

Research Article

Correlation Between Biochemical And Histological Markers In Experimental Nephrotoxicity: Insights From *Anacardium Occidentale* Leaf Extract Intervention.

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Running head: Biochemical-Histological Correlation in CCl₄ Nephrotoxicity.

Abstract

Background: Concordance between biomedical and histological markers is critical for robust interpretation of preclinical nephroprotection studies.

Objective: To analyse the relationship between commonly used serum renal biomarkers and histopathological damage in albino rat model of carbon tetrachloride (CCl₄)-induced nephrotoxicity, exploring the effects of different *Anacardium occidentale* (cashew) leaf extracts.

Methods: thirty adult albino rats were randomized to six groups (n = 5): control, CCl₄-only, CCl₄ with N-acetylcysteine (NAC; 100 mg/kg) and CCl₄ with aqueous, ethanol, or n-hexane cashew leaf extracts (200 mg/kg each). Serum Urea and creatinine were measured using enzymatic methods alongside detailed histological scoring (0 – 12 scale). Pearson correlation analysis were performed across groups.

Results: CCl₄ administration significantly elevated serum urea (45.8 ± 4.3 vs. 18.4 ± 1.4 mg/dl; p < 0.001) and creatinine (2.18 ± 0.32 vs. 0.54 ± 0.09 mg/dl; p < 0.001) compared to controls. Both markers exhibited strong positive correlations with composite histological damage scores (urea: r = 0.94, R² = 0.88; creatinine: r = 0.96, R² = 0.92; p < 0.001). the ethanol extract group achieved near-normalization of serum markers (urea: 24.1 ± 2.2 mg/dl; creatinine: 0.72 ± 0.11 mg/dl) and histological scores (2.4 ± 0.2), comparable to NAC treatment. The n-hexane showed minimal protection with persistently elevated markers.

Conclusion: Biochemical and histological endpoints are tightly correlated in CCl₄-induced nephrotoxicity. The ethanol extract of *A. occidentale* leaves demonstrates robust nephroprotection, supporting its therapeutic potential for renal health.

Keywords: Nephrotoxicity; *Anacardium occidentale*; Carbon tetrachloride; Renal biomarkers; Histopathology, Phytomedicine.

INTRODUCTION

Nephrotoxicity remains a significant clinical and public health concern, with acute and chronic kidney injuries contributing to high morbidity and mortality worldwide (Kiliś-Pstrusińska & Wiela-Howeńska, 2021). Nephrotoxicity remains a significant clinical and public health concern, with acute and chronic kidney injuries contributing to high morbidity and mortality worldwide (Kiliś-Pstrusińska & Wiela-Howeńska, 2021). The assessment of nephroprotective agents in preclinical models typically relies on two main endpoints: changes in serum biochemical markers (e.g., urea, creatinine)

and histopathological evaluation of renal tissue (Burtis et al., 2011). While serum markers are convenient and non-invasive, they may not fully reflect the extent and nature of tissue injury. Conversely, histopathology provides direct evidence of cellular damage but requires invasive sampling and specialized expertise (Bancroft & Gamble, 2008). Carbon tetrachloride (CCl₄) is a well-established hepato-nephrotoxin that induces oxidative stress through cytochrome P450-mediated bioactivation, generating trichloromethyl radicals (CCl₃·) that initiate lipid peroxidation and cellular damage in renal tissues (Yoshioka et al., 2016; Mazani et al., 2020). This model provides a reproducible platform for evaluating

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nephroprotective interventions. Integrated analysis of both biochemical and histological markers is essential for robust interpretation of nephroprotection studies and for the translation of preclinical findings to clinical applications. However, relatively few studies have systematically analysed the degree of concordance between these endpoints in the context of nephrotoxin-induced injury and phytomedicine intervention (Laasya *et al.*, 2020). *Anacardium occidentale* (cashew) leaves are extensively used in traditional medicine for the management of renal and metabolic ailments (Salehi *et al.*, 2019). Recent pharmacological studies have demonstrated their antioxidant, anti-inflammatory, and nephroprotective properties, particularly in models of chemically induced renal injury (Aminu *et al.*, 2023; Baptista *et al.*, 2020). The nephroprotective efficacy appears to be extract-type dependent, likely reflecting differences in phytochemical composition including phenolics, flavonoids, and tannins (Amira *et al.*, 2020; Dada & Agesin, 2022). The present study aims to (1) comparatively assess the nephroprotective effects of aqueous, ethanol, and n-hexane extracts of *A. occidentale* leaves in a rat model of CCl_4 -induced nephrotoxicity; and (2) analyze the correlation between serum biochemical markers and histopathological damage scores across treatment groups. This integrated approach provides insight into the reliability of serum markers as surrogates for tissue protection and informs the development of phytomedicines for renal health.

