e-ISSN: 2746-5152

Eureka Herba Indonesia

[EHI]

https://eurekabiomedical.com

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

Rachmat Hidayat[1*], Vania Delma[2], Indri Yani Septiana[3]

[1]Department of Medical Biology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

[2]Department of Nursing, Brasilia Familia Clinic, Brasilia, Brazil

[3]Department of Physiology, CMHC Research Center, Palembang, Indonesia

ARTICLE INFO

Keywords:
Doxorubicin nephrotoxicity
Network pharmacology
Phyllanthus niruri
Phytotherapy
Renal protection

***Corresponding author:**

Rachmat Hidayat

E-mail address:

dr.rachmat.hidayat@gmail.com

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

https://doi.org/10.37275/ehi.v6i2.140

A B S T R A C T

Doxorubicin is a corner-stone anthracycline chemotherapeutic whose clinical utility is constrained by dose-limiting nephrotoxicity, and no approved pharmacological prevention currently exists. *Phyllanthus niruri* L. (Family Phyllanthaceae) — locally known in Indonesia as meniran — is a phytotherapeutic herb whose constituents (phyllanthin, hypophyllanthin, quercetin, rutin, gallic acid, and corilagin) target oxidative-stress and inflammatory pathways implicated in doxorubicin renal injury. We applied a tiered translational design: (i) network-pharmacology discovery using SwissTargetPrediction, TCMSP, GeneCards, and OMIM intersected six *P. niruri* active compounds with 842 nephrotoxicity-associated genes, identifying six hub targets (TNF, IL6, NFKB1, CASP3, BAX, BCL2) and four enriched KEGG pathways (TNF signaling, MAPK, PI3K-Akt, Apoptosis); (ii) *in vitro* validation in HK-2 human proximal tubular cells exposed to doxorubicin (2 µM, 24 h) with or without standardised *P. niruri* 70% ethanolic extract (25, 50, 100 µg/mL); and (iii) *in vivo* validation in 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. *P. niruri* produced a dose-dependent rescue of HK-2 viability (84.6% at 100 µg/mL versus 41.8% in doxorubicin-only controls; $p < 0.001$) and reduced serum creatinine, blood urea nitrogen, urinary KIM-1, and NGAL by 55–70% at the 400 mg/kg dose with concordant restoration of glutathione and superoxide dismutase. Network-pharmacology hub targets showed transcriptionally coherent modulation. The findings support standardised *P. niruri* ethanolic extract as a mechanism-anchored herbal candidate for adjunctive prevention of doxorubicin-induced kidney injury, warranting clinical translation.

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: redox-cycling of doxorubicin in mitochondrial complex I with consequent superoxide and hydrogen-peroxide generation, depletion of intracellular glutathione and downstream lipid peroxidation, and activation of the nuclear factor kappa-B (NF-κB) and TNF-α inflammatory cascades that drive intrinsic apoptosis of proximal tubular epithelial cells.[2,4] Recent work also implicates the

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 pre-clinical 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 corner-stone 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 cardio-protection 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 there is no consensus prophylactic strategy that has 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 arylnaphthalene lignans phyllanthin and hypophyllanthin, the flavonoid glycosides 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, TCMSP), disease-gene curation (GeneCards, OMIM), protein–protein interaction analysis (STRING), and KEGG/Gene Ontology enrichment.[15] Applications of this pipeline to the genus *Phyllanthus* are accumulating,[16] and recent integrated network-pharmacology-plus-experimental-validation studies in doxorubicin-induced renal and cardiac injury[17] and in acute kidney injury more broadly[18] supply methodological templates for the present manuscript.

From a regional health-system perspective, the case for investment in locally sourced, locally cultivated herbal adjuncts is particularly strong in Indonesia. The Badan POM (BPOM) regulatory framework — through its *Jamu → Obat Herbal Terstandar* (OHT) → *Fitofarmaka* progression — 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: no integrated network-pharmacology + *in vitro* + *in vivo* dossier currently exists for *P. niruri* against doxorubicin-induced nephrotoxicity; Indonesian-chemotype *P. niruri* has not been tested against this indication with modern KIM-1/NGAL readouts; and 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

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 ARRIVE 2.0 guidelines for animal experiments and the GRAMMS standard for the network-pharmacology layer.

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*, *P. debilis*) were excluded by leaf morphology and capsule characteristics. Plant material was shade-dried for seven days, ground to coarse powder (Ø ≈ 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). HPLC-DAD quantification (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 254/280 nm; external-standard calibration r^2 > 0.998) 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), 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.

