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DOSE-RELATED AMELIORATIVE EFFECTS OF COCONUT WATER (*COCOS NUCIFERA* L.) ON DOXORUBICIN-INDUCED RENAL INJURY IN MALE WISTAR RATS: BIOCHEMICAL AND HISTOPATHOLOGICAL EVIDENCE

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ABSTRACT

Doxorubicin is an effective anthracycline whose clinical use is limited by organ toxicity, including nephrotoxicity involving oxidative and nitrosative stress, inflammation and tissue remodelling. Coconut water (*Cocos nucifera* L.) contains antioxidant constituents, but evidence of its effects on doxorubicin-induced renal injury is limited. Forty-eight male Wistar rats were initially assigned to six groups (n = 8 per group): control; doxorubicin (DOX, 10 mg/kg, single intraperitoneal dose); DOX plus coconut water (6 or 12 mL/kg); and coconut water alone (6 or 12 mL/kg). During the seven-day experimental period, 12 animals died in the doxorubicin-containing groups. From the surviving animals, four rats were randomly selected per group for the final biochemical and histopathological analyses (n = 4 per group; total analysed = 24). Coconut water was administered orally once daily for seven days. Serum nitric oxide (NO), superoxide dismutase (SOD), kidney injury molecule-1 (KIM-1) and transforming growth factor-β1 (TGF-β1) were measured, and kidney sections were examined histologically. Doxorubicin increased NO and TGF-β1 and caused moderate histological injury among surviving animals, but did not increase KIM-1 or deplete SOD. Coconut water produced graded histological improvement, reaching near-normal architecture at 12 mL/kg, with dose-related NO reduction. TGF-β1 remained elevated in the co-treated groups. ANOVA showed significant group differences for NO, SOD, KIM-1 and TGF-β1 (p ≤ 0.0155), and TGF-β1 correlated strongly with NO (r = 0.716, p < 0.0001). Coconut water produced partial, dose-related amelioration of doxorubicin-associated renal injury, strongest at 12 mL/kg.

Keywords: Coconut Water, *Cocos nucifera*, Doxorubicin, Nephrotoxicity, Nitric Oxide, Superoxide Dismutase, Kidney Injury Molecule-1, Transforming Growth Factor-B1.

1 INTRODUCTION

Doxorubicin is one of the most effective and widely used anthracycline antibiotics in clinical oncology, with established activity against haematological malignancies and a broad range of solid tumours, including breast cancer, lymphoma, leukaemia and sarcomas (1–3). Its therapeutic value is nonetheless constrained by cumulative, dose-limiting off-target toxicity. Cardiotoxicity is the most familiar of these adverse effects, but the kidney is also a recognised target of anthracycline injury, and doxorubicin-associated nephrotoxicity can limit the doses that may be delivered safely and add to the morbidity of cancer treatment (4,5). This concern is amplified in settings where the background burden of kidney disease is high and where renal replacement therapy is scarce and costly, as is the case across much of sub-Saharan Africa, including Nigeria (6–8).

The mechanisms underlying experimental doxorubicin nephrotoxicity are multifactorial and incompletely resolved, but oxidative and nitrosative stress, inflammation and tissue remodelling feature prominently. Doxorubicin undergoes redox cycling that generates reactive oxygen species and accumulates in mitochondria, where it disrupts electron transport and amplifies oxidant production (9–11). It also promotes the formation of reactive nitrogen species: the reaction of superoxide with nitric oxide yields peroxynitrite, a highly reactive oxidant implicated in anthracycline tissue injury (9). Superoxide dismutase (SOD) constitutes the first line of enzymatic antioxidant defence, catalysing the dismutation of superoxide and thereby limiting downstream oxidant formation, and its activity is frequently used as an index of antioxidant status in toxicological models (12–14). Nitric oxide occupies a genuinely dual position in renal biology, contributing to normal haemodynamic regulation under basal conditions while participating in cytotoxic nitrosative chemistry when generated in excess during inflammatory or oxidative insult (9,15). Alongside these oxidative changes, doxorubicin activates inflammatory and profibrotic signalling that can culminate in extracellular-matrix deposition and tubulointerstitial fibrosis in more chronic settings (16,17).

Two protein markers are of particular relevance to the renal response and were selected for this study. Kidney injury molecule-1 (KIM-1) is a type-1 transmembrane glycoprotein that is markedly up-regulated on the apical membrane of proximal tubular epithelial cells following injury and is regarded as a sensitive and relatively specific biomarker of proximal tubular damage (18,19). Transforming growth factor- β 1 (TGF- β 1) is a pleiotropic cytokine central to tissue remodelling, wound repair and immune regulation and is a principal driver of tubulointerstitial fibrosis when its signalling is sustained; it is therefore widely used as an index of a remodelling or profibrotic response to renal injury (20). Measuring KIM-1 and TGF- β 1 together with nitric oxide and SOD allows the tubular-injury, nitrosative-stress, antioxidant and remodelling dimensions of doxorubicin nephrotoxicity to be examined in parallel.

Interest in affordable, accessible natural products that might attenuate chemotherapy-associated organ toxicity has grown, particularly in resource-limited settings. Coconut water, the clear liquid endosperm of the fruit of *Cocos nucifera* L., is an inexpensive and widely available tropical beverage with a well-characterised composition that includes ascorbic acid, phenolic compounds, amino acids such as L-arginine, cytokinins and essential electrolytes including potassium, magnesium and calcium (21–23). Several of these constituents have documented antioxidant and anti-inflammatory activity *in vitro* and *in vivo* (24,25), and coconut-derived preparations have shown organ-protective effects in experimental models. Coconut inflorescence sap powder reduced oxidative stress, lipid peroxidation and pro-inflammatory cytokines and preserved renal tissue in gentamicin-induced nephrotoxicity (26); tender coconut water increased antioxidant-enzyme activity and lowered malondialdehyde in oxidatively challenged rats (27); and coconut juice extract improved antioxidant status, renal-function indices and the lipid profile in male Wistar rats (28). These observations provide a biologically plausible rationale for evaluating coconut water in the specific context of doxorubicin-induced renal injury.

Despite this rationale, direct experimental evidence on whether coconut water can ameliorate doxorubicin-induced renal injury is limited, and the few available natural-product studies in the doxorubicin model have generally emphasised classical antioxidant-enzyme depletion and serum renal-function indices rather than a combined biomarker-and-histology assessment that includes KIM-1 and TGF- β 1. The present study was therefore designed to evaluate, in a short-term male Wistar rat model, the effect of a single nephrotoxic dose of doxorubicin and the ameliorative potential of orally administered coconut water at two dose levels, using nitric oxide and SOD as markers of nitrosative and antioxidant status, KIM-1 as a marker of proximal tubular injury, TGF- β 1 as a marker of remodelling signalling, and renal histopathology as a structural readout. It was hypothesised that coconut water would attenuate doxorubicin-associated renal injury in a dose-related manner, with the totality of biochemical and structural evidence, rather than any single biomarker, providing the basis for interpretation.

2 MATERIALS AND METHODS

2.1 Study design

The study used a completely randomised experimental design in which male Wistar rats were allocated to one of six treatment or control groups and maintained under uniform laboratory conditions. The design was chosen to give each animal an equal probability of assignment to any group and to allow the ameliorative effect of coconut water against doxorubicin-induced renal injury to be assessed. In keeping with the ARRIVE 2.0 reporting recommendations, the species, sex, group sizes, doses, routes, frequency and study duration are reported below. Animals were randomly allocated to groups, and outcome assessors (including the histopathologist and the analyst) were blinded to group allocation, whereas the caregivers administering treatments were not blinded.

