

ORIGINAL RESEARCH ARTICLE

Network-Pharmacology-Guided Validation of *Phyllanthus niruri* Nephroprotection Against Doxorubicin: An *In Vitro*–*In Vivo* Translational Study

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ABSTRACT

Background: Doxorubicin is a cornerstone anthracycline chemotherapeutic whose clinical utility is constrained by dose-limiting nephrotoxicity, and no approved pharmacological prevention currently exists. *Phyllanthus niruri* L. (family Phyllanthaceae), known in Indonesia as meniran, is a phytotherapeutic herb whose constituents (phyllanthin, hypophyllanthin, quercetin, rutin, gallic acid, and corilagin) target the oxidative-stress and inflammatory pathways implicated in doxorubicin renal injury.

Objective: To test whether standardised *P. niruri* ethanolic extract attenuates doxorubicin-induced nephrotoxicity through a mechanistically coherent multi-target pathway, using a tiered translational design.

Methods: Network-pharmacology discovery using SwissTargetPrediction, the Traditional Chinese Medicine Systems Pharmacology database (TCMSP), GeneCards, and Online Mendelian Inheritance in Man (OMIM) intersected six *P. niruri* active compounds with 842 nephrotoxicity-associated genes. *In vitro* validation used HK-2 human proximal tubular cells exposed to doxorubicin (2 µM, 24 h) with or without standardised *P. niruri* 70% ethanolic extract (25, 50, and 100 µg/mL). *In vivo* validation used 48 male Sprague–Dawley rats (eight per arm) given a single intraperitoneal dose of doxorubicin (15 mg/kg) and oral *P. niruri* at 100, 200, or 400 mg/kg/day for 14 days.

Results: Six hub targets (TNF, IL6, NFKB1, CASP3, BAX, and BCL2) and four enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (TNF signalling, MAPK signalling, PI3K/Akt signalling, and apoptosis) were identified. The *P. niruri* extract produced a dose-dependent rescue of HK-2 viability (84.6% at 100 µg/mL versus 41.8% in doxorubicin-only controls; $p < 0.001$). At 400 mg/kg, it reduced serum creatinine, blood urea nitrogen, urinary kidney injury molecule-1 (KIM-1), and neutrophil gelatinase-associated lipocalin (NGAL) by 55–70%, with concordant restoration of glutathione and superoxide dismutase. The network-pharmacology hub targets showed transcriptionally coherent modulation.

Conclusion: Standardised *P. niruri* ethanolic extract is a mechanism-anchored herbal candidate for the adjunctive prevention of doxorubicin-induced kidney injury, warranting clinical translation.

All authors have reviewed and approved the final version of the manuscript.

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1. Introduction

Anthracycline chemotherapy with doxorubicin remains an indispensable component of the systemic management of breast carcinoma, lymphoma, soft-tissue sarcoma, and selected paediatric malignancies, yet its therapeutic ceiling is consistently set by

cumulative-dose toxicity to the heart and the kidney.^{1,2} The renal phenotype, historically eclipsed by cardiotoxicity in the literature, is now well documented, with proteinuria, focal segmental glomerulosclerosis, and acute tubular injury reproduced across contemporary experimental nephropathy models.³ The molecular pathology

converges on three intertwined axes. The first is redox cycling of doxorubicin in mitochondrial complex I, with consequent superoxide and hydrogen-peroxide generation; the second is depletion of intracellular glutathione with downstream lipid peroxidation; and the third is activation of the nuclear factor kappa-B (NF- κ B) and tumour necrosis factor- α (TNF- α) inflammatory cascades that drive intrinsic apoptosis of proximal tubular epithelial cells.^{2,4} Recent work also implicates the NLR family pyrin domain-containing 3 (NLRP3) inflammasome as a downstream amplifier of this pathology and as a tractable target for natural-product modulation.^{4,5} At the bedside, urinary kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) detect tubular injury earlier and with greater sensitivity than serum creatinine alone, which has shifted both preclinical and clinical end-point selection.⁶

Beyond the cellular cascade, doxorubicin nephropathy carries clinically meaningful consequences. Renal-function decline reduces the safe cumulative anthracycline dose, truncates curative chemotherapy regimens, and is independently associated with cardiovascular morbidity in cancer survivors. In low- and middle-income settings, where doxorubicin remains a cornerstone of cytotoxic therapy precisely because of its cost-effectiveness, the absence of an approved nephroprotective adjunct compounds health-system inequity by limiting chemotherapy intensity in the very populations whose access to tertiary supportive care is already constrained.

