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Acute and sub-chronic toxicity assessment of methanol leaf extract of *Persea americana* (avocado) in Wistar rats

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ABSTRACT: *Persea americana* (Avocado) leaves are widely used in traditional medicine, yet scientific data on their toxicological safety remains limited. This study evaluated the acute and sub-chronic toxicity profiles of the methanolic leaf extract of *P. americana* in Wistar rats. Acute toxicity was assessed using 28 rats (7 groups; n = 4 per group) administered single oral doses ranging from 500–5000 mg/kg. Sub-chronic toxicity involved 24 rats (4 groups; n = 6 per group) treated daily with doses of 400–1600 mg/kg for 28 days. Cardiovascular, haematological, biochemical, and histopathological parameters were measured. Data was statistically analysed using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) for post hoc comparisons, with significance set at $p<0.05$. The estimated median lethal dose (LD_{50}) was 3162.27 mg/kg, with signs of toxicity observed at 4000 mg/kg and 50% mortality at 5000 mg/kg. No significant changes in blood pressure were detected across dose groups. Sub-chronic administration resulted in dose-dependent alterations in weight gain and mild reductions in PCV, Hb, and RBC counts at the highest dose. Histopathological analysis revealed progressive cardiac, hepatic, and renal damage with increasing dosage, despite largely unaltered serum biochemical markers. These findings suggest that *P. americana* methanol leaf extract is relatively safe at lower doses but may pose organ-specific risks at higher concentrations and prolonged exposure. Further research is warranted to elucidate underlying mechanisms, particularly oxidative stress, and to guide the development of safe and standardized therapeutic applications.

Keywords: Dose-dependent toxicity, histopathology, methanol extract, medicinal plants, *Persea americana*, toxicity profile.

INTRODUCTION

Avocado (*Persea americana* Mill.), an evergreen tree native to Central America and southern Mexico, is widely recognised for its nutritional and culinary value (Maity *et al*., 2023). Beyond its edible fruit, various parts of the plant, including the leaves, have been traditionally used to manage conditions such as hypertension, diabetes, inflammation, and microbial infections (Juma, 2024; Agunloye *et al*., 2025). With growing global interest in

natural therapies, recent studies have intensified investigations into the pharmacological properties of *P. americana* leaves, revealing bioactive compounds with antioxidant, anti-inflammatory, and hypoglycaemic potential (Gavídia-Valencia *et al*., 2024).

Phytochemical analyses have revealed that methanol leaf extracts of *Persea americana* contain various bioactive compounds, notably flavonoids, tannins, saponins, and alkaloids, which are implicated in anti-inflammatory, hypoglycaemic, and hypotensive activities (Rahman *et al*., 2018; Olasunkanmi and Ogunyemi, 2023; Gavídia-Valencia *et al*., 2024). However, while therapeutic effects have been documented, concerns regarding potential toxicity, particularly with prolonged or high-dose exposure, have begun to surface. For example, Padilla-Camberos *et al*. (2013) reported adverse effects from the seed extract of avocado, raising questions about the safety of other plant components. More recently, Juma (2025) and Ojo *et al*. (2024) highlighted similar issues related to leaf extract toxicity, reinforcing the need for rigorous investigation.

Despite widespread traditional use, scientific data on the toxicological profile of *Persea americana* leaf preparations remain limited. Recent reviews and experimental studies have highlighted that most prior investigations either focused on other plant parts, such as seeds or lacked methodological rigor, including small sample sizes, narrow dose ranges, and short observation periods (Juma, 2025; Nwachukwu *et al*., 2023). These limitations hinder the development of standardized dosing protocols and safety thresholds. As interest in herbal remedies and self-medication continues to rise, the absence of robust toxicological data leaves healthcare providers and regulatory bodies without sufficient evidence to guide safe usage (Pușcaș *et al*., 2022).

Herbal medicines, though often perceived as safe due to their natural origin, may pose risks stemming from interactions among bioactive compounds, accumulation in target organs, contamination, or unanticipated side effects (Jităreanu *et al*., 2023; Khoobchandani, 2024). The absence of standardised manufacturing practices and inconsistent regulatory oversight further complicates efforts to ensure consumer safety across herbal formulations (Pawar *et al*., 2025; Amorim *et al*., 2024). In response, regulatory authorities now emphasise the importance of robust preclinical toxicity profiling as a prerequisite for phytotherapeutic drug development and market approval (Dubale *et al*., 2025; Munoz-Muriedas, 2021). This study contributes to these priorities by generating essential toxicological data that can inform public health policy, guide clinical recommendations, and support evidence-based integration of herbal medicines into mainstream healthcare.

Wistar rats, widely used in toxicological assessments, serve as a reliable and well-characterised model for evaluating the potential effects of bioactive substances. Their consistent physiological and biochemical responses enable comprehensive analysis of cardiovascular, hematological, biochemical, and histopathological markers (de Kort *et al*., 2020; Patel *et al*., 2024). Recent studies have reaffirmed their suitability for both acute and sub-chronic toxicity designs, which facilitate the identification of lethal dose thresholds, cumulative effects, and dose-dependent organ-specific responses (Akande *et al*., 2024; Selvestrel *et al*., 2022). These approaches are essential for establishing the safety margin of plant-derived extracts and informing risk assessment protocols.

The present study aims to evaluate the acute and sub-chronic toxicity profiles of methanolic extracts of *Persea americana* leaves in Wistar rats. By examining a range of physiological and biochemical parameters, this investigation seeks to determine dose-dependent effects and tissue-specific responses, thereby informing safe usage protocols for avocado leaf preparations. The findings are expected to contribute significantly to the evolving landscape of plant-derived therapeutics and provide evidence-based guidance for their integration into both traditional and modern healthcare systems.

MATERIALS AND METHODS

Plant material and extraction

Fresh leaves of *Persea americana* (avocado) were collected from a local farm at Ajibode, Ibadan, Nigeria. The leaves were identified and authenticated by a botanist at the University of Ibadan, Nigeria, and a voucher specimen (voucher number: UIH-22531) was deposited at the herbarium of the University of Ibadan.

The leaves were washed thoroughly with distilled water to remove dirt and debris, then air-dried at room temperature (25 ± 2°C) for 7 days. The dried leaves were cut into small pieces and soaked in n-hexane for 48 hours to remove fats and other non-polar compounds. After decanting the n-hexane, the leaves were air-dried again to remove residual solvent. The defatted leaves were then soaked in methanol for 72 hours with occasional stirring. The methanol extract was filtered using a sieve cloth, and the filtrate was concentrated using a rotary evaporator (Buchi Rotavapor R-200, Switzerland) at 40°C under reduced pressure. The resulting crude extract was reconstituted in corn oil to achieve concentrations of 100 mg/mL and 200 mg/mL for experimental use. Methanol was selected as the extraction solvent due to its high polarity, broad-spectrum solubilising capacity, and proven efficiency in extracting a wide range of bioactive phytochemicals, including flavonoids, tannins, saponins, and phenolic compounds known to exhibit pharmacological effects.

Experimental animals

Forty-four (44) healthy adult Wistar albino rats (weighing

90–150 g) of both sexes were obtained from the animal facility of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The rats were housed in standard polypropylene cages under controlled environmental conditions (temperature: 25 ± 2°C, humidity: 60 ± 5%, and a 12-hour light/dark cycle). They were acclimatised for two weeks prior to the experiment and provided standard rodent feed and water *ad libitum*. All experimental procedures were conducted in accordance with the guidelines of the Animal Care and Use Committee of the University of Ibadan.

Acute toxicity study

Twenty-eight (28) rats were randomly divided into 7 groups (n = 4 per group):

Group A (Control): Corn oil (vehicle) only
Groups B–G: Single oral doses of 500, 1000, 2000, 3000, 4000, and 5000 mg/kg methanol extract, respectively

Sub-chronic toxicity study

Twenty-four (24) rats were randomly divided into 4 groups (n = 6 per group):

Group A (Control): Corn oil (vehicle) only
Groups B–D: Daily oral doses of 400, 800, and 1600 mg/kg methanol extract for 28 consecutive days

Extract administration and sample collection

The extract was administered orally via gavage using a flexible, blunt-ended feeding cannula to ensure accurate dosing and minimise animal distress. Dosing occurred consistently at approximately the same time each day, with volumes not exceeding 1 mL per 100 g of body weight, in accordance with established safety guidelines for oral administration in rodents.