Animals were monitored daily throughout the experimental period for general health and signs of toxicity, including weight loss, reduced activity and behavioural changes. Animals that died before the scheduled terminal sample collection did not contribute to the final biochemical or histopathological analyses. After the mortality observed during the study, four surviving animals were randomly selected from each experimental group for final analysis, resulting in an analytical sample of 24 animals ($n = 4$ per group). Specific predefined humane endpoint thresholds and formal exclusion criteria were not separately documented in the primary experimental records; therefore, no retrospective thresholds or exclusion criteria have been assigned.

Sample-size justification. A total of 48 male Wistar rats were used, allocated equally to six groups ($n = 8$ per group). The group size was based on the six-group design and on established practice for multi-group rodent studies rather than on a formal power calculation. Applying Mead's resource equation ($E = N - T$, where N is the total number of animals and T the number of groups) gives $E = 48 - 6 = 42$ error degrees of freedom. Although this exceeds the classic ideal range of 10–20, the larger value was chosen deliberately for three reasons: (i) the six-group design increases the degrees of freedom required for a robust one-way ANOVA and for subsequent post-hoc comparisons; (ii) the seven-day observation period introduces day-to-day biological variability, and $n = 8$ per group provides adequate power to detect moderate-to-large treatment effects while allowing for possible attrition or technical loss; and (iii) group sizes of six to ten animals are widely reported as sufficient for comparable multi-group Wistar-rat designs, balancing statistical power against the 3Rs principle of Reduction. Only male rats were used, to minimise the hormonal variability associated with the oestrous cycle, which can increase data variance and inflate the required sample size.

Following mortality during the experimental period, the final analytical sample comprised 24 animals ($n = 4$ per group), randomly selected from the surviving animals in each group to maintain equal group sizes for analysis.

2.2 Animals, housing and ethical approval

A total of 48 male Wistar rats weighing 140–230 g were initially enrolled in the study. Animals were housed under standard laboratory conditions with adequate ventilation, controlled temperature and a 12-hour light/dark cycle, with free access to standard rodent feed and clean water *ad libitum*, and were acclimatised for seven days before the experiment. All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (29). Formal approval for the study was obtained from the institutional animal-care and use/ethics committee (IUO/ETHICS/086/626).

2.3 Coconut water

Mature coconuts (*Cocos nucifera* L.) were obtained from a coconut farm in Utese Community, Ikpoba-Okha Local Government Area, Edo State, Nigeria (latitude 6.21031° N; longitude 5.69201° E). The nuts were transported to the laboratory, dehusked and opened under sterile conditions, and the fresh liquid endosperm (coconut water) was collected and measured. Fresh coconut water was extracted on the morning of each administration.

2.4 Doxorubicin

The doxorubicin used was manufactured by Khandelwal Laboratories Private Limited (batch number DL25106) and supplied as a lyophilised powder for injection containing doxorubicin hydrochloride 50 mg with lactose (q.s.). For administration, it was reconstituted in sterile 0.9% sodium chloride to an appropriate working concentration, and the injection volume was adjusted to body weight before intraperitoneal administration.

2.5 Experimental groups and treatment protocol

Animals were randomly assigned to six groups of eight rats each, as summarised in Table 1. Renal injury was induced by a single intraperitoneal injection of doxorubicin at 10 mg/kg body weight, a dose reported to induce nephrotoxicity in rodents (15,17), after which animals were observed for seven days. Coconut water was administered by oral gavage

once daily for seven consecutive days following doxorubicin, at 6 mL/kg or 12 mL/kg body weight according to group; the control group received normal saline (10 mL/kg).

Table 1: Experimental groups and treatment protocol

Group	Treatment	Dose, route and frequency	n
I — Control	Normal saline	10 mL/kg; oral gavage; once daily × 7 days	8
II — DOX	Doxorubicin	10 mg/kg; intraperitoneal; single dose	8
III — DOX + CW 6	Doxorubicin + coconut water	DOX 10 mg/kg i.p. (single) + CW 6 mL/kg oral, once daily × 7 days	8
IV — DOX + CW 12	Doxorubicin + coconut water	DOX 10 mg/kg i.p. (single) + CW 12 mL/kg oral, once daily × 7 days	8
V — CW 6	Coconut water only	6 mL/kg; oral gavage; once daily × 7 days	8
VI — CW 12	Coconut water only	12 mL/kg; oral gavage; once daily × 7 days	8

DOX, doxorubicin; CW, coconut water; i.p., intraperitoneal. The n column indicates the number of animals initially allocated to each group. Following mortality during the experimental period, four surviving animals were randomly selected from each group for the final biochemical and histopathological analyses (n = 4 per group). Coconut water was freshly extracted from *Cocos nucifera* L. and administered by oral gavage.

Animals were monitored daily for signs of toxicity, including weight loss, reduced activity and behavioural change. No deaths occurred on days 1–2 or on day 7. Mortality was confined to the doxorubicin-containing groups: over days 3–6, four animals died in the doxorubicin-only group, four in the doxorubicin plus coconut water 6 mL/kg group and four in the doxorubicin plus coconut water 12 mL/kg group, whereas no deaths occurred in the control or coconut water-only groups (Table 2). Sample harvesting was carried out on the morning of day 7. A total of 36 animals survived until the scheduled termination of the experiment. To maintain equal group sizes for analysis, four surviving animals were randomly selected from each experimental group, yielding a final analytical sample of 24 animals (n = 4 per group).

Table 2: Daily mortality by group during the seven-day observation period

Group	Day 3	Day 4	Day 5	Day 6	Total deaths	Survivors (of 8)
I — Control	0	0	0	0	0	8
II — DOX	1	1	1	1	4	4
III — DOX + CW 6	1	1	1	1	4	4
IV — DOX + CW 12	0	2	1	1	4	4
V — CW 6	0	0	0	0	0	8
VI — CW 12	0	0	0	0	0	8

No deaths occurred on days 1, 2 or 7. DOX, doxorubicin; CW, coconut water. Following the mortality observed during the study, 36 animals survived. Four surviving animals were randomly selected from each experimental group for the final biochemical and histopathological analyses, resulting in a final analytical sample of 24 animals (n = 4 per group).

2.6 Sample collection

Body weight was measured on Day 0 (before commencement of treatment) and again on Day 7, before terminal sample collection. Group body weights at each time point were expressed as mean ± standard error of the mean (SEM). On day 7, four surviving animals were randomly selected from each experimental group for terminal sample collection and subsequent biochemical and histopathological analyses. At the end of the seven-day period, animals were anaesthetised with a combination of ketamine (91 mg/kg) and xylazine (9.1 mg/kg) administered intraperitoneally. After confirmation of deep anaesthesia by loss of the pedal-withdrawal reflex, the abdomen was opened, and blood was collected for biochemical and haematological analyses. Both kidneys were excised immediately, and portions were fixed in 10% neutral buffered formalin for histopathological examination.