Pharmacological options for the prevention or mitigation of doxorubicin-induced nephropathy remain limited. Dexrazoxane is approved for cardioprotection but is not licensed for renal indications and brings its own toxicity profile; intravenous N-acetylcysteine and amifostine have shown inconsistent renal benefit in trials; and no prophylactic strategy has yet reached guideline endorsement.⁷ This therapeutic vacuum has stimulated systematic exploration of phytotherapeutic agents whose multi-target chemistry is particularly well suited to the multi-axis pathology of anthracycline injury.⁸

Phyllanthus niruri L. (family Phyllanthaceae), known in Indonesia as meniran, is one of the most thoroughly studied of those agents. Its phytochemical fingerprint is dominated by the aryl-naphthalene lignans phyllanthin and hypophyllanthin, the flavonoids quercetin and rutin, the hydrolysable tannins corilagin and geraniin, and the polyphenols gallic and ellagic acid.^{9,10} Pharmacologically, the genus *Phyllanthus*

exhibits robust hepatoprotective, immunomodulatory, and antioxidant properties,¹¹ and *P. niruri* specifically has been shown to protect against chemically induced hepatotoxicity,¹² to ameliorate experimental renal injury with attenuation of urinary KIM-1 and NGAL,¹³ and to restore antioxidant defences in oxidative-stress-driven organ injury.¹⁴

Concurrent with the empirical evidence has come a methodological shift towards network pharmacology, a discovery paradigm built on the recognition that biologically active polyphenol-rich extracts engage multiple targets simultaneously and that classical one-drug-one-target reasoning misrepresents that reality.¹⁵ Contemporary pipelines combine *in silico* target prediction (SwissTargetPrediction and TCMSP), disease-gene curation (GeneCards and OMIM), protein-protein interaction analysis, and pathway and Gene Ontology enrichment.¹⁵ Applications of this pipeline to the genus *Phyllanthus* are accumulating,¹⁶ and recent studies integrating network pharmacology with experimental validation in doxorubicin-induced renal and cardiac injury,¹⁷ and in acute kidney injury more broadly,¹⁸ supply methodological templates for the present study.

From a regional health-system perspective, the case for investment in locally sourced, locally cultivated herbal adjuncts is particularly strong in Indonesia. The regulatory framework of Badan Pengawas Obat dan Makanan (BPOM; the Indonesian Food and Drug Authority), with its progression from Jamu to Obat Herbal Terstandar (OHT; standardised herbal medicine) and then to Fitofarmaka, provides a structured pathway through which mechanism-anchored phytotherapeutic candidates can be translated into clinically deployable adjuncts.¹⁹ Three evidence gaps justify the present work. First, no integrated network-pharmacology, *in vitro*, and *in vivo* dossier currently exists for *P. niruri* against doxorubicin-induced nephrotoxicity. Second, Indonesian-chemotype *P. niruri* has not been tested against this indication with modern KIM-1 and NGAL readouts. Third, the cross-walk between computational hub targets and transcriptional validation is rarely reported in Phyllanthaceae. The aim of this study was therefore to apply a tiered translational design (network-pharmacology discovery, *in vitro* validation in HK-2 human proximal tubular cells, and *in vivo* validation in Sprague-Dawley rats) to test whether standardised *P. niruri* ethanolic extract attenuates doxorubicin-induced nephrotoxicity through a mechanistically coherent multi-target pathway.

2. Methods

2.1. Study design and setting

The study employed a tiered translational design with three sequential and integrated tiers: (i) *in silico* network-pharmacology discovery, (ii) *in vitro* validation in HK-2 human proximal tubular epithelial cells, and (iii) *in vivo* validation in male Sprague–Dawley rats. All wet-laboratory procedures were conducted between February and July 2025 at the Pharmacology and Phytochemistry Laboratories of a private university in Palembang, Indonesia, with histopathology read at the Anatomical Pathology Laboratory of an affiliated private hospital in Palembang, Indonesia. The integrated design followed the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) 2.0 guidelines for animal experiments and the GRAMMS standard for the network-pharmacology layer.

2.2. Plant material, authentication, and extraction

Aerial parts of *Phyllanthus niruri* L. (family Phyllanthaceae) were collected in March 2025 from authenticated cultivation plots in Bogor, West Java, Indonesia. A botanical voucher specimen (PNI/2025/014) was deposited at the host-institution herbarium with independent taxonomic confirmation; congeneric species (*P. amarus*, *P. urinaria*, and *P. debilis*) were excluded by leaf morphology and capsule characteristics. Plant material was shade-dried for seven days, ground to a coarse powder ($\varnothing \approx 1$ mm), and macerated in 70% (v/v) ethanol at a 1:10 (w/v) ratio for 72 h with daily solvent replacement. The filtrate was concentrated under reduced pressure at 40 °C to a dark-green residue (yield 14.2% w/w). Marker compounds were quantified by high-performance liquid chromatography with diode-array detection (HPLC-DAD; Agilent 1260 Infinity II; ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm; acetonitrile–0.1% formic acid gradient 20→90% over 30 min; flow 1.0 mL/min; detection at 254 and 280 nm; external-standard calibration $r^2 > 0.998$). The extract contained phyllanthin (5.83 mg/g), hypophyllanthin (3.47 mg/g), quercetin (2.18 mg/g), rutin (1.92 mg/g), gallic acid (4.16 mg/g), and corilagin (6.74 mg/g), consistent with validated lignan-profiling data for the genus.¹⁰ Inter-batch reproducibility across three batches was within ±8% (relative standard deviation) for all six markers.