Following administration, rats were monitored over 72 hours for clinical signs of toxicity, including changes in behaviour, motor activity, respiratory function, and mortality. Daily observations were recorded throughout the study.

At the end of the observation period, blood samples were collected via retro-orbital puncture under mild anaesthesia for haematological and biochemical analyses. Euthanasia was performed by cervical dislocation, and vital organs (heart, liver, kidneys) were harvested, weighed, and subjected to histopathological examination to evaluate potential toxicological effects on the organs.

Determination of Median Lethal Dose (LD_{50}) Using Geometric Mean Method

The median lethal dose (LD_{50}) of the methanol extract of *Persea americana* leaves were estimated using the geometric mean approach.

For LD_{50} calculation, the highest non-lethal dose (D_1) and the lowest lethal dose (D_2) were identified.

The LD_{50} value was computed using the geometric mean formula:

$$LD_{50} = \sqrt{(D_1 \times D_2)}$$

Blood pressure measurement

Blood pressure was measured in all rats using a non-invasive tail-cuff plethysmography system (CODA, Kent Scientific, USA). Prior to measurement, the rats were acclimatised to the procedure for 10–15 minutes to minimise stress-induced variations. Measurements were taken in a quiet, temperature-controlled environment (25 ± 2°C) to ensure consistency.

For each rat, Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), and Mean Arterial Pressure (MAP) were recorded. Five consecutive readings were taken for each rat, and the average of these readings was used for analysis. Blood pressure measurements were performed:

In the acute toxicity study: Once, following the 72-hour observation period after a single dose administration.

In the sub-chronic toxicity study: At the end of the 28-day treatment period.

The CODA system was calibrated according to the manufacturer's instructions before each use to ensure accuracy. All measurements were performed by the researchers, assisted by a trained technician to minimise inter-observer variability.

Body weight monitoring

In the acute toxicity study, body weights were recorded immediately prior to dosing and again at the end of the 72-hour observation period. For the sub-chronic toxicity study, weights were measured on Day 0 prior to extract administration and subsequently weekly over the 28 days. All measurements were performed using a digital analytical balance with a precision of ±0.01 g. Percentage changes in body weight were calculated and compared across treatment groups to evaluate potential adverse effects on growth and metabolism.

Weight loss threshold

A ≥20% reduction in body weight from the baseline (Day 0) was predefined as a threshold indicative of significant toxicity. Animals exceeding this limit during the study were flagged for immediate clinical assessment and, if warranted,

humanely euthanised in accordance with institutional animal care and use protocols.

Organ collection and weighing

At the end of each study phase, i.e., 72 hours for the acute toxicity study and Day 29 for the sub-chronic study, rats were sacrificed under anesthesia. Internal organs, including the heart, kidneys, and liver, were excised, cleared of connective tissue, blotted to remove blood and excess moisture, and weighed immediately using the same precision balance. Relative organ weights (expressed as organ-to-body weight ratios) were calculated to normalize inter-group comparisons and account for size variability among animals.

Haematological analysis

Blood samples collected in EDTA-coated tubes were used for haematological analysis. Parameters assessed included packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) count, white blood cell (WBC) count, platelet count, and differential leukocyte count. These parameters were determined using an automated haematology analyser (Sysmex XN-1000, Japan) following standard protocols.

Biochemical analysis

Blood samples collected in plain tubes were allowed to clot at room temperature for 30 minutes and then centrifuged at 3000 rpm for 10 minutes to obtain serum. The serum was stored at +2 to +8°C and analysed within 7 days. Biochemical parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, and high-density lipoprotein (HDL), were analysed using commercially available diagnostic kits (Randox Laboratories, UK) according to the manufacturer's instructions (Akinniyi *et al*., 2025). Absorbance readings were taken using a spectrophotometer (Jenway 6305, UK) at the specified wavelengths for each assay.

Histopathological examination

Tissue samples from the heart, liver, and kidneys were fixed in 10% neutral buffered formalin, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a microtome (Leica RM2125 RTS, Germany), stained with haematoxylin and eosin (H&E), and examined under a light microscope (Olympus CX41, Japan) for histopathological changes.

Data analysis

All data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) for post-hoc comparisons. A p-value < 0.05 was considered statistically significant. All analyses were conducted using SPSS version 25.0 (IBM Corp., USA).

RESULTS

Observation of acute toxicity and LD_{50} determination

Rats administered 4000 mg/kg of the methanol extract of *Persea americana* leaves exhibited signs of acute toxicity, including exhaustion, incoordination, inappetence, and general feebleness. No observable toxicity symptoms were recorded in groups treated with doses ranging from 500 to 2000 mg/kg. Mortality occurred in the 5000 mg/kg group, with a rate of 50%, indicating that the extract is toxic and potentially lethal at this dosage.

The median lethal dose (LD_{50}) was calculated using the following formula:

$$LD_{50} = \sqrt{(2000 \times 5000)} = \sqrt{(10{,}000{,}000)} \approx 3162.27 \text{ mg/kg}$$

From these results, the highest non-lethal dose was determined to be 2000 mg/kg, while the lowest lethal dose was established at 5000 mg/kg.

Blood pressure response in acute toxicity study

Administration of the methanol extract of *Persea americana* leaves did not produce any statistically significant changes in blood pressure across treated groups when compared to controls ($p>0.05$). Systolic blood pressure values ranged from 109.33 ± 4.91 mmHg in the 2000 mg/kg group to 139.33 ± 12.91 mmHg in the control group. Diastolic pressure varied from 85.00 ± 14.74 mmHg (1000 mg/kg) to 120.00 ± 12.34 mmHg in controls. Mean arterial pressure (MAP) remained stable across all dose levels, with values between 88.00 ± 7.37 mmHg (2000 mg/kg) and 126.33 ± 12.67 mmHg in controls. These results suggest that acute administration of the extract did not significantly affect cardiovascular parameters (Table 1).

Body weight and organ weights in acute toxicity study

Acute exposure to the extract resulted in dose-dependent variations in body weight gain and organ mass. The control group recorded an average 18.3% increase in body weight, while the group receiving 5000 mg/kg had the lowest gain at 6.3%. Organ weights, including the heart, liver, and kidneys, showed no statistically significant

Table 1. Systolic, diastolic, and mean arterial blood pressure (mmhg) of rats administered methanol extract of *persea americana* leaves during acute toxicity study.

Group	Dose (mg/kg)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Mean Arterial Pressure (MAP) (mmHg)
A (Control)	0	139.33 ± 12.91[a]	120.00 ± 12.34[a]	126.33 ± 12.67[a]
B	500	132.67 ± 7.67[a]	111.33 ± 6.44[a]	117.33 ± 6.89[a]
C	1000	111.67 ± 15.76[a]	85.00 ± 14.74[a]	93.33 ± 15.25[a]
D	2000	109.33 ± 4.91[a]	85.33 ± 2.85[a]	88.00 ± 7.37[a]
E	3000	120.33 ± 7.31[a]	94.67 ± 9.40[a]	103.67 ± 8.41[a]
F	4000	126.67 ± 9.56[a]	103.67 ± 9.56[a]	111.00 ± 9.29[a]
G	5000	119.00 ± 9.64[a]	94.33 ± 8.82[a]	102.00 ± 9.07[a]

Values are expressed as mean ± SEM, Superscript (a) indicates no significant difference (P > 0.05) compared to the control group, BP: Blood Pressure; MAP: Mean Arterial Pressure.

Table 2. Percentage change in body weight and relative organ weights of rats following acute exposure to methanol extract of *Persea americana* leaves.