2.7 Biochemical assays

Serum nitric oxide was estimated from nitrite using the Griess reaction, in which nitrite reacts with sulphanilamide and N-(1-naphthyl)ethylenediamine to form a chromophore measured spectrophotometrically at 540 nm against a sodium nitrite standard curve. Superoxide dismutase activity was determined by the nitroblue tetrazolium (NBT) photoreduction method, in which SOD inhibits the reduction of NBT by photochemically generated superoxide radicals,

with absorbance read at 560 nm and activity expressed from the percentage inhibition of NBT reduction. KIM-1 was measured with a commercial rat-specific sandwich enzyme-linked immunosorbent assay (ELISA; R&D Systems Quantikine ELISA, catalogue no. RKM100; Minneapolis, MN, USA; validated for serum, EDTA plasma and blood), and TGF- β 1 was measured with a commercial rat ELISA (R&D Systems Quantikine, catalogue no. DB100C, or Boster PicoKine, catalogue no. EK0514; validated for serum and EDTA plasma), each according to the manufacturer's instructions, with absorbance read at 450 nm and concentrations interpolated from standard curves. Haematological parameters were analysed using an automated Sysmex XQ-320 haematology analyser (Sysmex Corporation, Kobe, Japan) with EDTA-anticoagulated whole blood. For biochemical analyses, venous blood samples were collected into plain sample tubes, allowed to clot, and centrifuged to obtain serum. Serum was used as the sample matrix for all biochemical assays. Lipid profile parameters, including total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol, were determined using commercially available reagent kits (Randox Laboratories Ltd., Crumlin, UK). HDL-cholesterol was measured using the Randox direct clearance method (Cat. No. CH3811), while LDL-cholesterol was determined using the Randox direct clearance method (Cat. No. CH3841).

2.8 Histopathological processing and assessment

Kidney tissues fixed in 10% neutral buffered formalin (24–48 h) were dehydrated through graded alcohols, cleared in xylene, embedded in paraffin wax, sectioned at approximately 5 μ m on a microtome, mounted on glass slides and stained with haematoxylin and eosin. Sections were examined by light microscopy at $\times$ 100 and $\times$ 400 magnification and assessed qualitatively for glomerular and tubular integrity, inflammatory-cell infiltration, congestion and architectural distortion. The assessor was blinded to group allocation. No numerical or semi-quantitative histological injury score was applied, and no statistical comparison of histological grades was undertaken.

2.9 Statistical analysis

Data are expressed as mean $\pm$ standard error of the mean (SEM) for the animals included in the final analyses ($n = 4$ per group; total $n = 24$). Differences among groups were assessed by one-way analysis of variance (ANOVA) followed by the Tukey post-hoc test for pairwise comparisons, and associations between markers were examined by Pearson correlation. A probability value of $p < 0.05$ was considered statistically significant. SPSS version 26 was used for statistical analysis.

3 RESULTS

3.1 Nitric oxide

Serum nitric oxide differed significantly among the groups ($F = 6.65$, $p = 0.0011$; Table 3, Figure 1A). Nitric oxide was higher in the doxorubicin-only group (53.10 ± 4.47 μ mol/L) than in the control group (45.55 ± 2.66 μ mol/L), consistent with a nitrosative response to the drug. In the doxorubicin plus coconut water groups, nitric oxide showed a dose-related decline: the 6 mL/kg co-treatment group remained elevated (53.63 ± 2.68 μ mol/L), whereas the 12 mL/kg co-treatment group returned toward the control value (46.23 ± 2.43 μ mol/L). The coconut water-only groups recorded the lowest concentrations (38.40 ± 1.53 μ mol/L at 6 mL/kg and 36.55 ± 1.88 μ mol/L at 12 mL/kg).

3.2 Superoxide dismutase

Superoxide dismutase activity also differed significantly among the groups ($F = 4.35$, $p = 0.0090$; Table 3, Figure 1B), but not in the direction of doxorubicin-induced depletion. SOD activity was marginally higher in the doxorubicin group (2.03 ± 0.13 U/mL) than in the control (1.85 ± 0.10 U/mL) and was comparable in the doxorubicin plus coconut water groups (2.10 ± 0.11 and 2.05 ± 0.23 U/mL). The lowest SOD activity occurred in the coconut water-only groups (1.45 ± 0.06 and 1.60 ± 0.07 U/mL). SOD therefore did not distinguish doxorubicin-exposed from unexposed animals in a manner indicative of antioxidant depletion.

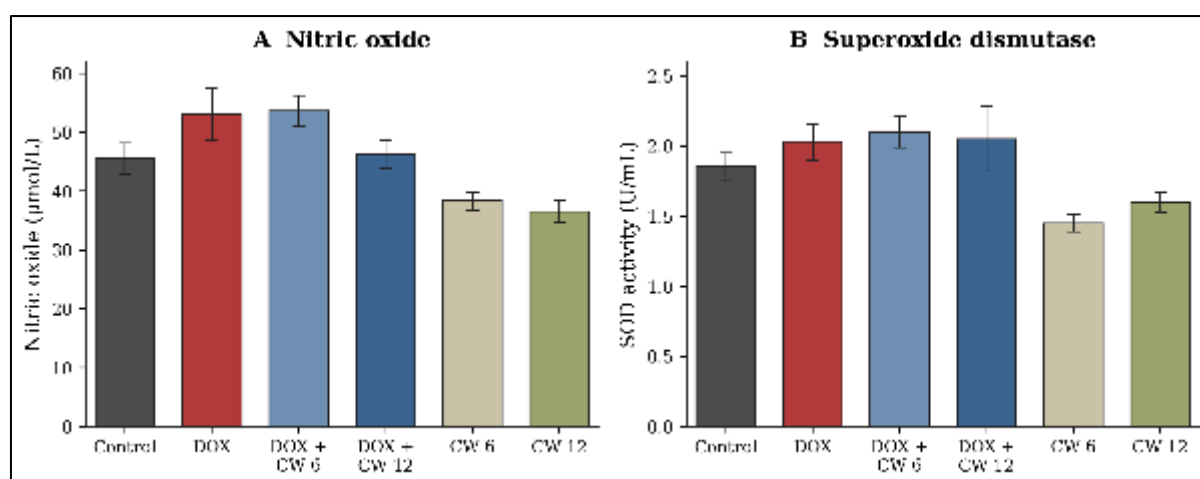


Figure 1: Effect of doxorubicin and coconut water on serum nitric oxide (A) and superoxide dismutase activity (B). Bars represent mean $\pm$ SEM (n = 4 per group). Overall one-way ANOVA: nitric oxide, $F = 6.65$, $p = 0.0011$; SOD, $F = 4.35$, $p = 0.0090$. Nitric oxide was higher in the doxorubicin group and fell in a dose-related manner with coconut water co-treatment, whereas SOD was not depleted by doxorubicin. DOX, doxorubicin; CW, coconut water.

3.3 Kidney injury molecule-1

KIM-1 differed significantly among the groups ($F = 3.82$, $p = 0.0155$; Table 3, Figure 2A); however, the pattern did not follow the direction expected of a tubular-injury marker. KIM-1 in the doxorubicin group (1.30 ± 0.15 ng/mL) was essentially the same as in the control group (1.33 ± 0.19 ng/mL), and the doxorubicin plus coconut water groups recorded similar or slightly higher values (1.33 ± 0.03 and 1.45 ± 0.19 ng/mL). The lower KIM-1 concentrations occurred in the coconut water-only groups, with the lowest value in the high-dose coconut water-only group (1.05 ± 0.15 and 0.70 ± 0.00 ng/mL). Thus doxorubicin did not produce a measurable increase in KIM-1 within the observation period.