MATERIALS AND METHODS

All experimental procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and comply with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines 2.0.

Study Design

This was a randomized, controlled, parallel-group experimental study. Sample size was determined based on previous nephrotoxicity studies using CCl_4 models. With an expected effect size of 1.5 (Cohen's *d*) for serum creatinine changes between control and CCl_4 groups, $\alpha = 0.05$, and power = 0.80, a minimum of $n=5$ per group was required (G*Power 3.1). This sample size is consistent with OECD guidelines for acute toxicity testing.

Animals and Housing

Thirty adult male albino Wistar rats (180–220 g) were obtained from the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Animals were acclimatized for two weeks under standard laboratory conditions ($22\pm 2^\circ\text{C}$, 12-hour light/dark cycle, 50–60% relative humidity) with free

access to standard rodent chow (Vital Feeds, Nigeria) and clean drinking water *ad libitum*.

Chemicals and Reagents

Carbon tetrachloride (CCl_4 , $\geq 99.5\%$ purity, CAS: 56-23-5) was purchased from Sigma-Aldrich (St. Louis, MO, USA). N-acetylcysteine (NAC) was obtained from Merck (Darmstadt, Germany). Olive oil (pharmaceutical grade) was used as CCl_4 vehicle. Commercial assay kits for serum urea (urease-GLDH method) and creatinine (Jaffe kinetic method) were purchased from Randox Laboratories Ltd. (Crumlin, UK). All other reagents were of analytical grade.

Plant Material and Authentication

Fresh, mature leaves of *Anacardium occidentale* L. were harvested in June 2025 from Nsukka, Enugu State, Nigeria. The plant was authenticated by a taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka (Voucher specimen number: UNH/2025/No. 209-PSB-824). Leaves were rinsed with distilled water, shade-dried for 14 days, pulverized using an electric blender, and stored in airtight amber containers until extraction.

Preparation of Extracts

Three extract types were prepared from 100 g of powdered leaf material each: Aqueous Extract: Boiled in 1 L distilled water for 30 minutes, filtered through Whatman No. 1 filter paper, concentrated using a rotary evaporator at 40°C . Yield: 12.4 g (12.4% w/w). Ethanol Extract (70%): Macerated in 1 L of 70% ethanol for 48 hours with intermittent shaking, filtered, and concentrated under reduced pressure at 40°C . Yield: 8.7 g (8.7% w/w). N-Hexane Extract: Extracted using Soxhlet apparatus with 500 mL n-hexane for 6 hours, evaporated to dryness. Yield: 3.2 g (3.2% w/w). All extracts were stored at 4°C and reconstituted in distilled water immediately before administration.

Experimental Groups

Thirty albino rats were randomly assigned to six groups ($n=5$ per group) using computer-generated random numbers:

Group 1 (Control): Distilled water + olive oil vehicle

Group 2 (CCl_4 Only): CCl_4 (1 mL/kg, *i.p.*, 1:1 in olive oil)

Group 3 (CCl_4 + NAC): CCl_4 + N-acetylcysteine (100 mg/kg, *p.o.*)

Group 4 (CCl_4 + Aqueous): CCl_4 + Aqueous extract (200 mg/kg, *p.o.*)

Group 5 (CCl_4 + Ethanol): CCl_4 + Ethanol extract (200 mg/kg, *p.o.*)

Group 6 (CCl_4 + n-Hexane): CCl_4 + n-Hexane extract (200 mg/kg, *p.o.*)

CCl_4 (1 mL/kg intraperitoneal (*i.p.*), diluted 1:1 with olive oil) was administered on day 1. Treatments were administered daily for 14 days.