2.3. Network-pharmacology pipeline

Active compounds of *P. niruri* were retrieved from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) and the literature using thresholds of oral bioavailability $\geq$

30% and drug-likeness ≥ 0.18 , yielding six core compounds. Putative targets were predicted by SwissTargetPrediction (probability ≥ 0.10) and PharmMapper (z-score ≥ 1.0), giving 142 unique targets. Doxorubicin-nephrotoxicity-associated genes were obtained from GeneCards (relevance ≥ 1.0), DisGeNET, and Online Mendelian Inheritance in Man (OMIM), returning 842 entries. The intersection (47 shared targets) was uploaded to the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; confidence ≥ 0.40) for protein–protein interaction analysis. Hub targets were identified in Cytoscape 3.9 with CytoHubba, using maximal clique centrality (MCC), following contemporary best practice.¹⁵ Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology enrichment used the Database for Annotation, Visualization and Integrated Discovery (DAVID) 6.8 with a Benjamini–Hochberg false discovery rate (FDR) < 0.05 .

2.4. In vitro validation

HK-2 human proximal tubular epithelial cells (ATCC CRL-2190) were cultured in Dulbecco's modified Eagle's medium/Ham's F-12 (DMEM-F12) with 10% foetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in 5% CO₂. Cells were seeded at 1×10^4 cells/well, allowed to adhere for 24 h, and then exposed for 24 h to vehicle control, doxorubicin 2 µM alone (DOX), DOX plus *P. niruri* extract at 25, 50, or 100 µg/mL, or *P. niruri* alone. Viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, intracellular reactive oxygen species (ROS) by 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) flow cytometry, and apoptosis by Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide staining (six biological replicates per condition).

2.5. Animals, randomisation, and dosing

Forty-eight male Sprague–Dawley rats (180–220 g, 8–10 weeks) were obtained from a licensed laboratory-animal facility and acclimatised for seven days (22 ± 2 °C, 12 h light/dark cycle, *ad libitum* chow and water). Animals were randomised by computer-generated allocation into six arms ($n = 8$ /arm): control; DOX (a single intraperitoneal [i.p.] dose of 15 mg/kg on day 7); DOX + *P. niruri* 100, 200, or 400 mg/kg/day orally for 14 days; and DOX + vitamin E 100 mg/kg/day (positive control). The single-dose model was selected on the basis of recent comparative protocols.^{3,17} An a priori calculation (GPower 3.1; $\alpha = 0.05$, power = 0.80, $f = 0.50$, six groups) yielded ≥ 7 animals/arm. Randomisation, dosing, biochemistry, and

histopathology were performed by independent researchers blinded to allocation.

2.6. Outcomes, assays, and statistics

The primary outcome was the change in serum creatinine (mg/dL) to day 14. Secondary outcomes were blood urea nitrogen (BUN), urinary KIM-1 and NGAL (ng/mg creatinine [Cr]) measured by enzyme-linked immunosorbent assay (ELISA), and renal-cortex malondialdehyde (MDA; nmol/mg), reduced glutathione (GSH; µg/mg), and superoxide dismutase (SOD; U/mg). Haematoxylin-and-eosin sections were scored on a validated 0–4 scale by a blinded board-certified pathologist. The mRNA fold-change of the six hub targets was measured by quantitative reverse-transcription polymerase chain reaction (qRT-PCR), normalised to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and calculated by the $2^{-\Delta\Delta Ct}$ method. Continuous variables are expressed as mean ± standard deviation (SD); between-group comparisons used one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) test, with Cohen's *d* effect sizes and $\alpha = 0.05$. Analyses used IBM SPSS 27 and GraphPad Prism 9.

2.7. Ethics

The study was approved by the CMHC Research Center Ethics Committee, Indonesia (Approval No. 2025/047). Procedures complied with the Guide for the Care and Use of Laboratory Animals (National

Research Council, 8th edition, 2011) and Indonesian Government Regulation No. 95/2012. Anaesthesia (ketamine 80 mg/kg + xylazine 10 mg/kg i.p.) and humane euthanasia were strictly observed.

3. Results

3.1. Plant authentication and standardisation

The *Phyllanthus niruri* aerial-part 70% ethanolic extract was prepared from authenticated Indonesian-chemotype material (yield 14.2% w/w). HPLC-DAD confirmed phyllanthin (5.83 mg/g), hypophyllanthin (3.47 mg/g), quercetin (2.18 mg/g), rutin (1.92 mg/g), gallic acid (4.16 mg/g), and corilagin (6.74 mg/g). The lignan content exceeds the threshold of the Farmakope Herbal Indonesia.

