depletion elisa. *Lab on a Chip*, 23(2): 251-260.

Lekjing, S., Venkatachalam, K., Charoenphun, N., and Noonim, P. (2024). Effect of different drying methods on the phytochemical and antioxidant properties of soursop leaves at two stages of maturity. *Acs Omega*, 4(6): 71.

Monica, D. and Handayani, E. (2023). The subchronic effect of water extract leaves ofsoursop (*Annona muricata* l.) bax expression of neuron rat *hippocampus gyrus dentatus* (rattus norvegicus). *Journal of Social Science (Joss),* 2(9): 798-806.

Moronkeji, A., Adeniyi, T. D., Oyeleke, A., Igunbor, C. M., Ayeni, J. O., Adelabl, T. O., & Moronkeji, A. (2024). Cymbopogon citratus: a modulator of Tnf-α and IL-10 in NSAID-induced hepatorenal toxicity in adult Wistar rats. *Bulletin of Pharmaceutical Sciences Assiut University*, *47*(2), 1251-1262.

Moronkeji, A., Adeniyi, T., Olubunmi, E., Kayode, I., Ajala, O. J., Moronkeji, A. I., & Idowu, D. (2025). Modulatory effects of Aframomum melegueta and Rauvolfia vomitoria in cadmium-induced renal toxicity in adult rats. *Journal of Cellular Biotechnology*, *11*(1), 3-13.

Moronkeji, Akinpelu and O. Akinbo, Frederick (2024). Genotoxic Response and Histological Alterations in Rat Lungs Exposed to Gasoline Generator Exhaust, *Sultan Qaboos University Journal for Science*:29(1):15-27

Moronkeji, Akinpelu, Adeniyi Temidayo, Akinnagbe Benita, and Oladele Latifat. 2024. 'Methanolic Leaf Extract of Vernonia Amygdalina Mitigates Cadmium-Induced Oxidative Stress in the Liver and Kidney of Adult Male Wistar Rats'. *Egyptian Journal of Basic and Applied Sciences* 11(1): 469–78. https://doi.org/10.1080/2314808X.2024.2360364.

Nonso, E., Serah, N., Uchefuna, N., Uche, N., U., N., Chinwendu, A., and Chisom, U. (2024). The effect of ethanolic soursop leaf extract on caffeine-induced anxiety in female wistar rats. *International Journal of Research and Scientific Innovation,* 11(12): 195-204.

Oladele, A., Olumayowa, A., and Temitope, O. (2020). Phytochemical and antimicrobial activity of ethnomedicinal leaf extract of selected plants in nigeria. *World Journal of Advanced Research and Reviews,* 7(1): 253-262.

Pieme, C., Kumar, S., Dongmo, S., Moukette, B., Boyoum, F., Ngogang, J., and Saxena, A. (2014). Antiproliferative activity and induction of apoptosis by *Annona muricata* (annonaceae) extract on human cancer cells. *BMC Complementary and Alternative Medicine*, 14(1): 14-72.

Rady, I., Bloch, M., Chamcheu, R., Mbeumi, S., Anwar, M., Mohamed, H., and Chamcheu, J. (2018). Anticancer properties of graviola (*Annona muricata*): A comprehensive mechanistic review. *Oxidative Medicine and Cellular Longevity*, 8(1): 18-26.

Rasyid, F., Amin, A., Sukmawati, S., Djakariani, K., Riska, R., Aliansyah, M., and Sari, C. (2023). Toxicity activity and total phenolic content of soursop leaves from three regions in south sulawesi, indonesia. *Journal of Experimental and Clinical Pharmacy (Jecp),* 3(2): 116.

Solomon, B. A., & Akinbo, F. O. (2023). Histomorphological Observations in Reproductive Organs of Wistar Rats Administered with Aqueous Leaf Extract of Momordica charantia. *European J Med Plants*.

Sunday, R. and Ilesanmi, O. (2023). Toxicity studies of *physalis angulata* leaves extract on biochemical and haematological parameters in albino rats. *Journal of Pharmacology and Toxicology*, 18(2): 83-88.

Usunobun, U. (2014). Hepatoprotective potential of *Annona muricata* leaves against hepatotoxicity induced by carbon tetrachloride in rats. *International Journal of Medicine and Biomedical Research*, 3(2): 99-108.

Usunobun, U., and Okolie, P. N. (2016). Phytochemical, trace and mineral composition of *Annona muricata* (soursop) leaves. *International Journal of Biological and Chemical Sciences*, 9(4): 2168-2177.