3.4 Transforming growth factor- β 1

Table 3: Renal injury, nitrosative and oxidative-stress markers (mean $\pm$ SEM; n = 4 per group)

Marker	Control	DOX	DOX + CW 6	DOX + CW 12	CW 6	CW 12	F	P value
Nitric oxide (μmol/L)	45.55 $\pm$ 2.66	53.10 $\pm$ 4.47	53.63 $\pm$ 2.68	46.23 $\pm$ 2.43	38.40 $\pm$ 1.53	36.55 $\pm$ 1.88	6.65	0.0011
SOD (U/mL)	1.85 $\pm$ 0.10	2.03 $\pm$ 0.13	2.10 $\pm$ 0.11	2.05 $\pm$ 0.23	1.45 $\pm$ 0.06	1.60 $\pm$ 0.07	4.35	0.0090
KIM-1 (ng/mL)	1.33 $\pm$ 0.19	1.30 $\pm$ 0.15	1.33 $\pm$ 0.03	1.45 $\pm$ 0.19	1.05 $\pm$ 0.15	0.70 $\pm$ 0.00	3.82	0.0155
TGF- β 1 (pg/mL)	7.20 $\pm$ 0.89	7.95 $\pm$ 0.59	8.30 $\pm$ 0.11	8.13 $\pm$ 0.76	5.35 $\pm$ 0.27	4.60 $\pm$ 0.26	7.93	0.00042

DOX, doxorubicin; CW, coconut water; SOD, superoxide dismutase; KIM-1, kidney injury molecule-1; TGF- β 1, transforming growth factor- β 1. F and P values are from one-way ANOVA across the six groups; pairwise comparisons used the Tukey post-hoc test. Values are mean $\pm$ SEM (n = 4 per group). The four animals analysed per group were randomly selected from the surviving animals.

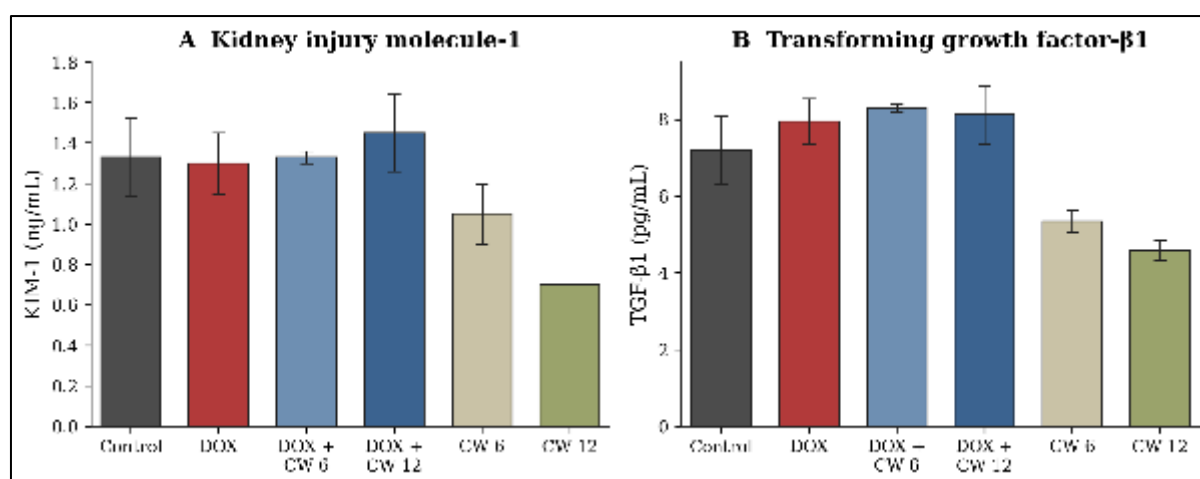


Figure 2: Effect of doxorubicin and coconut water on kidney injury molecule-1 (KIM-1; A) and transforming growth factor-β1 (TGF-β1; B). Bars represent mean $\pm$ SEM (n = 4 per group). Overall one-way ANOVA: KIM-1, $F = 3.82$, $p = 0.0155$; TGF-β1, $F = 7.93$, $p = 0.00042$. KIM-1 did not increase with doxorubicin; TGF-β1 was elevated in the doxorubicin and doxorubicin + coconut water groups and low only in the coconut water-only groups. DOX, doxorubicin; CW, coconut water.

TGF-β1 showed the most marked group differences ($F = 7.93$, $p = 0.00042$; Table 3, Figure 2B). TGF-β1 was higher in the doxorubicin group (7.95 ± 0.59 pg/mL) than in the control (7.20 ± 0.89 pg/mL) and remained elevated in the doxorubicin plus coconut water groups (8.30 ± 0.11 and 8.13 ± 0.76 pg/mL). Only the coconut water-only groups, which were never exposed to doxorubicin, showed low TGF-β1 concentrations (5.35 ± 0.27 and 4.60 ± 0.26 pg/mL). Coconut water co-treatment therefore did not lower TGF-β1 in the presence of doxorubicin.

3.5 Correlations among markers

Pearson correlation analysis (Table 4) revealed a moderate positive association between KIM-1 and nitric oxide ($r = 0.555$, $p = 0.0044$), a moderate positive association between KIM-1 and SOD ($r = 0.459$, $p = 0.022$), and a strong positive association between TGF-β1 and nitric oxide ($r = 0.716$, $p < 0.0001$). These associations are correlational and indicate that the markers tended to vary together across animals; they do not establish causation or the direction of any relationship.

Table 4: Pearson correlations among renal markers

Variable pair	r	P value	Strength / direction
KIM-1 vs nitric oxide	0.555	0.0044	Moderate, positive
KIM-1 vs SOD	0.459	0.022	Moderate, positive
TGF-β1 vs nitric oxide	0.716	< 0.0001	Strong, positive

Correlations are descriptive of covariation across animals and do not imply causation or directionality. SOD, superoxide dismutase; KIM-1, kidney injury molecule-1; TGF-β1, transforming growth factor-β1. Correlation analyses were performed using the 24 animals included in the final analytical dataset (n = 4 per group).

3.6 Histopathological findings

Qualitative histological assessment provided the clearest and most internally consistent evidence in the study (Figure 3). Control kidneys showed normal architecture, with well-defined glomeruli, intact Bowman's capsules, preserved tubular epithelial linings and no inflammatory infiltration, congestion or structural distortion. The doxorubicin-only group showed moderate alterations, comprising interstitial infiltration of inflammatory cells, vascular and interstitial congestion, and distortion of the renal architecture relative to the control. A graded improvement accompanied coconut water co-treatment: the doxorubicin plus coconut water 6 mL/kg group showed improved architecture with largely preserved glomeruli and tubules but persisting mild interstitial congestion, whereas the doxorubicin plus coconut water 12 mL/kg group showed near-normal architecture with intact glomeruli and tubules and no significant inflammatory infiltration. The coconut water-only groups showed essentially normal renal histology comparable to the control, although mild interstitial congestion was noted. Because assessment was qualitative, no statistical significance is attached to the histological comparisons.