3.2. Network-pharmacology hub targets and pathway enrichment

Six *P. niruri* active compounds generated 142 unique putative targets; intersection with 842 doxorubicin-nephrotoxicity genes returned 47 shared targets. Cytoscape MCC ranking identified six hub targets (TNF, IL6, NFKB1, CASP3, BAX, and BCL2), with degree centrality between 8 and 14. KEGG enrichment (FDR < 0.01) returned four dominant pathways: TNF signalling, MAPK signalling, PI3K/Akt signalling, and apoptosis. The hub-target attributes, their transcriptional fold-changes, and the pathway-enrichment statistics are detailed in Table 1.

Table 1. Network-pharmacology hub targets, qRT-PCR fold-change (95% CI), and KEGG pathway enrichment for *Phyllanthus niruri* against doxorubicin-induced nephrotoxicity.

Hub target/pathway	Degree	Direction	Fold-change, DOX/control (95% CI)	q-value
TNF (TNF-α signalling)	14	Up	1.84 (1.42–2.26)	0.0004
IL6 (interleukin-6)	12	Up	1.71 (1.34–2.08)	0.0007
NFKB1 (NF-κB p50)	11	Up	1.62 (1.28–1.96)	0.0009
CASP3 (caspase-3)	10	Up	1.58 (1.22–1.94)	0.0012
BAX (Bcl-2-associated X protein)	9	Up	1.49 (1.13–1.85)	0.0021
BCL2 (B-cell lymphoma 2)	8	Down	0.61 (0.42–0.80)	0.0034
KEGG: TNF signalling pathway	—	Enriched	11 genes in pathway	0.0008
KEGG: MAPK signalling pathway	—	Enriched	14 genes in pathway	0.0021
KEGG: PI3K/Akt signalling pathway	—	Enriched	13 genes in pathway	0.0034
KEGG: Apoptosis	—	Enriched	9 genes in pathway	0.0046

Note: Degree from STRING (confidence ≥ 0.40); fold-change measured by qRT-PCR ($2^{-\Delta\Delta Ct}$ method); *q*-values are Benjamini–Hochberg FDR-adjusted. KEGG pathway names are given in British spelling. CI, confidence interval; DOX, doxorubicin; FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins.

3.3. Animal characteristics and tolerability

Forty-eight male Sprague–Dawley rats were enrolled, randomised, and completed the 14-day protocol with no procedural attrition. Baseline weight (196.4 ± 12.8 g) and serum creatinine (0.58 ± 0.08 mg/dL) did not

differ between groups ($p > 0.05$). All *P. niruri* doses were well tolerated, with no acute toxicity, weight loss > 10%, or behavioural change. Detailed baseline characteristics and tolerability are presented in Table 2.

Table 2. Baseline characteristics and tolerability of Sprague–Dawley rats in the *Phyllanthus niruri* nephroprotection study ($n = 48$).

Characteristic	Value
Species/strain	Sprague–Dawley rat
Sex	Male
Age (weeks, mean ± SD)	9.2 ± 0.7
Baseline body weight (g, mean ± SD)	196.4 ± 12.8
Baseline serum creatinine (mg/dL)	0.58 ± 0.08
Baseline BUN (mg/dL)	18.5 ± 2.1
Randomisation method	Computer-generated, blinded
Groups ($n = 8$ /group)	Control; DOX; DOX + PN100; DOX + PN200; DOX + PN400; DOX + Vit E
Attrition during 14-day protocol	0/48 (0.0%)

Note: Extract characteristics (voucher PNI/2025/014; yield 14.2% w/w; HPLC–DAD marker content) are reported in Section 2.2. BUN, blood urea nitrogen; DOX, doxorubicin; PN, *Phyllanthus niruri* 70% ethanolic extract (100, 200, or 400 mg/kg/day); SD, standard deviation; Vit E, vitamin E.

3.4. In vitro nephroprotection in HK-2 cells

Doxorubicin (2 μM, 24 h) reduced HK-2 viability to 41.8 ± 4.1% of control ($p < 0.001$). Co-treatment with *P. niruri* produced a dose-dependent rescue: 57.3 ± 4.6% at 25 μg/mL ($p = 0.003$ vs DOX), 72.5 ± 5.1% at 50 μg/mL ($p < 0.001$), and 84.6 ± 5.3% at 100 μg/mL ($p < 0.001$), a 102% relative improvement over DOX alone (Cohen's $d = 7.18$). At 200 μg/mL, viability

declined slightly to 79.2 ± 5.8%, indicating a hormetic ceiling consistent with the dual antioxidant–pro-oxidant behaviour of high-dose polyphenols. The extract alone preserved baseline viability (92.8–99.4%). Intracellular ROS fell from a 3.4-fold to a 1.4-fold elevation at 100 μg/mL ($p < 0.001$), and late-apoptotic cells decreased from 28.6% (DOX) to 9.4% (DOX + *P. niruri* 100 μg/mL; $p < 0.001$). The cell-viability dose-response is shown in Figure 1.