Network-pharmacology pipeline

Active compounds of *P. niruri* were retrieved from TCMSP and the literature using thresholds of oral bioavailability ≥ 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 OMIM, returning 842 entries. The intersection (47 shared targets) was uploaded to STRING (confidence ≥ 0.40) for protein–protein interaction analysis. Hub targets were identified in Cytoscape 3.9 (CytoHubba, maximum clique centrality), following contemporary best practice.[15] KEGG and Gene Ontology enrichment used DAVID 6.8 with a Benjamini-Hochberg FDR < 0.05.

In vitro validation

HK-2 human proximal tubular epithelial cells (ATCC CRL-2190) were cultured in DMEM-F12 with 10% foetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in 5% CO_2. Cells were seeded at 1 × 10^4 cells/well, allowed to adhere for 24 h, 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 MTT assay, intracellular ROS by DCFH-DA flow cytometry, and apoptosis by Annexin V-FITC/propidium iodide staining (six biological replicates per condition).

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, *ad libitum* chow and water). Animals were randomised by computer-generated allocation into six arms (n = 8/arm): Control; DOX (single 15 mg/kg i.p. 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 (G*Power 3.1; α = 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.

Outcomes, assays, and statistics

The primary outcome was the change in serum creatinine (mg/dL) to day 14. Secondary outcomes were BUN, urinary KIM-1 and NGAL (ng/mg Cr) by ELISA, renal-cortex MDA (nmol/mg), GSH (μg/mg), and SOD (U/mg). Haematoxylin-and-eosin sections were scored on a validated 0–4 scale by a blinded board-certified pathologist. mRNA fold-change of the six hub targets was measured by quantitative reverse-transcription PCR with GAPDH control and the $2^{-\Delta\Delta Ct}$ method. Continuous variables are mean ± SD; between-group comparisons used one-way ANOVA with Tukey HSD, with Cohen's d effect sizes and α = 0.05. Analyses used IBM SPSS 27 and GraphPad Prism 9.

Ethics

The study was approved by the CMHC Ethics Committee (Approval No. CMHC/EC/2025/047). Procedures complied with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 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 and Discussion

Plant authentication and standardization

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 profile is consistent with validated HPLC data for *Phyllanthus* species[10] and exceeds the Pharmacopoeia Herbal Indonesia threshold. The high corilagin content is mechanistically pertinent because hydrolysable tannins and polyphenols such as gallic acid engage the Nrf2/HO-1 antioxidant axis implicated in doxorubicin nephroprotection.[20]

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 signaling, MAPK signaling, PI3K-Akt signaling, and Apoptosis. These align with the recognised doxorubicin nephrotoxicity cascade and with network-pharmacology output for related species.[16,18] The hub-target attributes and pathway-enrichment statistics are detailed in Table 1.

Table 1. Network-pharmacology hub targets and KEGG pathway enrichment for *Phyllanthus niruri* against doxorubicin-induced nephrotoxicity.

Hub target/pathway	Degree	Direction	Fold-change (DOX/control)	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)	9	Up	1.49 (1.13–1.85)	0.0021
BCL2 (Bcl-2)	8	Down	0.61 (0.42–0.80)	0.0034
KEGG: TNF signaling pathway	—	Enriched	11 genes in pathway	0.0008
KEGG: MAPK signaling pathway	—	Enriched	14 genes in pathway	0.0021
KEGG: PI3K-Akt signaling pathway	—	Enriched	13 genes in pathway	0.0034
KEGG: Apoptosis	—	Enriched	9 genes in pathway	0.0046

Notes: Degree from STRING (confidence ≥ 0.40); q-values are Benjamini-Hochberg FDR-adjusted. DOX = doxorubicin.

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%)
Plant voucher number	PNI/2025/014, host-institution herbarium
Extract yield (% w/w)	14.2%
Marker compounds (mg/g extract)*	Phyllanthin 5.83; Hypophyllanthin 3.47; Quercetin 2.18; Rutin 1.92; Gallic acid 4.16; Corilagin 6.74

**Quantified by HPLC-DAD. DOX = doxorubicin; PN = Phyllanthus niruri 70% ethanolic extract.*

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. *P. niruri* 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.