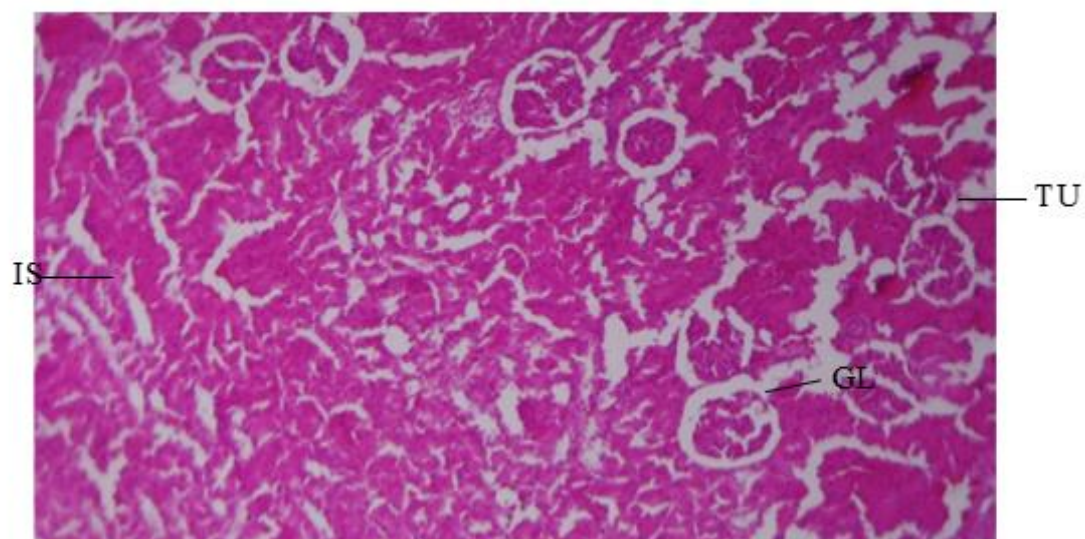


Plate (a). Rat kidney administered normal food and normal Saline show (Control group): normal architecture: tubules (TU), interstitial space (IS) and glomeruli (GL): H&E 100 X

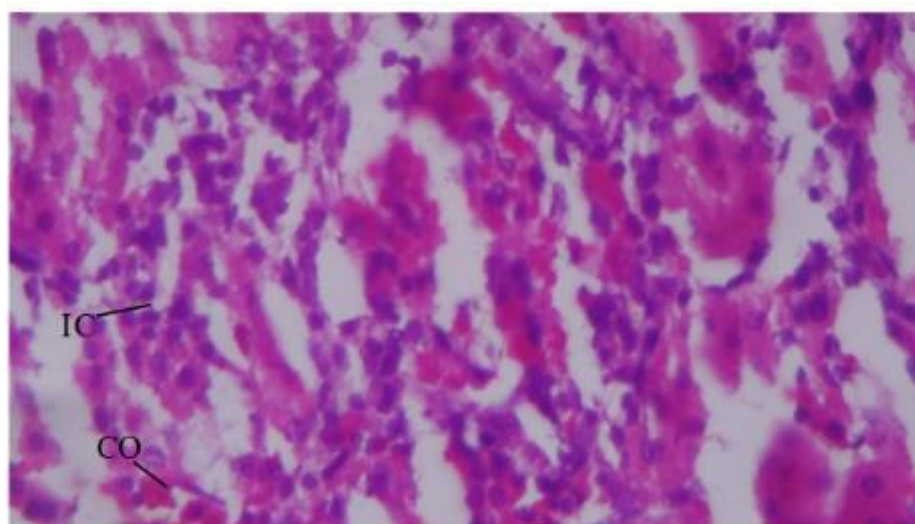


Plate (b). Rat kidney administered Doxorubicin only show (DOX group): moderate interstitial infiltrates of inflammatory cells (IC) and interstitial congestion (CO): H&E 100 X

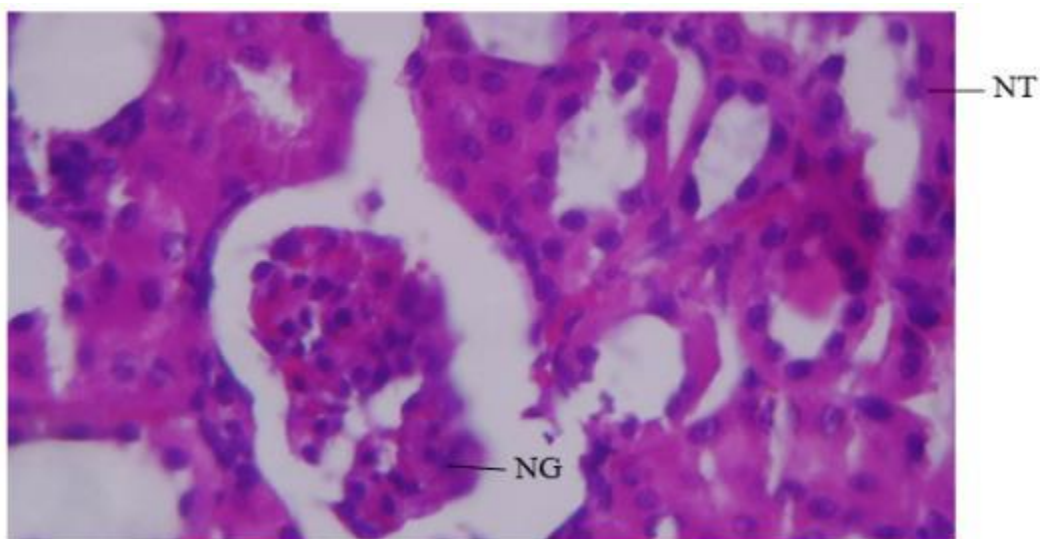


Plate (c). Rat kidney administered Doxorubicin followed by 6ml/kg Coconut water show (DOX + CW 6 ml/kg group): normal glomeruli (NG), tubules (NT) and active interstitial congestion (AC): H&E 100 X

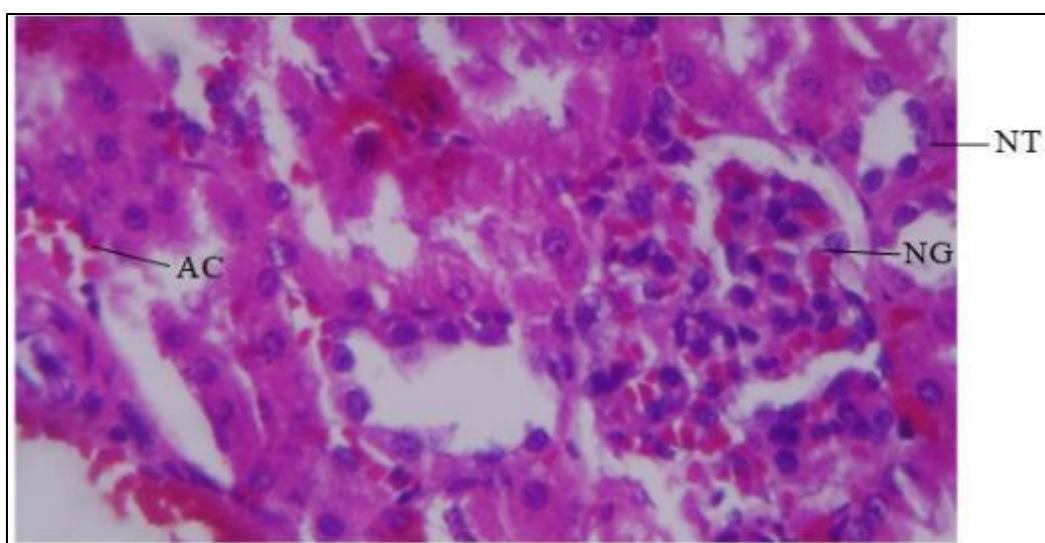


Plate (d): Rat kidney administered Doxorubicin followed by 12ml/kg Coconut water show (DOX + CW 12 ml/kg group): normal tubules (NT), normal glomerulus (NG): H&E 100 X