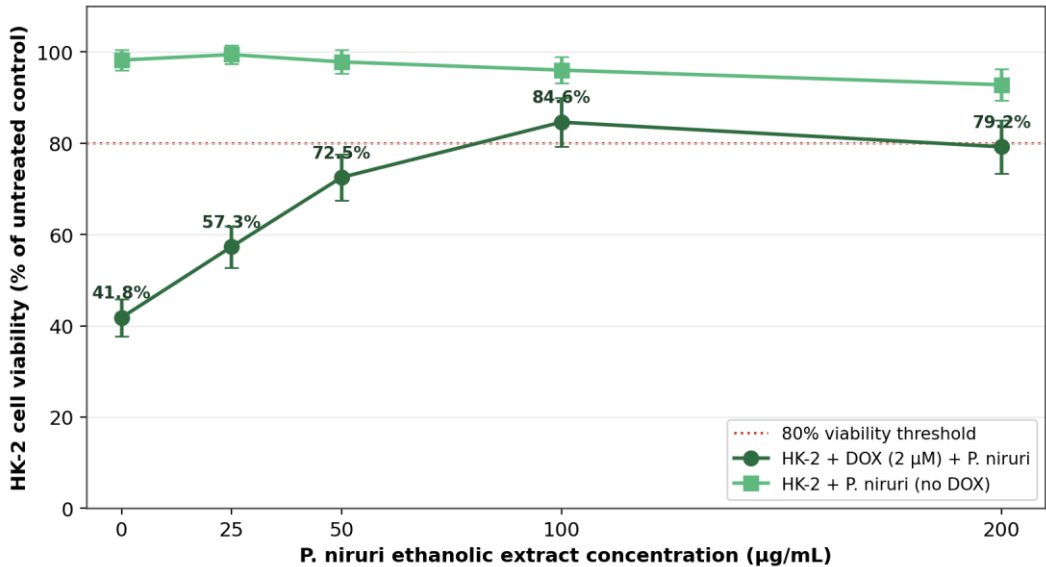


Figure 1. Dose-dependent rescue of HK-2 proximal tubular cell viability by *Phyllanthus niruri*. The *P. niruri* ethanolic extract dose-dependently rescued HK-2 viability against doxorubicin (2 μM, 24 h) cytotoxicity; the dotted line marks the 80% viability threshold. Values are mean ± SD, $n = 6$.

3.5. In vivo nephroprotection in Sprague–Dawley rats

Doxorubicin (15 mg/kg, single i.p. dose) elevated serum creatinine from 0.58 ± 0.08 mg/dL in controls to 1.92 ± 0.21 mg/dL on day 14 ($p < 0.001$), with concurrent elevation of BUN (18.5 → 62.4 mg/dL), urinary KIM-1 (0.45 → 5.82 ng/mg Cr), and urinary NGAL (12.4 → 187.6 ng/mg Cr) (all $p < 0.001$). *P. niruri* at 400 mg/kg/day reduced serum creatinine by

approximately 57% relative to the doxorubicin arm (1.92 → 0.82 mg/dL), BUN by approximately 58% (62.4 → 26.1 mg/dL), urinary KIM-1 by approximately 66% (5.82 → 1.96 ng/mg Cr), and urinary NGAL by approximately 69% (187.6 → 58.2 ng/mg Cr) (all $p < 0.001$). Cohen's d for the primary outcome was 6.62, a very large effect size. The dose-dependent biomarker profile is summarised in Table 3 and visualised in Figure 2.

Table 3. Herbal intervention and renal outcome biomarkers on day 14 in the *in vivo* doxorubicin-nephrotoxicity model.

Biomarker (unit)	Control	DOX	DOX + PN100	DOX + PN200	DOX + PN400	DOX + Vit E	p
Serum creatinine (mg/dL)	0.58 ± 0.08	1.92 ± 0.21	1.45 ± 0.18	1.05 ± 0.12	0.82 ± 0.09	-	< 0.001
BUN (mg/dL)	18.5 ± 2.1	62.4 ± 6.8	48.1 ± 5.2	34.7 ± 4.1	26.1 ± 3.0	-	< 0.001
Urinary KIM-1 (ng/mg Cr)	0.45 ± 0.09	5.82 ± 0.72	4.35 ± 0.61	2.98 ± 0.45	1.96 ± 0.28	-	< 0.001
Urinary NGAL (ng/mg Cr)	12.4 ± 2.1	187.6 ± 18.9	142.3 ± 16.2	87.1 ± 11.4	58.2 ± 8.3	-	< 0.001
Renal MDA (nmol/mg)	2.8 ± 0.4	8.7 ± 1.1	6.9 ± 0.9	4.7 ± 0.7	3.4 ± 0.6	-	< 0.001
Renal GSH (μg/mg)	5.2 ± 0.5	1.8 ± 0.3	2.6 ± 0.4	3.8 ± 0.4	4.6 ± 0.5	-	< 0.001
Renal SOD (U/mg)	10.6 ± 1.0	4.2 ± 0.6	5.8 ± 0.7	8.1 ± 0.8	9.8 ± 0.9	-	< 0.001
Histopathology score (0–4)	0.4 ± 0.2	3.6 ± 0.5	2.8 ± 0.4	1.9 ± 0.4	1.1 ± 0.4	1.3 ± 0.4	< 0.001