Group	Dose (mg/kg)	Initial Body Weight (g)	Final Body Weight (g)	% Weight Change	Heart Weight (g)	Liver Weight (g)	Left Kidney Weight (g)	Right Kidney Weight (g)	Total Kidney Weight (g)
A	0	100.00±4.41[a]	118.33±4.41[a]	18.3%	0.49±0.03[a]	5.10±0.52[a]	0.45± 0.05	0.39±0.04[a]	0.84±0.05[ab]
B	500	113.00±1.67[a]	133.33±1.67[a]	18.0%	0.49±0.03[a]	5.32±0.40[a]	0.44±0.003[a]	0.43±0.009[a]	0.87±0.01[a]
C	1000	103.00±8.66[bc]	115.00±8.66[bc]	11.7%	0.47±0.06[a]	3.96±0.68[a]	0.32±0.03[b]	0.35± 0.04[a]	0.65±0.04[cd]
D	2000	107.00±4.41[ab]	123.33±4.41[ab]	15.3%	0.45±0.01[a]	4.40±0.40[a]	0.34 ±0.02[b]	0.36±0.02[a]	0.69±0.04[c]
E	3000	110.00±1.67[ab]	126.67±1.67[ab]	15.2%	0.46±0.02[a]	4.54±0.40[a]	0.34±0.003[b]	0.37±0.01[a]	0.72±0.02[bc]
F	4000	107.00±4.41	116.67±4.41[b]	9.0%	0.49±0.03[a]	3.96±0.22[a]	0.32±0.02[b]	0.32±0.02[a]	0.71±0.06[bc]
G	5000	95.00±3.33[c]	101.67±3.33[c]	6.3%	0.44±0.01[a]	4.18±0.31[a]	0.32±0.05[b]	0.34±0.03[a]	0.56±0.03[d]

Note: Values are expressed as **mean ± standard error of the mean (SEM)**. Superscripts (**a, b, c, d**) denote statistically significant differences between groups (**p<0.05**). *% Weight Change* refers to the percentage change in body weight from Day 0 to Day 5.

Table 3. Haematological parameters of rats following acute exposure to methanol extract of *Persea americana* Leaves (PCV, Hb, RBC, WBC).

Group	Dose (mg/kg)	PCV (%)	Hb (g/dL)	RBC (x10^6/mm^3)	WBC (x10^3/mm^3)
A (Control)	0	47.00 ± 1.73[a]	7.27 ± 0.18[a]	7.27 ± 0.18[a]	8133.33 ± 1017.08[a]
B	500	38.67 ± 4.84[a]	6.13 ± 0.72[a]	6.13 ± 0.72[a]	6800.00 ± 472.58[a]
C	1000	41.33 ± 4.26[a]	6.90 ± 0.57[a]	6.90 ± 0.57[a]	8366.67 ± 726.48[a]
D	2000	45.33 ± 2.33[a]	7.37 ± 0.44[a]	7.37 ± 0.44[a]	12333.33 ± 2034.15[a]
E	3000	48.50 ± 0.50[a]	7.50 ± 0.10[a]	7.50 ± 0.10[a]	11000.00 ± 400.00[a]
F	4000	45.25 ± 2.10[a]	7.10 ± 0.37[a]	7.10 ± 0.37[a]	11350.00 ± 1583.51[a]
G	5000	41.67 ± 5.61[a]	6.47 ± 0.74[a]	6.47 ± 0.74[a]	9466.67 ± 2684.73[a]

Note: Values are presented as **mean ± standard error of the mean (SEM)**. Superscript **(a)** denotes **no statistically significant difference (p>0.05)** compared to the control group. Abbreviations: **PCV** – Packed Cell Volume; **Hb** – Haemoglobin; **RBC** – Red Blood Cell; **WBC** – White Blood Cell.

differences except for kidney weight. Rats administered 5000 mg/kg exhibited a significant reduction in total kidney weight (0.56 ± 0.03 g) compared to the control (0.84 ± 0.05 g, p<0.05), indicating a potential mild nephrotoxic effect at higher doses (Table 2).

Haematological parameters in acute toxicity study

Analysis of haematological indices revealed no significant differences (p>0.05) among treatment groups for packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) count, or white blood cell (WBC) count. PCV values ranged from 38.67 ± 4.84% to 48.50 ± 0.50%, while Hb concentrations spanned 6.13 ± 0.72 g/dL to 7.50 ± 0.10 g/dL. RBC counts ranged from 6.13 ± 0.72 × 10^6/mm^3 to 7.50 ± 0.10 × 10^6/mm^3, with WBC counts remaining stable across all groups (Table 3).

Differential leukocyte analysis also showed no statistically significant variation in platelet count, lymphocyte,

Table 4. Differential leukocyte counts and platelet levels in rats following acute exposure to methanol extract of *Persea americana* leaves.

Group	Dose (mg/kg)	Platelets (x10³/mm³)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophil (%)
A (Control)	0	701333.33 ± 104811.47[a]	49.00 ± 1.00[a]	41.67 ± 1.67[a]	8.33 ± 1.67[a]	1.00 ± 1.00[a]
B	500	625000.00 ± 60893.35[a]	49.33 ± 0.33[a]	44.67 ± 1.86[a]	5.33 ± 1.45[a]	0.67 ± 0.67[a]
C	1000	764000.00 ± 9609.02[a]	47.83 ± 5.93[a]	43.33 ± 2.85[a]	6.67 ± 2.03[a]	2.67 ± 1.45[a]
D	2000	804000.00 ± 107034.26[a]	49.67 ± 1.67[a]	41.00 ± 1.00[a]	9.33 ± 0.67[a]	0.00 ± 0.00[a]
E	3000	581000.00 ± 55000.00[a]	50.50 ± 1.50[a]	40.50 ± 0.50[a]	9.00 ± 1.00[a]	0.00 ± 0.00[a]
F	4000	717750.00 ± 111019.05[a]	52.50 ± 0.87[a]	40.00 ± 0.91[a]	7.00 ± 0.71[a]	0.50 ± 0.50[a]
G	5000	657333.33 ± 89856.06[a]	44.33 ± 6.17[a]	45.67 ± 4.81[a]	8.33 ± 0.88[a]	1.67 ± 0.88[a]

Note: Data are presented as **mean ± standard error of the mean (SEM)**. Superscript **(a)** denotes **no statistically significant difference (P > 0.05)** compared to the control group.

Table 5. Serum biochemical indices of rats following acute exposure to methanol extract of *Persea americana* Leaves

Group	Dose (mg/kg)	ALP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Creatinine (mg/dL)	HDL (mg/dL)
A (Control)	0	112.00 ± 9.34[a]	30.00 ± 0.82[a]	39.50 ± 3.12[a]	16.60 ± 0.89[a]	0.68 ± 0.05[a]	19.93 ± 1.18[a]
B	500	110.50 ± 5.43[a]	34.33 ± 0.52[a]	47.83 ± 2.64[a]	16.90 ± 1.08[a]	0.80 ± 0.21[a]	25.60 ± 0.84[a]
C	1000	104.00 ± 9.06[a]	30.50 ± 1.00[a]	41.75 ± 1.26[a]	16.40 ± 0.71[a]	0.83 ± 0.12[a]	23.02 ± 2.27[a]
D	2000	107.67 ± 13.05[a]	30.00 ± 1.73[a]	41.00 ± 1.73[a]	17.53 ± 0.91[a]	0.87 ± 0.12[a]	23.17 ± 1.63[a]
E	3000	100.00 ± 17.69[a]	35.00 ± 1.00[a]	50.33 ± 2.31[a]	17.93 ± 0.31[a]	1.00 ± 0.20[a]	24.77 ± 0.72[a]
F	4000	112.50 ± 8.19[a]	34.00 ± 1.41[a]	48.50 ± 4.65[a]	16.50 ± 1.49[a]	0.85 ± 0.10[a]	22.85 ± 1.72[a]
G	5000	112.00 ± 12.00[a]	28.00 ± 2.00[a]	39.33 ± 2.08[a]	17.33 ± 0.96[a]	0.77 ± 0.05[a]	20.00 ± 2.07[a]

Note: Data are presented as **mean ± standard error of the mean (SEM)**. Superscript **(a)** denotes **no statistically significant difference (p>0.05)** compared to the control group. Abbreviations: **ALP** – Alkaline Phosphatase; **ALT** – Alanine Aminotransferase; **AST** – Aspartate Aminotransferase; **BUN** – Blood Urea Nitrogen; **HDL** – High-Density Lipoprotein.

neutrophil, monocyte, or eosinophil percentages between treated and control groups (P > 0.05). Platelet counts ranged from 581,000 ± 55,000 × 10³/mm³ to 804,000 ± 107,034 × 10³/mm³, suggesting that the extract did not trigger acute haematological toxicity at the administered doses (Table 4).