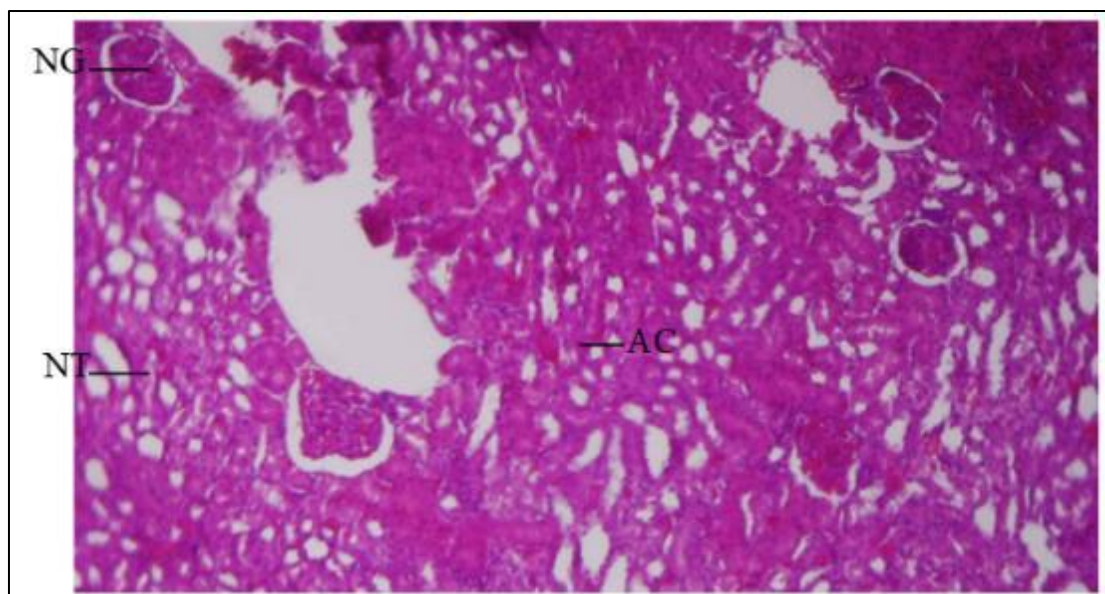


Plate (e). Rat kidney administered Coconut water 6ml/kg show (CW 6 ml/kg group): normal glomerulus (NG), tubules (NT), active interstitial congestion (AC): H&E 100 X

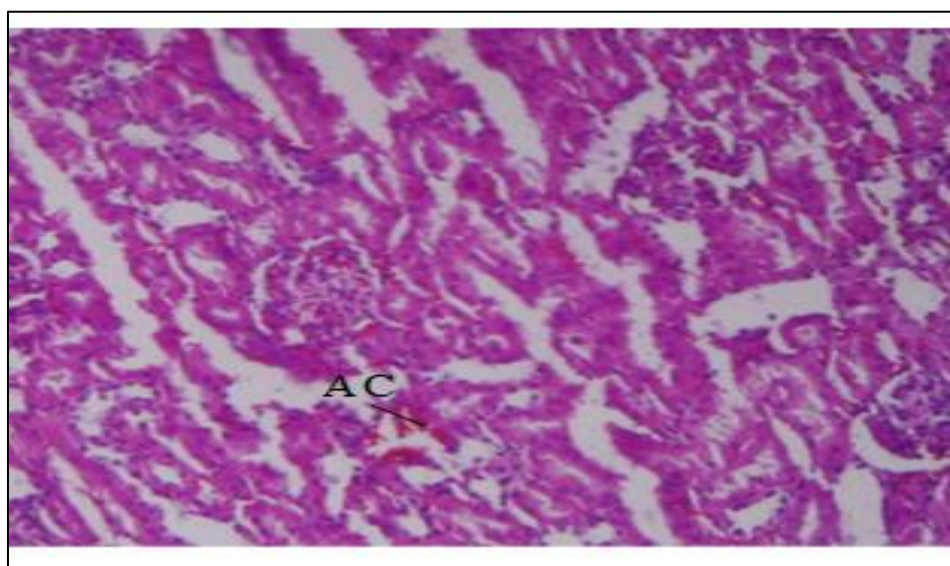


Plate (f): Rat kidney administered Coconut water 12ml/kg show (CW 12 ml/kg group): normal tubules (NT), glomerulus (NG), active interstitial congestion (AC): H&E 100 X

Figure 3: Representative photomicrographs of kidney sections (haematoxylin and eosin) across the six groups. Panels should show: (a) Control — normal architecture with well-defined glomeruli (GL), tubules (TU) and interstitial space (IS); (b) DOX — moderate interstitial inflammatory-cell infiltration (IC) and interstitial congestion (CO) with architectural distortion; (c) DOX + CW 6 mL/kg — improved architecture with largely normal glomeruli (NG) and tubules (NT) but persisting interstitial congestion; (d) DOX + CW 12 mL/kg — near-normal architecture with intact glomeruli (NG) and tubules (NT) and no significant infiltration; (e) CW 6 mL/kg and (f) CW 12 mL/kg — essentially normal architecture with mild interstitial congestion. Original magnifications $\times 100$

3.7 3.7 Secondary systemic observations (haematology and lipid profile)

Haematological and lipid-profile indices were measured as secondary systemic parameters and are summarised in Supplementary Table S1. Doxorubicin did not produce frank anaemia or leucopenia within the seven-day window; significant group differences were observed for red-blood-cell count ($F = 256.42$, $p < 0.0001$), mean corpuscular haemoglobin ($F = 3.94$, $p = 0.0138$), mean corpuscular haemoglobin concentration ($F = 4.70$, $p = 0.0064$), platelet count ($F = 4.90$, $p = 0.0053$) and monocyte count ($F = 3.88$, $p = 0.0147$), with the coconut water-treated groups showing lower white-cell counts and highly variable platelet counts. For the lipid profile, significant differences in total cholesterol (F

= 4.01, $p = 0.0127$), triglycerides ($F = 3.28$, $p = 0.0279$), HDL-cholesterol ($F = 3.79$, $p = 0.0160$) and LDL-cholesterol ($F = 7.12$, $p = 0.00078$) were driven mainly by the coconut water-only groups, which showed lower total cholesterol, triglycerides and LDL-cholesterol than the control and doxorubicin groups; the doxorubicin and doxorubicin plus coconut water groups were close to control. These systemic findings are reported for completeness and are secondary to the renal endpoints.

4 DISCUSSION

In this short-term male Wistar rat model, a single 10 mg/kg intraperitoneal dose of doxorubicin produced a coherent but moderate pattern of renal disturbance among the surviving animals: an increase in nitric oxide indicative of a nitrosative component, an increase in the remodelling cytokine TGF- β 1, and moderate structural injury on histology comprising interstitial inflammation, congestion and architectural distortion. Importantly, this occurred without a rise in the proximal tubular marker KIM-1 and without depletion of SOD, indicating that the injury reflected in the measured biomarkers within seven days was real but not severe and did not overwhelm the first-line antioxidant defences. Against this background, coconut water appeared to modify parts of the response in a dose-related manner, with the strongest evidence of amelioration being structural and supported by the reduction of nitric oxide; the evidence was not, however, uniform across every biochemical endpoint.