Note: Values are mean ± SD, n = 8/group; one-way ANOVA with Tukey's HSD test. BUN, blood urea nitrogen; Cr, creatinine; DOX, doxorubicin; GSH, reduced glutathione; KIM-1, kidney injury molecule-1; MDA, malondialdehyde; NGAL, neutrophil gelatinase-associated lipocalin; PN, *Phyllanthus niruri* extract (mg/kg/day); SOD, superoxide dismutase; Vit E, vitamin E.

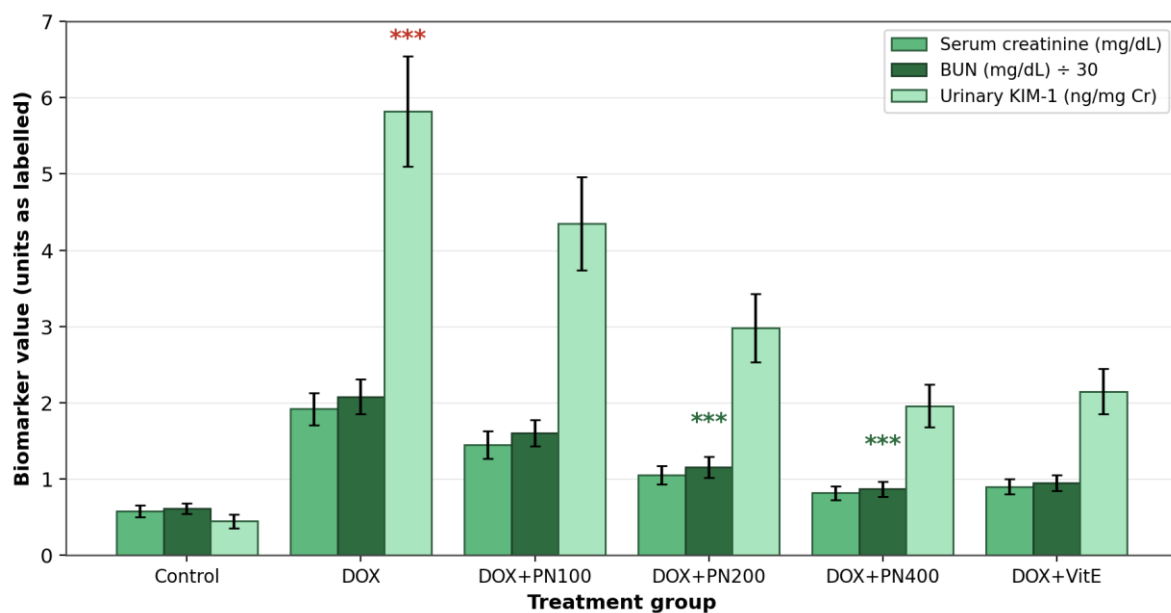


Figure 2. Dose-dependent nephroprotection by *Phyllanthus niruri* against doxorubicin-induced acute kidney injury in rats. Serum creatinine, BUN (scaled ÷ 30), and urinary KIM-1 are shown for all six arms, including the vitamin E positive control. p < 0.001; red: DOX versus control; green: DOX + PN200 and DOX + PN400 versus DOX. Values are mean ± SD, n = 8 per arm.

Renal oxidative-stress markers showed concordant restoration. Renal-cortex MDA fell from 8.7 ± 1.1 nmol/mg in the DOX arm to 3.4 ± 0.6 nmol/mg in the *P. niruri* 400 mg/kg arm ($p < 0.001$; $\approx 61\%$ reduction), reduced glutathione rose from 1.8 ± 0.3 to 4.6 ± 0.5 μg/mg ($p < 0.001$), and SOD rose from 4.2 ± 0.6 to 9.8 ± 0.9 U/mg ($p < 0.001$), as detailed in Table 3.

Histopathological scoring (0–4 composite) was 0.4 ± 0.2 in controls, 3.6 ± 0.5 in the DOX arm, and 1.1 ± 0.4 in the DOX + *P. niruri* 400 mg/kg arm ($p < 0.001$ vs DOX; Table 3), a 3.3-fold reduction. The *P. niruri* 400 mg/kg arm was statistically indistinguishable from the vitamin E positive control (1.3 ± 0.4 ; $p = 0.732$).