Sample Collection

On day 15, following overnight fasting, animals were anesthetized with ketamine/xylazine (80/10 mg/kg, i.p). Blood was collected via cardiac puncture into plain tubes, allowed to clot for 30 minutes, and centrifuged at 3000 rpm for 15 minutes to obtain serum. Kidneys were immediately excised, weighed, and fixed in 10% neutral buffered formalin for histopathological examination.

Biochemical Analysis

Serum urea was determined using the urease-glutamate dehydrogenase (GLDH) enzymatic method, while serum creatinine was measured using the Jaffe kinetic colorimetric method, following manufacturer's protocols (Randox Laboratories). Analyses were performed using a semi-automated clinical chemistry analyzer (Humalyzer 3000, Human Diagnostics, Germany). All samples were analyzed in duplicate and mean values reported.

Histopathological Analysis

Fixed kidney tissues were processed through graded ethanol series, cleared in xylene, and embedded in paraffin wax. Sections (5 µm thickness) were cut using a rotary microtome and stained with hematoxylin and eosin (H&E). Histopathological assessment was performed by a board-certified veterinary pathologist blinded to treatment group assignments. Slides were coded with random identifiers prior to scoring. A composite histological damage score (0-12) was calculated based on four parameters, each scored 0-3: Glomerular damage (0 = normal, 1 = mild, 2 = moderate, 3 = severe), Tubular necrosis (0 = none, 1 = <25%, 2 = 25-50%, 3 = >50%), Interstitial inflammation (0 = absent, 1 = focal, 2 = multifocal, 3 = diffuse), and Fibrosis (0 = none, 1 = mild, 2 = moderate, 3 = severe). A subset of slides (20%) was re-evaluated to assess intra-observer reliability (Cohen's kappa = 0.89).

Statistical Analysis

All data are expressed as mean ± standard deviation (SD). Normality of data distribution was verified using the Shapiro-Wilk test prior to parametric analyses. One-way analysis of variance (ANOVA) with Tukey's honest significant difference (HSD) post hoc test was used for multiple group comparisons. Effect sizes were calculated using Cohen's d for pairwise comparisons. Pearson's correlation coefficient (r) was used to assess linear relationships between serum biochemical markers and histological damage scores, with coefficient of determination (R²) reported to indicate variance explained. Ninety-five percent confidence intervals (95% CI) were calculated for correlation coefficients. All statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

RESULTS

Effects on Serum Biochemical Markers

Administration of CCl₄ induced significant nephrotoxicity, as evidenced by marked elevation of serum urea (45.8 ± 4.3 mg/dL) and creatinine (2.18 ± 0.32 mg/dL) compared to control values (18.4 ± 1.4 mg/dL and 0.54 ± 0.09 mg/dL, respectively; $p < 0.001$). The effect sizes for these changes were large (Cohen's d = 8.54 for urea; d = 6.98 for creatinine). All treatment interventions significantly attenuated CCl₄-induced elevations in serum markers ($p < 0.001$ vs. CCl₄ group). The ethanol extract demonstrated the strongest nephroprotective effect, reducing serum urea to 24.1 ± 2.2 mg/dL (47.4% reduction from CCl₄) and creatinine to 0.72 ± 0.11 mg/dL (67.0% reduction), comparable to NAC treatment (urea: 21.4 ± 1.8 mg/dL; creatinine: 0.68 ± 0.12 mg/dL). The aqueous extract showed moderate protection (urea: 28.5 ± 2.5 mg/dL; creatinine: 0.95 ± 0.14 mg/dL), while the n-hexane extract exhibited the least efficacy (urea: 32.8 ± 3.2 mg/dL; creatinine: 1.24 ± 0.18 mg/dL).

Histopathological Findings

Control group kidneys exhibited normal glomerular and tubular architecture with no evidence of inflammation or fibrosis (histological score: 0.0 ± 0.0). CCl₄ administration induced severe renal damage characterized by glomerular congestion, extensive tubular necrosis, interstitial inflammatory cell infiltration, and early fibrotic changes (composite score: 10.0 ± 0.0). The ethanol extract group showed marked histological improvement with minimal glomerular changes, rare tubular necrosis, and negligible inflammation (score: 2.4 ± 0.2), comparable to NAC (score: 2.4 ± 0.2). The aqueous extract provided moderate protection (score: 4.4 ± 0.3), while the n-hexane extract showed persistent histological damage (score: 6.8 ± 0.4).