Serum biochemical parameters in acute toxicity study

Evaluation of serum biomarkers related to hepatic and renal function revealed the elevation of the serum biomarkers of liver and kidney function, although the differences observed between treated and control groups were not statistically significant (p>0.05) (Table 5).

Histopathological assessment of the heart, kidneys, and liver in acute toxicity study

Heart tissue

Control animals exhibited intact myocardial architecture with no visible lesions (Figure 1A). Low-dose groups (≤1000 mg/kg) showed no significant histological abnormalities (Figure 1B, C), indicating cardiovascular safety at these levels. However, mid-dose groups (2000–3000 mg/kg) displayed mild myocardial congestion and necrosis, suggestive of early cardiac stress (Figure 1D, E). High-dose groups (4000–5000 mg/kg) revealed focal thrombosis and extensive necrotic areas, consistent with significant cardiac damage and correlating with observed mortality rates (Figure 1F, G).

Kidney tissue

Kidney sections from control animals showed normal glomerular and tubular architecture (Figure 2A). Low-dose groups (500–1000 mg/kg) exhibited mild congestion and localised renal casts, indicating early renal irritation (Figure 2B, C). Mid-dose groups showed mesangial hypercellularity and inflammation, reflecting moderate renal injury (Figure 2D, E). High-dose groups presented with glomerular fusion, marked hypercellularity, and red cell casts, demonstrating severe renal damage (Figure 2F, G). These findings are consistent with subtle biochemical elevations in creatinine.

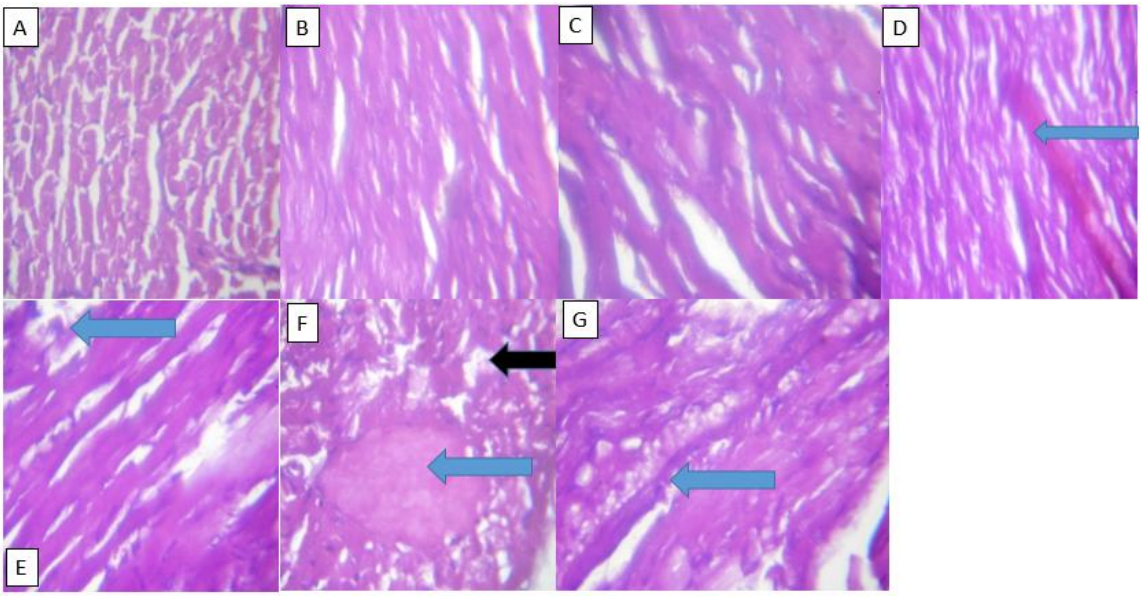

Figure 1. Histopathological Sections of the Heart in Rats Administered Methanol Extract of *Persea americana* Leaves During Acute Toxicity Study (H&E, 40×). (a) Control group (Group A) showing normal myocardial architecture with no observable lesions.. (b) 500 mg/kg (Group B) showing no significant histological alterations. (c) 1000 mg/kg (Group C) showing no visible myocardial damage. (d) 2000 mg/kg (Group D) displaying mild vascular congestion (blue arrow). (e) 3000 mg/kg (Group E) showing mild myocardial necrosis (blue arrow). (f) 4000 mg/kg (Group F) exhibiting focal thrombosis occluding blood vessels (blue arrow) and necrotic areas (black arrow). (g) 5000 mg/kg (Group G) showing extensive necrotic areas and focal thrombosis (blue arrow), consistent with severe cardiac damage.

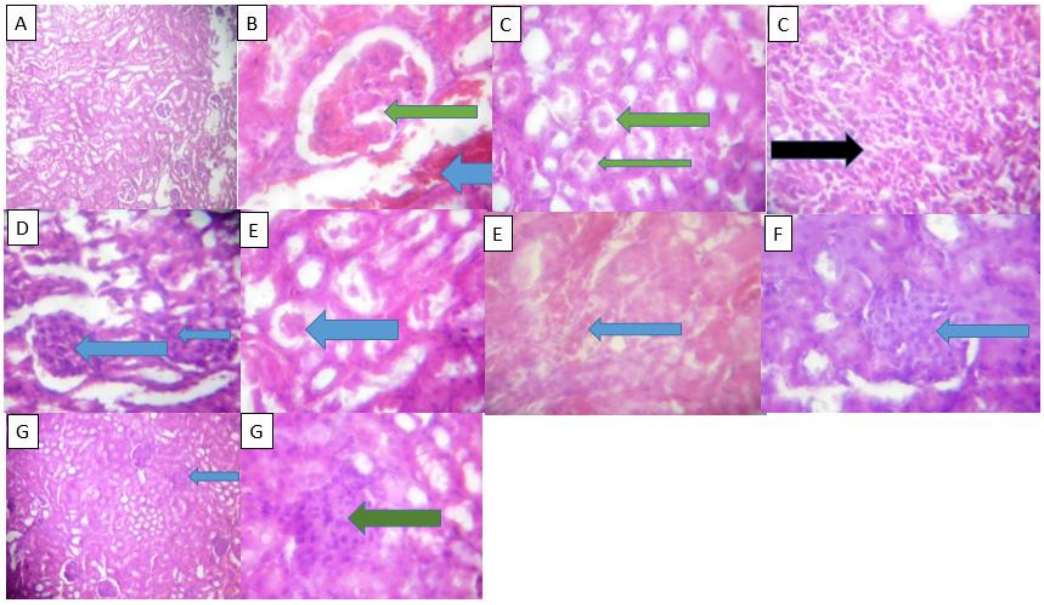

Figure 2. Histopathological sections of the kidney in rats administered methanol extract of *Persea americana* leaves during acute toxicity study (H&E, 40×). (a) Control rat (Group A) showing normal glomerular and tubular architecture with no pathological lesions. (b) 500 mg/kg (Group B) showing mild disseminated vascular congestion (blue arrow) and glomerular congestion (green arrow). (c) 1000 mg/kg (Group C) displaying focal renal casts (green arrow) and pronounced interstitial infiltration by inflammatory cells (black arrow). (d) 2000 mg/kg (Group D) showing glomerular hypercellularity of mesangial cells (blue arrows). (e) 3000 mg/kg (Group E) showing focal inflammation (bolder blue arrow) and red cell casts (blue arrow). (f) 4000 mg/kg (Group F) exhibiting hypercellularity of mesangial cells and fusion of glomeruli with Bowman's capsule (blue arrow). (g) 5000 mg/kg (Group G) presenting continued glomerular hypercellularity (blue arrow) and glomerular fusion with Bowman's capsule (green arrow).