Nitric oxide occupies a genuinely dual position in renal biology, and its increase should not be equated automatically with injury. Under basal conditions the constitutive endothelial and neuronal nitric oxide synthases regulate renal haemodynamics, whereas during inflammatory or oxidative insult the inducible isoform can generate large amounts of nitric oxide that react with superoxide to form peroxynitrite, a potent cytotoxic species (9). Doxorubicin has been shown to enhance inducible nitric oxide synthase expression and peroxynitrite formation (9), so the elevated nitric oxide in the doxorubicin group is consistent with a nitrosative-stress response, and the dose-related normalisation of nitric oxide with coconut water suggests attenuation of that response. Ascorbic acid, one of the antioxidant constituents of coconut water, has been reported to modulate nitric oxide synthase activity and mitigate doxorubicin-associated nitrosative stress (15), which offers a plausible, though not directly demonstrated, mechanistic link for this observation.

Contrary to the common assumption that doxorubicin uniformly depletes antioxidant enzymes, SOD activity here was slightly higher in the doxorubicin group than in the control and was not reduced by co-treatment. A modest early rise in SOD is more plausibly a compensatory up-regulation of the first-line antioxidant enzyme in response to increased superoxide generation than evidence of protection (12). The lower SOD activity in the coconut water-only groups, which were not exposed to doxorubicin, is consistent with a reduced demand for enzymatic induction in the absence of an oxidant challenge and should not be read as antioxidant impairment. This pattern differs from studies in which doxorubicin clearly reduced renal SOD activity (14,17); the discrepancy is most plausibly explained by the single, lower doxorubicin dose and short sampling interval used here, since overt enzymatic depletion tends to follow more severe or more sustained oxidative injury.

The KIM-1 findings require careful interpretation, and it would be incorrect to state that doxorubicin significantly increased KIM-1 in this study, because the data do not support such a claim. KIM-1 is up-regulated specifically on the apical surface of proximal tubular epithelial cells following injury (18,19). Several considerations may account for the flat response. First, the histological changes in the doxorubicin group were predominantly interstitial and vascular rather than florid proximal tubular necrosis, so the specific stimulus for marked KIM-1 shedding may have been limited. Second, the seven-day interval between dosing and sampling may not have coincided with the peak of KIM-1 expression, which is characteristically rapid and can be transient. Third, biochemical and morphological endpoints measure different aspects of injury and need not move in parallel. These explanations are biologically plausible rather than proven. The lower KIM-1 values in the coconut water-treated groups are at least consistent with an absence of tubular injury in those animals but must be regarded as suggestive rather than conclusive.

TGF- β 1 is best understood not simply as an inflammatory marker but as a pleiotropic cytokine central to tissue remodelling, extracellular-matrix regulation and repair, and a principal driver of tubulointerstitial fibrosis when its signalling is sustained (20). Its elevation by doxorubicin is consistent with activation of a remodelling programme. A finding that must not be overstated in the direction of the study hypothesis is that coconut water co-treatment did not lower TGF- β 1; the doxorubicin plus coconut water groups had TGF- β 1 concentrations at least as high as the doxorubicin-only group, and only the coconut water-only groups, never exposed to doxorubicin, showed low values. The persistence of elevated TGF- β 1 in the co-treated groups, at a time when their histology was improved relative to the doxorubicin-only group, represents a genuine discordance between a biochemical remodelling signal and the structural appearance of the tissue. One cautious interpretation, offered as a hypothesis, is that within the seven-day window TGF- β 1 signalling may have been serving a reparative role that accompanies rather than opposes structural recovery; whether the elevation would resolve, persist or progress to fibrosis over a longer period cannot be determined from these data.

The correlations observed that KIM-1 with nitric oxide ($r = 0.555$), KIM-1 with SOD ($r = 0.459$) and, most strongly, TGF- β 1 with nitric oxide ($r = 0.716$), are consistent with a relationship in which oxidative and nitrosative change accompanies both tubular stress and remodelling signalling, but they are correlational and do not establish causation. The strong TGF- β 1–nitric oxide association is the most coherent of the three and is compatible with the reported linkage between reactive nitrogen species and remodelling signalling in the tissue response to doxorubicin (9,20).

The histopathological gradient, from normal control kidneys, through moderate interstitial injury with doxorubicin, to progressively preserved architecture with 6 mL/kg and then near-normal architecture with 12 mL/kg coconut water, is the strongest and most internally consistent line of evidence, and it aligns closely with the dose-related normalisation of nitric oxide. This morphological improvement is broadly concordant with the histological dimension of previous natural-product studies in the doxorubicin model, in which co-treatment improved renal architecture (14,17), and with reports that coconut-derived preparations preserved renal tissue against other nephrotoxic insults such as gentamicin (26) and reduced oxidative markers in oxidatively challenged animals (27,28). The present study nonetheless reaches a more restrained conclusion than much of this literature, because its biochemical markers were not uniformly protective; where earlier work often reported clear doxorubicin-induced SOD depletion and serum creatinine/urea elevation (14,17,31), the moderate, single-dose insult used here did not reproduce that enzymatic footprint. Comparable Wistar-rat work using the same 10 mg/kg doxorubicin dose has likewise shown that the haematological and systemic toxicity profile depends strongly on dosing schedule and sampling time (30), underscoring that model design shapes which endpoints move.

The mechanisms by which coconut water might have ameliorated the injury can only be inferred from its known composition and are framed here as hypotheses rather than demonstrated pathways. Coconut water contains ascorbic acid, phenolic compounds, amino acids including L-arginine, cytokinins and electrolytes, and has documented antioxidant and anti-inflammatory activity (21–25). Plausible contributions include direct antioxidant and free-radical scavenging that could reduce reactive oxygen and nitrogen species (consistent with the fall in nitric oxide and with the reported action of vitamin C on nitrosative stress (15)); attenuation of lipid peroxidation and stabilisation of cellular membranes, as reported for coconut-derived preparations in other models (26,27); and anti-inflammatory effects compatible with the reduced interstitial inflammation seen histologically (26). It must be emphasised that no molecular signalling pathway was measured in this study, and no claim is made that coconut water acted through the Nrf2/HO-1, NF- κ B, SIRT1 or apoptotic/pyroptotic pathways; such pathway-level effects, reported for other agents against doxorubicin nephrotoxicity (16,32,33), were not investigated here and would require direct testing before any mechanistic attribution. Taken together, the findings are most consistent with partial, dose-related amelioration of doxorubicin-induced renal injury by coconut water, with the 12 mL/kg dose producing clearer benefit than the 6 mL/kg dose, and with the histological and nitric oxide findings carrying the greatest evidential weight; they do not support a claim of complete protection, and the persistently elevated TGF- β 1 signal counsels against overstating the degree of protection achieved.