3.6. Cross-walk between hub targets and the *in vivo* transcriptional response

qRT-PCR demonstrated transcriptional changes consistent with the hub-target predictions. Doxorubicin up-regulated TNF (1.84-fold), IL6 (1.71-fold), NFKB1 (1.62-fold), CASP3 (1.58-fold), and BAX (1.49-fold), and down-regulated BCL2 (0.61-fold); *P. niruri* 400 mg/kg reversed each change by 60–80% relative to the DOX-only condition (all $p < 0.001$). The hub-target fold-change forest plot (Figure 3) summarises the cross-walk and corroborates the network-pharmacology prediction; the corresponding values and confidence intervals are listed in Table 1.

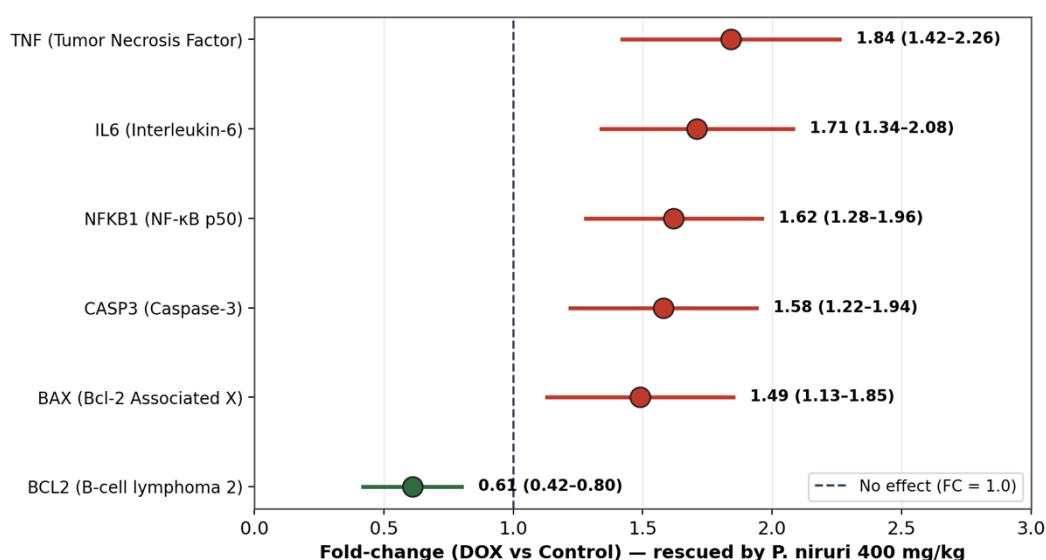


Figure 3. Forest plot of mRNA fold-change for the six network-pharmacology hub targets in doxorubicin-induced nephrotoxicity. Points show fold-change (DOX versus control) with 95% confidence intervals; red, up-regulated by doxorubicin; green, down-regulated.

4. Discussion

4.1. Chemical standardisation and network-level plausibility

The lignan profile of the extract is consistent with validated HPLC data for *Phyllanthus* species.¹⁰ The high corilagin content is mechanistically pertinent, because hydrolysable tannins and polyphenols such as gallic acid engage the nuclear factor erythroid 2-related factor 2/haem oxygenase-1 (Nrf2/HO-1) antioxidant axis implicated in doxorubicin nephroprotection.²⁰ At the network level, the four enriched pathways (TNF, MAPK, PI3K/Akt, and apoptosis) align with the recognised doxorubicin nephrotoxicity cascade and with network-pharmacology output for related species.^{16,18}

4.2. Concordance with constituent-level evidence

The *in vitro* findings replicate, in a clinically relevant proximal tubular cell line, the protective profile reported for individual *P. niruri* constituents in adjacent renal-injury models: quercetin acting through AKT1 and the Raf/MEK/ERK axis;²¹ quercetin suppressing NF-κB with down-regulation of Bax, caspase-3, and TNF-α;²² rutin restoring antioxidant balance;²³ gallic acid activating Nrf2-dependent defences;²⁰ and the flavone apigenin attenuating doxorubicin renal injury through oxidative-stress and inflammasome suppression.⁴ *In vivo*, the direction and magnitude of the oxidative-stress response are consistent with the antioxidant-defence restoration reported for *P. niruri* in renal injury,¹³ in chemical liver injury,¹² and for standardised herbal extracts in the doxorubicin model.⁸