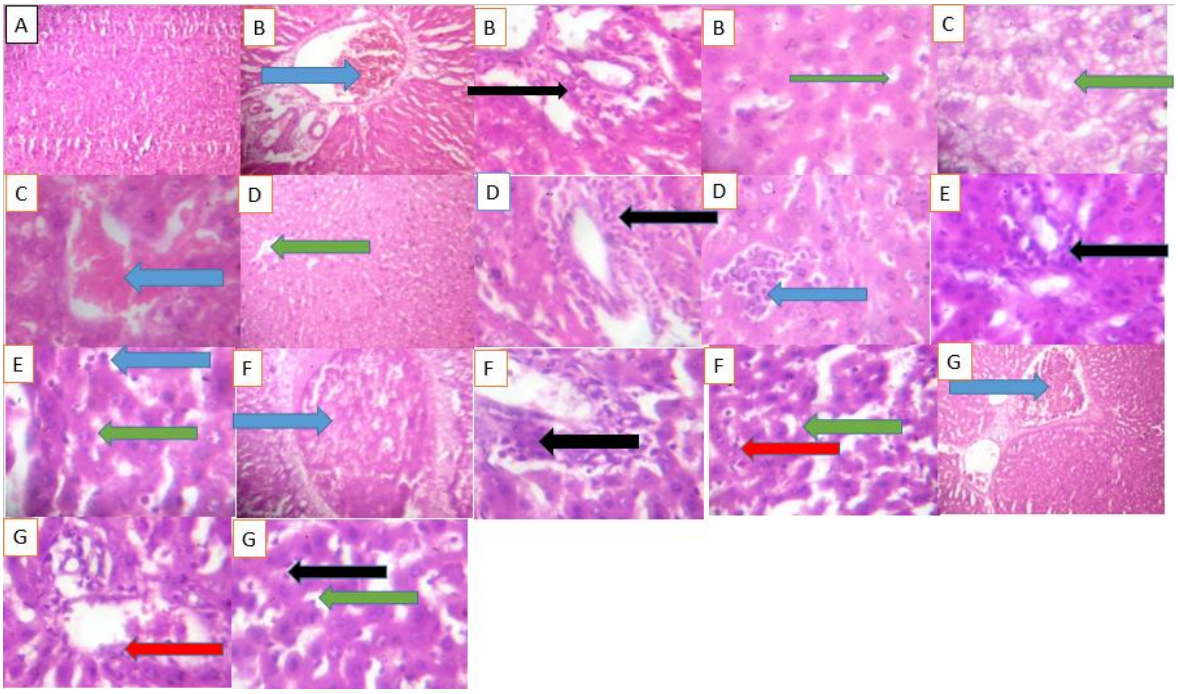

Figure 3. Histopathological sections of the liver in rats administered methanol extract of *Persea americana* leaves during an acute toxicity study (H&E, 40×). (a) Control group (Group A) showing normal hepatic architecture with no observable lesions. (b) 500 mg/kg (Group B) showing vascular congestion (blue arrow), mild periportal inflammation (black arrow), and mild disseminated macro- and microvesicular steatosis (green arrow). (c) 1000 mg/kg (Group C) presenting vascular congestion (blue arrow) and moderate macro- and microvesicular steatosis (green arrow). (d) 2000 mg/kg (Group D) exhibiting mild periportal inflammation (black arrow), focal lymphoid aggregates, and mild inflammatory infiltration of zone 2 (blue arrow). (e) 3000 mg/kg (Group E) showing vascular congestion, widespread steatosis (green arrow), mild zone 2 infiltration (slender arrow), and mild periportal inflammation (black arrow). (f) 4000 mg/kg (Group F) revealing vascular congestion (blue arrow), very mild periportal inflammation (black arrow), mild zone 2 infiltration by inflammatory cells (red arrow), and mild macrovesicular steatosis (green arrow). (g) 5000 mg/kg (Group G) showing vascular congestion (blue arrow), very mild periportal inflammation (red arrow), mild zone 2 infiltration by inflammatory cells (black arrow), and mild microvesicular steatosis (green arrow).

Liver tissue

Hepatic tissue from control animals displayed normal lobular structure with no pathological features (Figure 3A). Low-dose treatments resulted in mild periportal inflammation and steatosis, indicating early hepatic stress (Figure 3B, C). Mid-dose groups exhibited moderate congestion, inflammatory infiltration, and lipid accumulation (Figure 3D, E). Severe liver damage was evident in high-dose groups, characterised by pronounced inflammation, congestion, and steatosis, aligning with elevated ALT and AST levels observed biochemically (Figure 3F, G).

Blood pressure response in sub-chronic toxicity study

After 28 days of oral administration of the methanol extract of *Persea americana* leaves, no statistically significant changes ($p>0.05$) were observed in systolic, diastolic, or mean arterial blood pressure values across treated groups relative to controls. Systolic blood pressure ranged from 131.33 ± 10.40 mmHg in controls to 143.33 ± 3.84 mmHg in the 400 mg/kg group, while diastolic values varied from 100.00 ± 9.61 mmHg (control) to 122.00 ± 2.65 mmHg (400 mg/kg). These results suggest that sub-chronic exposure to the extract does not significantly affect cardiovascular function (see Table 6).

Body weight and organ weights in sub-chronic toxicity study

Significant differences ($p<0.05$) were noted in body weight changes among dose groups. The control group exhibited the highest percentage weight gain (37.6%), while the 1600 mg/kg group showed a slight weight reduction (−0.4%), indicating potential metabolic suppression at higher doses. Although heart, liver, and kidney weights remained relatively stable, a statistically significant

Table 6. Systolic, diastolic, and mean arterial blood pressure (mmHg) of rats following sub-chronic administration of methanol extract of *Persea americana* Leaves.

Group	Dose (mg/kg)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Mean Arterial Pressure (MAP) (mmHg)
A (Control)	0	131.33 ± 10.40[a]	100.00 ± 9.61[a]	115.33 ± 9.94[a]
B	400	143.33 ± 3.84[a]	122.00 ± 2.65[a]	115.33 ± 11.80[a]
C	800	135.67 ± 8.69[a]	102.58 ± 4.06[a]	111.33 ± 14.99[a]
D	1600	141.33 ± 5.21[a]	113.67 ± 8.41[a]	122.67 ± 7.07[a]

Note: Values are presented as **mean ± standard error of the mean (SEM)**. Superscript **(a)** denotes **no statistically significant difference (p>0.05)** compared to the control group. Abbreviations: **BP** – Blood Pressure; **MAP** – Mean Arterial Pressure.

Table 7. Percentage change in body weight and relative organ weights of rats following sub-chronic administration of methanol extract of *Persea americana* Leaves.

Group	Dose (mg/kg)	Initial Body Weight (g)	Final Body Weight (g)	% Weight Change	Heart Weight (g)	Liver Weight (g)	Left Kidney Weight (g)	Right Kidney Weight (g)	Total Kidney Weight (g)
A	0	109.00±8.85[ab]	150.00±8.85[ab]	37.6%	0.61±0.04[a]	5.84±0.15[a]	0.43±0.01[a]	0.43±0.01[ab]	0.87±0.02[ab]
B	400	132.00±6.01[ab]	151.67±6.01[ab]	14.9%	0.62±0.04[a]	5.82±0.28[a]	0.43±0.03[a]	0.45±0.02[ab]	0.88±0.05[ab]
C	800	158.00±6.63[a]	162.00±6.63[a]	2.5%	0.62±0.01[a]	6.13±0.39[a]	0.46±0.01[a]	0.48±0.02[a]	0.94±0.03[a]
D	1600	128.00±9.68[b]	127.50±9.68[b]	-0.4%	0.52±0.02[a]	5.29±0.35[a]	0.42±0.02[a]	0.40±0.02[b]	0.82±0.03[b]

Note: Values are presented as mean ± standard error of the mean (SEM). Superscripts (a, b) denote statistically significant differences between groups (p<0.05). *% Weight Change* refers to the percentage change in body weight from Day 0 to Day 28; negative values indicate a decrease in body weight.

Table 8. Haematological indices of rats following sub-chronic administration of methanol extract of *Persea americana* Leaves (PCV, Hb, RBC, WBC).