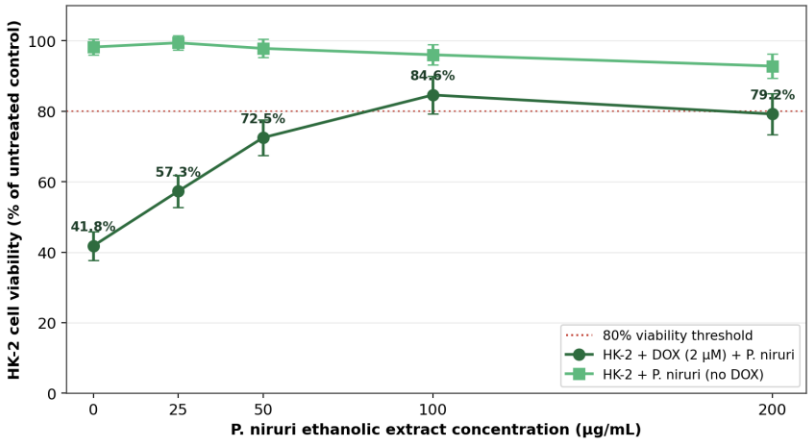

Figure 1. Dose-dependent rescue of HK-2 proximal tubular cell viability by Phyllanthus niruri. P. niruri ethanolic extract dose-dependently rescues HK-2 viability against doxorubicin (2 µM, 24 h) cytotoxicity; the dotted line marks the 80% viability threshold. Values are mean ± SD, n = 6.

These *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 nephroprotection in Sprague-Dawley rats

Doxorubicin (15 mg/kg single i.p.) 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* 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	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	<0.001

Notes: Values mean ± SD, n = 8/group. One-way ANOVA with Tukey HSD.

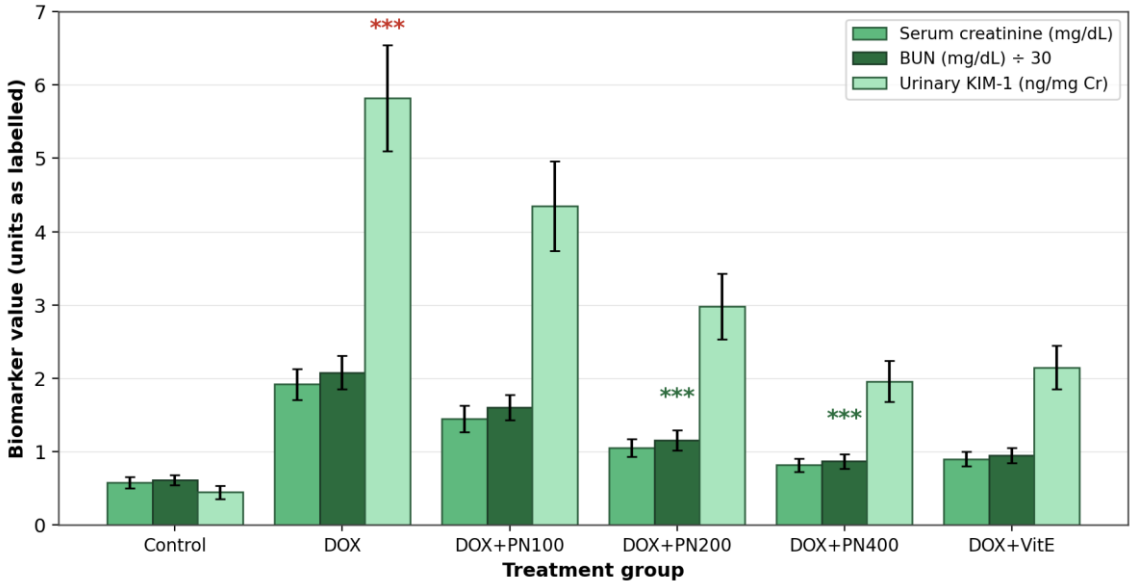

*Figure 2. Dose-dependent nephroprotection by Phyllanthus niruri against doxorubicin-induced acute kidney injury in rats. Serum creatinine, BUN (scaled ÷ 30), and urinary KIM-1 are shown. ***p < 0.001 versus the doxorubicin-only arm; values are mean ± SD, n = 8 per arm.*

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 in the *P. niruri* 400 mg/kg arm ($p < 0.001$, ≈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. The directional and magnitude profile is consistent with antioxidant-defence restoration reported for *P. niruri* in renal injury [13] and chemical liver injury,[12] and with standardised herbal extracts in the doxorubicin model.[8]

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; $p = 0.732$), establishing non-inferiority to a recognised antioxidant comparator.