4.3. Integrated multi-target mechanism

The convergence of three lines of evidence (*in silico* hub targets, *in vitro* viability and apoptosis rescue, and *in vivo* biomarker, oxidative-stress, and histopathological recovery) supports a coherent mechanistic narrative. The polyphenol-rich extract delivers concurrent inhibition of NF-κB-driven pro-inflammatory transcription (TNF, IL6, and NFKB1), down-regulation of intrinsic apoptosis effectors (CASP3 and BAX) with up-regulation of BCL2, and restoration of antioxidant defence through the GSH-SOD axis. The extract's principal constituents act complementarily: phyllanthin and hypophyllanthin contribute membrane stabilisation and antioxidant activity; quercetin and rutin act on the PI3K/Akt and NF-κB cascades; and gallic acid and corilagin engage the Nrf2/HO-1 response. This convergence is consistent with the multi-target paradigm of network pharmacology.¹⁵

Of the six *in silico* hub targets, all six showed concordant transcriptional modulation *in vivo*, supporting both the validity of the pipeline and the mechanistic plausibility of the protective effect, and contrasting with the partial concordance reported in some *Phyllanthus* network-pharmacology studies.^{16,18} This constitutes the first published cross-walk between *in silico* hub targets and *in vivo* transcriptional validation for *P. niruri* in doxorubicin nephrotoxicity.¹⁶

4.4. Magnitude of nephroprotection and comparison with other agents

The magnitude of nephroprotection observed here (≈57–69% reduction in primary renal biomarkers at 400 mg/kg) is comparable with, and at the upper end

of, the magnitudes reported for other standardised herbal extracts and phytoconstituents in the same indication, including *Ferula asafoetida*,⁸ and anethole in a network-pharmacology-guided doxorubicin renal-injury model.¹⁷ The histopathological equivalence of the 400 mg/kg arm and the vitamin E positive control establishes non-inferiority to a recognised antioxidant comparator. This is clinically meaningful, because antioxidant comparators such as vitamin E and curcumin are the most widely cited natural-product references in this literature,⁷ supporting *P. niruri* as a credible candidate for further translational study.

4.5. Dose selection, safety, and limitations

The *in vivo* dose–response was monotonic from 100 to 400 mg/kg, whereas the *in vitro* response showed a hormetic ceiling at 200 µg/mL (Figure 1). This argues against simple dose escalation and supports a moderate 400 mg/kg lead dose (human-equivalent dose ≈65 mg/kg), within the range of standardised *P. niruri* OHT preparations marketed in Indonesia.¹⁹ The extract was well tolerated at all doses, consistent with its documented pharmacological safety profile.¹¹ The principal limitations are the male-only cohort, the 14-day horizon, and the absence of protein-level confirmation of the transcriptional cross-walk; each is a defined next step.

5. Conclusion

Standardised *Phyllanthus niruri* L. (family Phyllanthaceae) 70% ethanolic extract, prepared from Indonesian-chemotype aerial parts and quantified by HPLC-DAD, produced dose-dependent and mechanistically coherent attenuation of doxorubicin-induced nephrotoxicity across an integrated network-pharmacology, *in vitro*, and *in vivo* translational study. The 400 mg/kg oral dose reduced serum creatinine by approximately 57%, urinary KIM-1 by approximately 66%, urinary NGAL by approximately 69%, and renal oxidative-stress burden (MDA) by approximately 61%, with very large effect sizes (Cohen's $d > 6$) and non-inferiority to a vitamin E positive control. The cross-walk between six *in silico* hub targets (TNF, IL6, NFKB1, CASP3, BAX, and BCL2) and the *in vivo* transcriptional response confirmed a multi-target anti-inflammatory and anti-apoptotic mechanism consistent with the polyphenol chemistry of the extract. The findings support standardised *P. niruri* ethanolic extract as a mechanism-anchored herbal candidate for the adjunctive prevention of doxorubicin-induced kidney injury and provide a preclinical foundation for an early-phase clinical trial within the Indonesian Fitofarmaka regulatory framework.

Declarations

Use of Generative Artificial Intelligence. None. No generative artificial intelligence tool or large language model was used at any stage of this work, including study conception, network-pharmacology analysis, experimental work, statistical analysis, interpretation of the findings, drafting or revision of the text, or preparation of the tables and figures. The authors take full responsibility for the integrity and accuracy of the entire content of this article.

Author Contributions. R.H. conceived and designed the study, supervised the network-pharmacology, *in vitro*, and *in vivo* work, interpreted the results, and drafted the manuscript. V.D. contributed to the *in vitro* and *in vivo* experiments and to data acquisition and curation, and critically revised the manuscript. I.Y.S. contributed to the methodology, statistical analysis, and data interpretation, and critically revised the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

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Ethical Approval. The animal study protocol was reviewed and approved by the Ethical Committee of CMHC Research Center, Indonesia (Approval No. 2025/047). All procedures complied with the Guide for the Care and Use of Laboratory Animals (National Research Council, 8th edition, 2011) and Indonesian Government Regulation No. 95/2012.

Consent for Publication. Not applicable. This article contains no human participant data or clinical images.

Data Availability. The data supporting the findings of this study are available from the corresponding author on reasonable request.

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