Group	Dose (mg/kg)	PCV (%)	Hb (g/dL)	RBC (x10⁶/mm³)	WBC (x10³/mm³)
A (Control)	0	37.00 ± 1.03[a]	12.05 ± 0.38[ab]	6.20 ± 0.23[a]	6183.33 ± 1186.78[b]
B	400	34.17 ± 1.40[ab]	11.10 ± 0.53[ab]	5.48 ± 0.29[ab]	9485.00 ± 753.50[a]
C	800	37.40 ± 1.03[a]	12.36 ± 0.35[a]	6.14 ± 0.22[a]	7030.00 ± 576.33[ab]
D	1600	32.75 ± 0.85[b]	10.88 ± 0.28[b]	5.33 ± 0.03[b]	7287.50 ± 470.98[ab]

Note: Values are expressed as mean ± standard error of the mean (SEM). Superscripts (a, b) indicate statistically significant differences between groups (P < 0.05). Abbreviations: PCV – Packed Cell Volume; Hb – Haemoglobin; RBC – Red Blood Cell; WBC – White Blood Cell.

reduction in kidney weight was observed in the 1600 mg/kg group (0.82 ± 0.03 g) compared to the control (0.87 ± 0.02 g). This suggests potential nephrotoxicity with prolonged high-dose exposure (Table 7).

Haematological parameters in sub-chronic toxicity study

Haematological analysis revealed significant reductions (P < 0.05) in packed cell volume (PCV), haemoglobin concentration (Hb), and red blood cell (RBC) count in the 1600 mg/kg group: - PCV: 32.75 ± 0.85% vs. control 37.00 ± 1.03%, Hb: 10.88 ± 0.28 g/dL vs. control 12.05 ± 0.38 g/dL and RBC: 5.33 ± 0.03 × 10^6/mm³ vs. control 6.20 ± 0.23 × 10^6/mm³. These findings point to a mild anaemic effect at higher doses.

No significant differences (p>0.05) were found in platelet counts or differential leukocyte indices (lymphocytes, neutrophils, monocytes, eosinophils), indicating no major extract-induced impact on immune cell populations (see Tables 8 and 9).

Serum biochemical parameters in sub-chronic toxicity study

Biochemical assessment showed variable changes in the serum liver and kidney function markers, including alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine, and high-density lipoprotein (HDL). Although the differences observed were not statistically significant. These results imply that extended exposure to the extract at the doses administered did not significantly compromise hepatic or renal function (Table 10).

Table 9. Differential leukocyte counts and platelet levels in rats following sub-chronic administration of methanol extract of *Persea americana* leaves.

Group	Dose (mg/kg)	Platelets (x10³/mm³)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophil (%)
A (Control)	0	169666.67 ± 25877.49[a]	67.50 ± 1.18[a]	28.33 ± 1.12[a]	1.83 ± 0.40[a]	2.33 ± 0.33[a]
B	400	256166.67 ± 38780.08[a]	68.50 ± 2.20[a]	27.50 ± 2.23[a]	2.00 ± 0.37[a]	2.00 ± 0.37[a]
C	800	235600.00 ± 39581.06[a]	69.80 ± 2.80[a]	26.80 ± 2.63[a]	1.80 ± 0.37[a]	1.60 ± 0.40[a]
D	1600	144500.00 ± 45993.66[a]	64.50 ± 1.85[a]	31.50 ± 2.33[a]	2.00 ± 0.41[a]	2.00 ± 0.41[a]

Note: Values are expressed as mean ± standard error of the mean (SEM). Superscripts (a, b) denote statistically significant differences between groups ($p<0.05$). Abbreviations: PCV – Packed Cell Volume; Hb – Haemoglobin; RBC – Red Blood Cell; WBC – White Blood Cell.

Table 10. Serum biochemical indices of rats following sub-chronic administration of methanol extract of *Persea americana* leaves.

Group	Dose (mg/kg)	ALP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Creatinine (mg/dL)	HDL (mg/dL)
A (Control)	0	113.25±7.54[a]	32.25±1.26[a]	42.25±2.87[a]	17.90 ± 0.67[a]	0.85 ± 0.10[a]	21.03 ± 0.90[a]
B	400	113.33±9.07[a]	32.33±1.53[a]	44.33±3.21[a]	16.57 ± 0.67[a]	0.77 ± 0.06[a]	22.77 ± 0.64[a]
C	800	112.50±7.20[a]	31.83±2.14[a]	42.33±2.42[a]	17.72 ± 0.65[a]	0.90 ± 0.11[a]	22.40 ± 1.38[a]
D	1600	115.00±12.76[a]	33.00±2.00[a]	43.33±1.53[a]	18.57 ± 0.46[a]	1.07 ± 0.12[a]	23.13 ± 0.47[a]

Note: Values are expressed as **mean ± standard error of the mean (SEM)**. Superscript **(a)** indicates **no statistically significant difference ($p>0.05$)** compared to the control group. Abbreviations: **ALP** – Alkaline Phosphatase; **ALT** – Alanine Aminotransferase; **AST** – Aspartate Aminotransferase; **BUN** – Blood Urea Nitrogen; **HDL** – High-Density Lipoprotein.

Histopathological assessment of heart, kidney, and liver in sub-chronic toxicity study

Heart tissue

The cross-section of heart tissue in control rats displayed normal myocardial histology (Figure 4a), indicating that the vehicle (corn oil) did not adversely affect cardiac tissue. In the 400 mg/kg group (Figure 4b), focal vascular congestion was observed, suggesting early signs of cardiac stress. At 800 mg/kg (Figure 4c), blood vessel congestion and focal fatty infiltration of the myocardium were evident, indicating moderate cardiac damage. In the 1600 mg/kg group (Figure 4d), marked myocardial inflammation and congestion were present, reflecting significant dose-dependent cardiac injury. These findings demonstrate that prolonged exposure to the methanol extract of *Persea americana* leaves induces progressive cardiac damage in a dose-dependent manner.

Kidney tissue

Kidney tissue from control rats exhibited intact glomerular and tubular structures (Figure 5a), confirming that the vehicle (corn oil) had no adverse effects. In the 400 mg/kg group (Figure 5b), blood vessel congestion, glomerular hypercellularity of mesangial cells, and focal renal casts were observed, indicating early renal stress. The 800 mg/kg group (Figure 5c) showed similar vascular congestion and glomerular hypercellularity, suggestive of moderate renal damage. At 1600 mg/kg (Figure 5d), prominent renal casts and glomerular hypercellularity were evident, indicating significant kidney injury at this higher dose.

Liver tissue

Liver tissue from control rats exhibited normal hepatic architecture (Figure 6a), confirming the absence of toxicity from the vehicle (corn oil). In the 400 mg/kg group (Figure 6b), marked disseminated microvesicular steatosis and mild periportal infiltration by inflammatory cells were observed, indicating early hepatic stress. The 800 mg/kg group (Figure 6c) showed blood vessel congestion, widespread macro- and microvesicular steatosis, and mild periportal inflammatory infiltration, suggesting moderate liver damage. In the 1600 mg/kg group (Figure 6d), similar congestion, marked microvesicular steatosis, and persistent periportal infiltration were present, indicating significant hepatic injury at this dose.

These histopathological changes indicate a dose-dependent progression of organ damage, particularly in the liver and kidney, and align with the subtle trends observed in organ weight variation and haematological parameters.

DISCUSSION

This study provides a comprehensive evaluation of the acute and sub-chronic toxicological profile of the methanol extract of *Persea americana* (avocado) leaves in Wistar