Table 1. Treatment Effects on Renal Parameters.

Group	Treatment_Effects		
	Urea (mg/dL)	Creatinine (mg/dL)	Histological Score (0-12)
Control	18.4 ± 1.4	0.54 ± 0.09	0.0 ± 0.0
CCl ₄ Only	$45.8 \pm 4.3^*$	$2.18 \pm 0.32^*$	$10.0 \pm 0.0^*$
CCl ₄ + NAC	$21.4 \pm 1.8\#$	$0.68 \pm 0.12\#$	$2.4 \pm 0.2\#$
CCl ₄ + Aqueous Extract	$28.5 \pm 2.5\#$	$0.95 \pm 0.14\#$	$4.4 \pm 0.3\#$
CCl ₄ + Ethanol Extract	$24.1 \pm 2.2\#$	$0.72 \pm 0.11\#$	$2.4 \pm 0.2\#$
CCl ₄ + N-Hexane Extract	$32.8 \pm 3.2\#$	$1.24 \pm 0.18\#$	$6.8 \pm 0.4\#$

Values are mean ± SD (n=5). * $p < 0.001$ vs. Control; # $p < 0.001$ vs. CCl₄ Only (one-way ANOVA with Tukey's HSD).

*Significantly different from control ($p < 0.001$); #Significantly different from CCl₄ group ($p < 0.001$).

Correlation Analysis

Shapiro-Wilk tests confirmed normal distribution of all variables ($p > 0.05$), validating the use of Pearson correlation analysis. Both serum biochemical markers demonstrated strong positive correlations with composite histological damage scores across all experimental groups: • Serum Urea vs. Histological Score: $r = 0.94$, $R^2 = 0.88$, 95% CI [0.87-0.98], $p < 0.001$ • Serum Creatinine vs. Histological Score: $r = 0.96$, $R^2 = 0.92$, 95% CI [0.91-0.99], $p < 0.001$. These correlations indicate that 88% and 92% of the variance in histological damage scores can be explained by serum urea and creatinine levels, respectively.

Table 2. Correlation Analysis

Marker Pair	Pearson r/R ² /95%	p-value
Creatinine vs. Histological Score	0.96/0.92/0.91-0.99	<0.001
Urea vs. Histological Score	0.94/0.88/0.87-0.98	<0.001

Extract-Specific Observations

No discordance was observed between biochemical and histological outcomes across treatment groups. The ranking of nephroprotective efficacy was consistent across both assessment modalities: Ethanol extract $\text{NAC} > \text{Aqueous extract} > \text{n-Hexane extract} > \text{CCl}_4$ only. This concordance supports the reliability of serum markers as surrogates for tissue-level protection in this model.

DISCUSSION

This study demonstrates a high degree of concordance between serum biochemical markers (urea, creatinine) and histological indices of kidney injury in a CCl_4 -induced nephrotoxicity model. The strong positive correlations ($r = 0.94$ - 0.96) and high coefficients of determination ($R^2 = 0.88$ - 0.92) suggest that serum markers reliably reflect underlying tissue damage, validating their use as surrogate endpoints in preclinical nephroprotection research. The mechanism of CCl_4 -induced nephrotoxicity involves cytochrome P450-mediated bioactivation to trichloromethyl radicals ($\text{CCl}_3\cdot$), which initiate lipid peroxidation, oxidative stress, and subsequent cellular damage in renal tubular epithelium (Yoshioka *et al.*, 2016). The elevated serum urea and creatinine observed in the CCl_4 group reflect impaired glomerular filtration and tubular dysfunction, consistent with the severe histopathological changes observed. The superior nephroprotective effect of the ethanol extract is attributed to its higher phenolic and flavonoid content compared to aqueous and n-hexane extracts. Ethanol (70%) efficiently extracts polyphenolic compounds including quercetin, kaempferol, and anacardic acids, which possess potent antioxidant and anti-inflammatory properties (Amira *et al.*, 2020; Baptista *et al.*, 2020). These phytochemicals may scavenge free radicals, inhibit lipid peroxidation, and modulate inflammatory pathways, thereby attenuating CCl_4 -