LIMITATIONS

Several limitations should be borne in mind when interpreting these findings. First, four of the eight animals in each doxorubicin-containing group died before the end of the study, whereas no deaths occurred in the control or coconut water-only groups. Thus, 12 of the 48 initially enrolled animals died, leaving 36 survivors. To maintain equal group sizes for the final analyses, four surviving animals were randomly selected from each experimental group, resulting in a final analytical sample of 24 animals ($n = 4$ per group). The biochemical and histopathological findings therefore reflect the randomly selected surviving animals and may be subject to survivor bias. Notably, coconut water co-treatment did not reduce mortality relative to doxorubicin alone, which is consistent with the study's central message of partial rather than complete protection. Second, the observation period was short (seven days) and followed a single doxorubicin exposure, so the model captures an early phase of injury rather than a severe or chronic one; this probably contributes to both the absence of SOD depletion and the flat KIM-1 response among survivors, and it precludes any inference about long-term fibrosis from the short-term TGF- β 1 signal. Third, the oxidative-stress panel was limited to nitric oxide and SOD and did not include malondialdehyde, reduced glutathione or catalase, and conventional renal-function markers (serum creatinine and blood urea nitrogen) and urinary KIM-1 were not measured, so functional and tubular injury could not be characterised fully. Fourth, histological assessment was qualitative, without a semi-quantitative injury score, and the original photomicrograph plates were not available at publication resolution (see Author Action Items). Finally, no molecular signalling pathway was examined, and the phytochemical composition of the coconut water used was not analysed, limiting mechanistic attribution.

5 CONCLUSION

Coconut water attenuated selected renal structural and nitrosative abnormalities among surviving animals, but did not mitigate the substantial systemic toxicity associated with doxorubicin. Doxorubicin produced evidence of moderate renal injury among the randomly selected surviving animals included in the final analysis, elevated nitric oxide,

increased TGF- β 1 and moderate histopathological change, without an increase in KIM-1 or depletion of SOD, indicating a real but not severe insult at the biomarker level, although the associated mortality in the doxorubicin-containing groups indicates meaningful whole-animal toxicity. The strongest evidence of amelioration was the graded improvement in renal histoarchitecture and the dose-related reduction of nitric oxide, particularly at 12 mL/kg. Because SOD and TGF- β 1 did not uniformly demonstrate protection, KIM-1 showed no clear doxorubicin-induced increase, and coconut water did not reduce mortality, the findings do not support claims of complete renal protection, reversal or cure. Future studies should employ larger sample sizes, longer follow-up, additional renal-function and urinary biomarkers, a broader oxidative-stress panel, fibrosis and molecular-pathway analyses, formal dose-response designs and blinded quantitative histological scoring. On the strength of the present preclinical evidence, coconut water cannot be recommended as an established nephroprotective therapy for patients receiving doxorubicin, and the results should not be extrapolated directly to clinical treatment in humans.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

Statement of ethical approval

All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (29) and were approved by the institutional animal-care and use/ethics committee (IUO/ETHICS/086/626).

Availability of Data and Materials

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

Authors' Contributions

SCN-conceptualisation, PGE-methodology, PGE-investigation, HUI-formal analysis, HUI-writing (original draft), SCN-writing (review and editing). All authors read and approved the final manuscript.

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APPENDIX

Supplementary Table S1. Haematological and lipid-profile indices (mean $\pm$ SEM; n = 4 per group)

Parameter	Control	DOX	DOX + CW 6	DOX + CW 12	CW 6	CW 12
WBC ($\times 10^9$ /L)	7.23 $\pm$ 0.52	7.10 $\pm$ 0.71	3.73 $\pm$ 1.24	3.93 $\pm$ 2.09	5.13 $\pm$ 0.89	3.55 $\pm$ 0.35
RBC ($\times 10^{12}$ /L)	6.63 $\pm$ 0.29	7.13 $\pm$ 0.20	6.13 $\pm$ 0.57	5.30 $\pm$ 1.17	6.25 $\pm$ 0.41	4.34 $\pm$ 0.22
HGB (g/dL)	13.13 $\pm$ 0.55	13.93 $\pm$ 0.54	14.33 $\pm$ 0.89	10.15 $\pm$ 2.18	11.93 $\pm$ 0.61	10.85 $\pm$ 0.50
HCT (%)	38.00 $\pm$ 1.41	39.50 $\pm$ 1.55	33.25 $\pm$ 3.50	31.00 $\pm$ 6.35	33.50 $\pm$ 2.02	31.25 $\pm$ 1.49
MCV (fL)	57.00 $\pm$ 0.41	55.50 $\pm$ 2.06	54.25 $\pm$ 1.25	58.00 $\pm$ 1.12	53.25 $\pm$ 1.03	56.50 $\pm$ 1.29
MCH (pg)	19.50 $\pm$ 0.29	19.50 $\pm$ 0.65	23.50 $\pm$ 1.50	20.00 $\pm$ 0.71	19.25 $\pm$ 0.25	20.25 $\pm$ 0.48
MCHC (g/dL)	35.00 $\pm$ 0.41	35.00 $\pm$ 0.41	44.25 $\pm$ 3.19	31.50 $\pm$ 2.87	36.00 $\pm$ 0.41	35.00 $\pm$ 0.41
RDW (%)	29.00 $\pm$ 0.82	29.25 $\pm$ 0.85	27.50 $\pm$ 0.96	35.25 $\pm$ 4.97	27.00 $\pm$ 0.00	34.25 $\pm$ 1.55
PLT ($\times 10^9$ /L)	716.50 $\pm$ 40.02	562.25 $\pm$ 155.20	127.25 $\pm$ 25.81	858.00 $\pm$ 252.60	197.50 $\pm$ 65.54	224.50 $\pm$ 22.18
LYM (%)	60.50 $\pm$ 1.66	56.50 $\pm$ 0.65	59.50 $\pm$ 4.05	61.25 $\pm$ 5.57	57.00 $\pm$ 1.87	60.50 $\pm$ 0.65
MON (%)	10.50 $\pm$ 1.50	16.25 $\pm$ 0.75	14.50 $\pm$ 1.50	16.00 $\pm$ 2.06	14.50 $\pm$ 0.65	11.25 $\pm$ 0.48
GRA (%)	28.50 $\pm$ 1.55	26.50 $\pm$ 1.55	26.00 $\pm$ 4.74	22.50 $\pm$ 3.78	28.25 $\pm$ 1.25	28.00 $\pm$ 1.08
Total cholesterol (mg/dL)	167.00 $\pm$ 9.79	159.25 $\pm$ 12.64	166.25 $\pm$ 1.32	160.50 $\pm$ 16.18	121.25 $\pm$ 15.08	119.25 $\pm$ 0.85
Triglycerides (mg/dL)	133.00 $\pm$ 8.51	135.50 $\pm$ 11.16	120.50 $\pm$ 10.74	123.50 $\pm$ 13.48	95.25 $\pm$ 3.19	100.00 $\pm$ 2.58
HDL-C (mg/dL)	45.50 $\pm$ 1.32	47.25 $\pm$ 3.32	46.50 $\pm$ 1.55	44.00 $\pm$ 3.54	35.50 $\pm$ 1.84	39.50 $\pm$ 1.12
LDL-C (mg/dL)	94.50 $\pm$ 10.31	83.25 $\pm$ 6.40	93.00 $\pm$ 1.48	91.00 $\pm$ 9.52	55.25 $\pm$ 3.00	59.50 $\pm$ 1.04

These systemic indices are secondary to the renal endpoints. Significant ANOVA differences were noted for RBC (F = 256.42, p < 0.0001), MCH (F = 3.94, p = 0.0138), MCHC (F = 4.70, p = 0.0064), platelet count (F = 4.90, p = 0.0053), monocytes (F = 3.88, p = 0.0147), total cholesterol (F = 4.01, p = 0.0127), triglycerides (F = 3.28, p = 0.0279), HDL-C (F = 3.79, p = 0.0160) and LDL-C (F = 7.12, p = 0.00078); LDL-C was calculated using the Friedewald equation. Abbreviations as conventional.