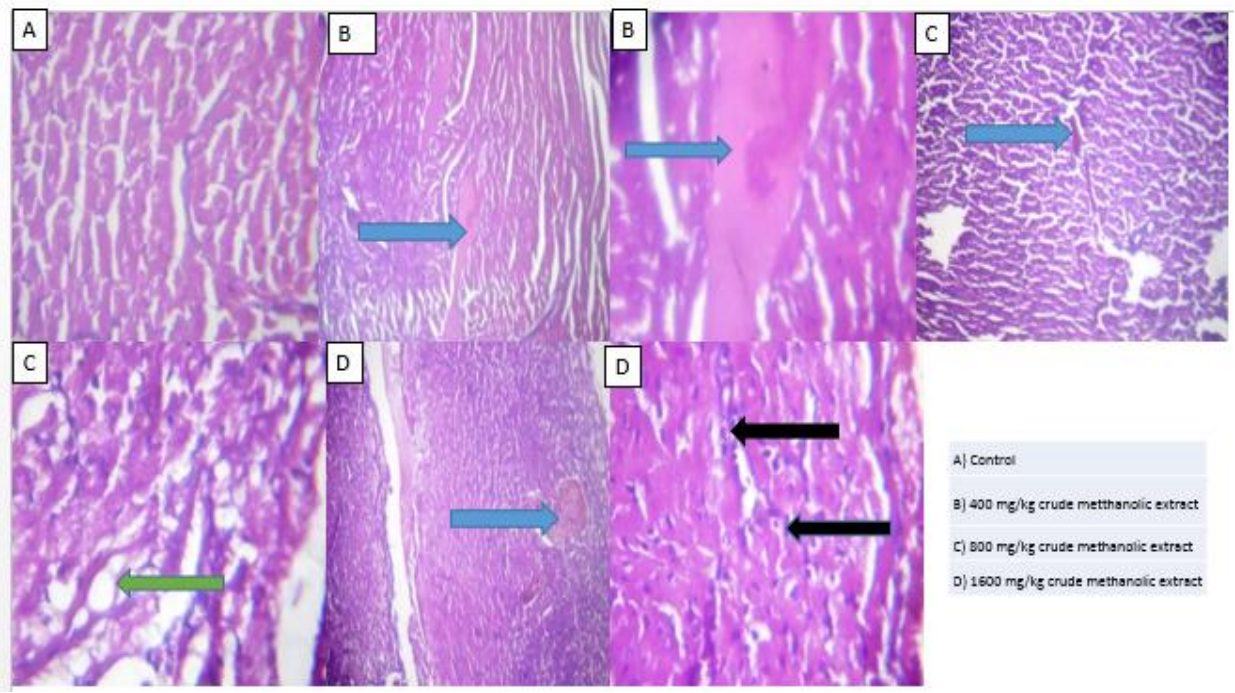

Figure 4. Histopathological sections of the heart in rats following sub-chronic administration of methanol extract of *Persea americana* leaves (H&E, 40×). (a) Control rat (Group A) showing normal myocardial architecture with no observable lesions. (b) 400 mg/kg (Group B) displaying focal vascular congestion (blue arrow). (c) 800 mg/kg (Group C) showing vascular congestion (blue arrow) and focal fatty infiltration of the myocardium (green arrow). (d) 1600 mg/kg (Group D) revealing vascular congestion (blue arrow) and focal myocardial infiltration by inflammatory cells (black arrow).

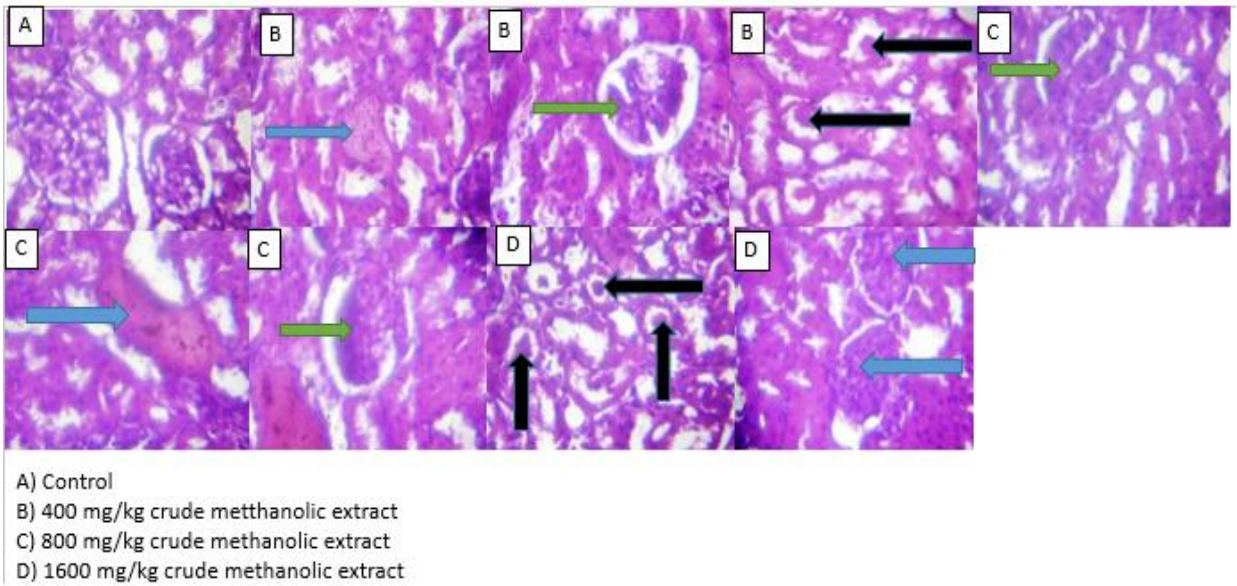

Figure 5. Histopathological Sections of the Kidney in Rats Following Sub-Chronic Administration of Methanol Extract of *Persea americana* Leaves (H&E, 40×). (a) Control group (Group A) showing normal glomerular and tubular architecture with no observable lesions. (b) 400 mg/kg (Group B) displaying vascular congestion (blue arrow), glomerular hypercellularity of mesangial cells (green arrow), and focal renal casts (black arrow). (c) 800 mg/kg (Group C) showing vascular congestion (blue arrow) and glomerular hypercellularity of mesangial cells (green arrow). (d) 1600 mg/kg (Group D) exhibiting focal renal casts (black arrow) and glomerular hypercellularity of mesangial cells (blue arrow).

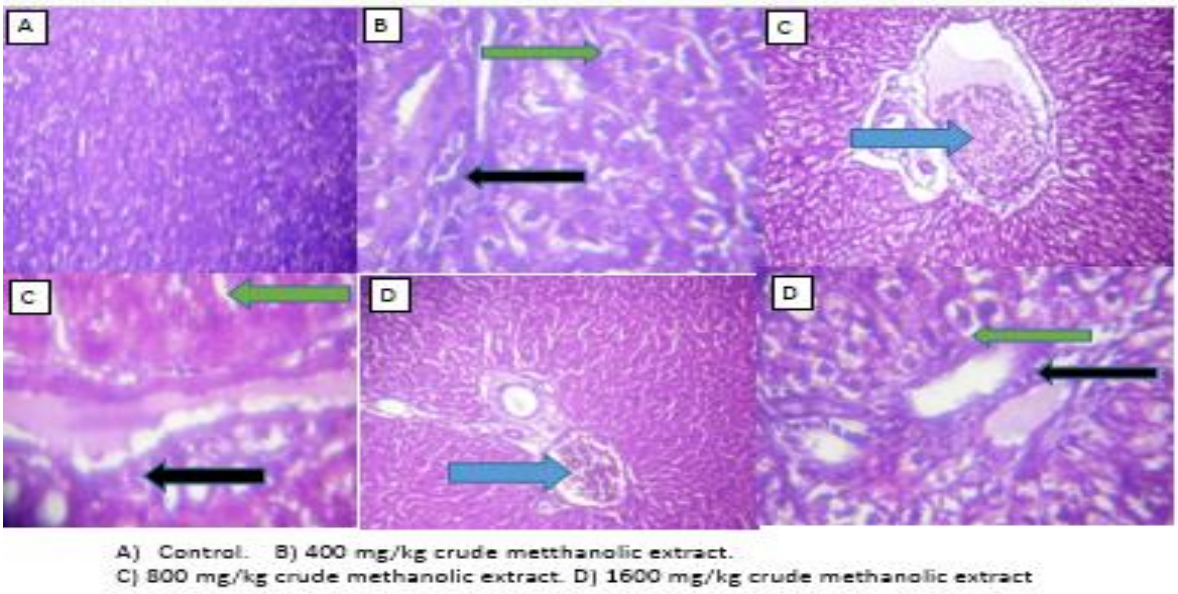

Figure 6. Histopathological Sections of the Liver in Rats Following Sub-Chronic Administration of Methanol Extract of *Persea americana* Leaves (H&E, 40×). (a) Control group (Group A) showing normal hepatic architecture with no observable lesions. (b) 400 mg/kg (Group B) exhibiting marked disseminated microvesicular steatosis (green arrow) and very mild periportal inflammatory cell infiltration (black arrow). (c) 800 mg/kg (Group C) showing vascular congestion (blue arrow), marked disseminated macro- and microvesicular steatosis (green arrow), and mild periportal inflammatory infiltration (black arrow). (d) 1600 mg/kg (Group D) presenting vascular congestion (blue arrow), marked disseminated microvesicular steatosis (green arrow), and mild periportal infiltration by inflammatory cells (black arrow).

rats. The findings demonstrate a clear pattern of dose- and duration-dependent toxicity, with more pronounced adverse effects observed at elevated concentrations and with extended exposure periods.