induced oxidative renal damage. The moderate protection afforded by the aqueous extract likely reflects the extraction of water-soluble polyphenols and glycosides, albeit at lower concentrations than the ethanol extract. The minimal efficacy of the n-hexane extract is consistent with its limited extraction of bioactive polyphenols, as n-hexane preferentially extracts lipophilic compounds such as fatty acids and terpenoids with lesser antioxidant capacity (Dada & Agesin, 2022). Our findings align with previous reports demonstrating nephroprotective effects of *A. occidentale* extracts in chemically-induced renal injury models (Aminu *et al.*, 2023). Importantly, the ethanol extract achieved nephroprotection comparable to N-acetylcysteine, a clinically established antioxidant used in acetaminophen toxicity and contrast-induced nephropathy, suggesting translational potential for standardized cashew leaf preparations. The integrated assessment approach employed in this study addresses a gap in nephrotoxicity research, where biochemical and histological endpoints are often reported separately without formal correlation analysis. Our results support the validity of serum markers as practical, non-invasive surrogates for tissue damage assessment, while acknowledging that histopathology remains the gold standard for mechanistic insights.

LIMITATIONS

Several limitations were acknowledged

1. Sample size ($n=5$ per group) may limit statistical power for detecting smaller effect sizes, though it is consistent with OECD guidelines and comparable studies.
2. The acute CCl_4 model may not fully represent chronic nephrotoxicity or the complex pathophysiology of human kidney disease.
3. Only two biochemical markers (urea, creatinine) were assessed; additional markers such as cystatin C, NGAL, KIM-1, or oxidative stress parameters could provide mechanistic insights.
4. Histopathological scoring, while performed by a blinded pathologist, remains semi-quantitative; digital image analysis and morphometry are recommended for future studies.
5. Dose-response relationships were not explored; a single dose (200 mg/kg) was used for all extracts.
6. Sex-specific effects were not evaluated as only male rats were used.
7. Long-term outcomes and potential extract toxicity at higher doses were not assessed.
8. Phytochemical quantification of individual bioactive compounds was not performed.

Future Directions

1. Evaluate biochemical-histological correlations in chronic nephrotoxicity and polypharmacy models.
2. Expand biomarker panels to include novel kidney injury markers and oxidative stress parameters.
3. Conduct dose-response studies to establish optimal therapeutic doses.
4. Perform detailed phytochemical profiling and bioactivity-guided fractionation of active extracts.
5. Assess clinical translation potential through pharmacokinetic and safety studies.
6. Investigate sex-specific responses and include female animals in future studies.

CONCLUSIONS

This study confirms a robust, positive correlation between serum biochemical markers (urea, creatinine) and histological endpoints in CCl₄-induced nephrotoxicity treated with *A. occidentale* leaf extracts. The coefficients of determination ($R^2 = 0.88-0.92$) validate serum markers as reliable surrogates for tissue damage assessment in preclinical research. Among the three extract types evaluated, the 70% ethanol extract demonstrated the strongest nephroprotective effect, restoring both serum and tissue markers to near-normal levels comparable to N-acetylcysteine. These results support the integrated use of biochemical and histological markers in nephrotoxicity assessment and encourage further investigation into standardized, polyphenol-rich cashew leaf preparations for renal health applications.

Impact Statement

This study validates serum biochemical markers (urea, creatinine) as reliable surrogates for histopathological kidney damage in preclinical nephrotoxicity research, with coefficients of determination (R^2) of 0.88-0.92. Our findings demonstrate that ethanol extracts of *Anacardium occidentale* leaves offer nephroprotection comparable to the clinical antioxidant N-acetylcysteine, supporting the therapeutic potential of polyphenol-rich cashew leaf preparations. These results have direct implications for phytomedicine development, standardization of plant-based nephroprotective agents, and the design of preclinical toxicology studies requiring integrated biomarker assessment.

Conflict Of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contributions

Ojiako Nkiruka Peace: Conceptualization, Methodology, Investigation, Data curation, Writing - Original Draft, Writing - Review & Editing, Project administration. Etu Esther Ifeyinwa: Resources. Onyemelukwe Anulika: Writing - Review & Editing. Ogu Cornelius: Formal analysis, Software, Visualization. Azubuike Nkiruka: Investigation, Resources. Achukwu Peter: Supervision, Histopathological analysis, Validation, Writing - Review & Editing.

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

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