The calculated median lethal dose (LD_{50}) of 3162.27 mg/kg suggests a relatively broad therapeutic safety margin at lower doses, though toxicity becomes evident at doses ≥4000 mg/kg. These findings align with previous literature, including Padilla-Camberos *et al*. (2013), who reported an LD_{50} of 1200.75 mg/kg for seed extract with increasing mortality at doses as low as 500 mg/kg. Iweala *et al.* (2025) corroborated this dose-dependent toxicity for avocado seed oil, noting complete mortality at 3000 mg/kg alongside neurobehavioral alterations at 1000 mg/kg. Such discrepancies between seed and leaf preparations may stem from differences in phytochemical composition, solvent polarity, and extraction methodology (Juma, 2025; Olasunkanmi and Ogunyemi, 2023).

Conversely, other studies have reported notably higher safety thresholds, revealing the role of extract type and formulation. For instance, Kamagate *et al*. (2016) observed no toxicity in rats administered up to 2000 mg/kg of leaf extract, estimating an LD_{50} above 5000 mg/kg. Likewise, Ozolua *et al*. (2009) documented no mortality even at 10 g/kg using aqueous seed extracts, while Bhila *et al*. (2024) reported an LD_{50} of 25.4 g/kg for avocado oil in mice, suggesting that lipophilic fractions may exhibit lower toxicity compared to crude or methanol extracts.

These divergent LD_{50} values reveal a critical need to contextualise safety assessments based on plant part, extraction method, and preparation type. As emphasised in Jităreanu *et al*. (2023) and Selvestrel *et al*. (2022), such variables can significantly influence pharmacodynamic and toxicological outcomes, calling for standardised protocols in herbal toxicology. Moreover, the integration of computational models as suggested by Amorim *et al*. (2024) could support predictive toxicology by simulating exposure outcomes across formulation types, enhancing risk assessment beyond empirical dosing.

In our acute toxicity assessment, no significant changes were observed in blood pressure across all treatment groups, indicating that the methanol leaf extract of *Persea americana* did not exert acute cardiovascular effects. This is consistent with findings from Kamagate *et al*. (2016), who also reported cardiovascular neutrality in rats treated with leaf extract. However, it contrasts with Bhila *et al*. (2024), who noted heart rate alterations in mice administered avocado oil, suggesting that differences in formulation (oil vs. methanol extract) and plant part may influence cardiovascular outcomes.

Sub-chronic haematological analysis revealed reductions in packed cell volume (PCV), haemoglobin (Hb), and red blood cell (RBC) counts at 1600 mg/kg, pointing toward a mild anaemic response. These findings

mirror those of Iweala *et al*. (2025), who observed similar declines with *Persea americana* seed oil (PASO). However, unlike their study, which also reported elevated WBC, platelet, and lymphocyte counts, our data did not show such changes, supporting the notion proposed by Juma (2025) and Olasunkanmi and Ogunyemi (2023) that toxicological profiles may be extract-specific and influenced by phytochemical variability.

Histopathological analyses revealed dose-dependent tissue damage in the heart, liver, and kidneys. Specifically, hepatic steatosis and inflammatory infiltration, as well as increased glomerular cell density and renal casts, were observed. These align closely with Iweala *et al*. (2025), who reported hepatocellular swelling and renal inflammation following PASO exposure. Additional support comes from Selvestrel *et al*. (2022), whose Monte Carlo modelling of repeated-dose toxicity highlighted liver and kidney as commonly affected organs in phytocompound testing. These converging observations suggest that *P. americana* preparations may pose organ-specific risks at higher doses or with prolonged use.

Interestingly, despite visible histopathological lesions, our sub-chronic biochemical assays showed no significant elevation in liver enzymes (ALT, AST, ALP) or kidney markers (BUN, creatinine). This contrasts with Iweala *et al*. (2025), who found substantial biomarker shifts at 400 mg/kg PASO. Such disparities could stem from differences in exposure duration, compound concentration, or assay sensitivity (Amorim *et al*., 2024).

Additional complexity arises when exploring alternative bioassay systems. For example, Amado *et al.* (2019) reported no toxicity in *Artemia salina* exposed to peel extract, but documented significant haemolytic activity, suggesting cytotoxicity not evident in whole-organism testing. This reveals the value of multi-model evaluation frameworks, echoing sentiments from Munoz-Muriedas (2021) and Jităreanu *et al*. (2023), who advocate for cross-platform toxicological screening in herbal research.

The phytochemical diversity of *P. americana*, identified by Gavídia-Valencia *et al*. (2024) and Kamagate *et al.* (2016), includes saponins, alkaloids, flavonoids, tannins, and polyphenols, many of which can have dose-dependent duality, acting as antioxidants at low concentrations but promoting oxidative stress at higher levels. Iweala *et al*. (2025) linked reduced levels of antioxidant biomarkers (GSH, SOD, CAT) and elevated malondialdehyde (MDA) with tissue injury, reinforcing oxidative stress as a key mechanism. Future studies incorporating biomarker profiling and ROS quantification would be valuable additions.

Notably, Iweala *et al*. (2025) also reported lipid profile improvements with PASO, reduced total cholesterol (TC), triglycerides (TAG), and low-density lipoprotein cholesterol (LDL-C), alongside increased HDL-C, suggesting potential cardioprotective effects. This paradoxical duality reflects the complexity of *P. americana* pharmacology, where therapeutic effects may coexist with toxic liabilities, depending on dose, duration, and extract composition.

Divergences in reported safety outcomes of *Persea americana* reveal the inherent complexity of natural product toxicology. Parameters such as the plant part used (leaf, seed, peel, fruit), extraction solvent (aqueous, methanolic, oil), animal model, and duration of exposure significantly influence toxicity profiles. Studies such as Bhila *et al*. (2024) and Amado *et al*. (2019) support the relative safety of avocado-derived products at low doses or in select formulations (e.g. oil or peel), whereas evidence from Padilla-Camberos *et al*. (2013), Iweala *et al*. (2025), and the present study indicates potential harm under sustained or high-dose conditions.

These discrepancies reinforce the urgent need for standardized extraction protocols and methodological rigor, as advocated by Jităreanu *et al*. (2023) and Pawar *et al*. (2025). Histopathological evaluation remains critical for identifying organ-specific risks and should be incorporated consistently in future investigations. Additionally, integrating computational toxicology tools (Amorim *et al*., 2024; Munoz-Muriedas, 2021) and Monte Carlo predictive models (Selvestrel *et al*., 2022) may enhance preclinical screening accuracy and support risk stratification before clinical translation.

Future research should emphasize the isolation and characterization of key bioactive compounds, including flavonoids, alkaloids, and saponins (Gavídia-Valencia *et al*., 2024; Kamagate *et al*., 2016), which may exert both therapeutic and toxic actions depending on dosage and synergy. Network pharmacology approaches, such as those presented in Ojo *et al*. (2024), offer promising frameworks to explore multi-target interactions and molecular mechanisms of action, especially as herbal therapies evolve toward integrative clinical applications.

One limitation of our study was the absence of post-treatment recovery analysis, which would have provided valuable data on the reversibility of tissue damage and long-term safety of extract withdrawal. Such follow-up assessments are vital for developing safe dosing regimens and understanding chronic exposure risks.

Conclusion

This study presents key insights into the acute and sub-chronic toxicological profile of methanol leaf extract of *Persea americana*, estimating an LD_{50} of 3162.27 mg/kg and suggesting relative safety at doses up to 2000 mg/kg. However, dose-dependent toxicity was evident at higher concentrations and with prolonged exposure, as reflected by histopathological alterations in cardiac, hepatic, and renal tissues. These changes occurred despite stable blood pressure and unremarkable biochemical parameters, revealing the need for caution when administering *P. americana* preparations.

To advance safety and efficacy, future investigations should focus on elucidating molecular mechanisms

underlying toxicity, particularly oxidative stress pathways and on identifying specific bioactive constituents driving both therapeutic and adverse effects. Such efforts will pave the way toward the development of standardised, clinically viable formulations and support the evidence-based integration of avocado-derived products into modern phytomedicine.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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