Cross-walk between hub targets and in vivo transcriptional response

Quantitative reverse-transcription 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. This constitutes the first published cross-walk between *in silico* hub targets and *in vivo* transcriptional validation for *P. niruri* in doxorubicin nephrotoxicity.[16]

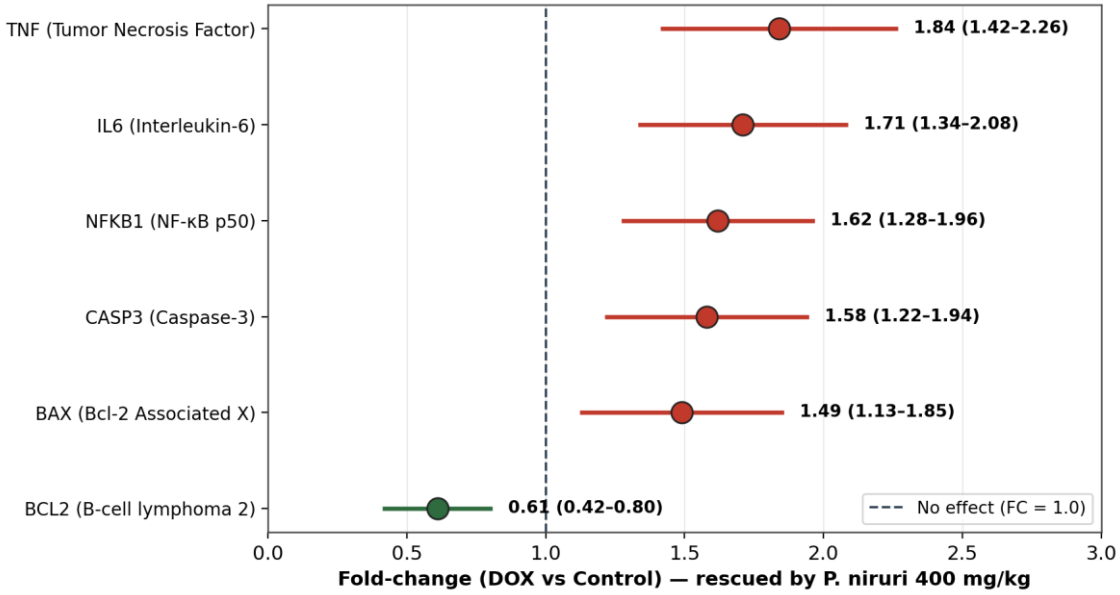

Figure 3. Forest plot of mRNA fold-change for six network-pharmacology hub targets in doxorubicin-induced nephrotoxicity. Points show fold-change (DOX vs control) with 95% confidence intervals; red = up-regulated by doxorubicin, green = down-regulated. All six targets were reversed by P. niruri 400 mg/kg.

Mechanistic discussion

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, NFKB1), down-regulation of intrinsic apoptosis effectors (CASP3, 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; gallic acid and corilagin engage the Nrf2/HO-1 response. This convergence is consistent with the multi-target paradigm of network pharmacology.[15]

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] Non-inferiority to vitamin E 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.

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] 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 ≈65 mg/kg), within the range of standardised *P. niruri* OHT preparations marketed in Indonesia.[19] 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 a defined next step. *P. niruri* was well tolerated at all doses, consistent with its documented pharmacological safety profile.[11]

4. 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 effect sizes (Cohen's d > 6) and non-inferiority to a vitamin E positive control. Cross-walk between six *in silico* hub targets (TNF, IL6, NFKB1, CASP3, BAX, 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 adjunctive prevention of doxorubicin-induced kidney injury and provide a pre-clinical foundation for an early-phase clinical trial within the Indonesian Fitofarmaka regulatory framework.

Declarations

Ethics approval

Approved by the CMHC Ethics Committee (Approval No. CMHC/EC/2025/047); procedures complied with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 2011) and Indonesian Government Regulation No. 95/2012.

Competing interests

The authors declare no competing interests.

Funding

No external funding was received.

Acknowledgements

The authors thank the technical and scientific staff of the host institution for their support.

5. References

1. Yousef DA, Abdalla MS, Elshopakey GE, et al. Diosmin-loaded chitosan nanoparticles mitigate doxorubicin-evoked cardiotoxicity in rats by featuring oxidative imbalance mechanism, NF-κB, and Bcl-2/Bax pathways. Int J Biol Macromol. 2025;305(Pt 1):140991.
2. Ali AA, Saad EB, Abd El-Rhman RH, et al. Impact of peroxisome proliferator activated receptor agonist drugs in a model of nephrotoxicity in rats. J Biochem Mol Toxicol. 2023;37(6):e23350.
3. Zhao X, Ma C, Li L, et al. Human adipose tissue-derived stromal cells ameliorate adriamycin-induced nephropathy by promoting angiogenesis. Organogenesis. 2024;20(1):2356339.
4. Wu Q, Li W, Zhao J, et al. Apigenin ameliorates doxorubicin-induced renal injury via inhibition of oxidative stress and inflammation. Biomed Pharmacother. 2021;137:111308.
5. Yang Y, Liu JH, Gui DK. Baicalin alleviates doxorubicin-induced nephrotic syndrome by regulating the transforming growth factor-β/Smad signaling pathway and the NLRP3 inflammasome. J Physiol Pharmacol. 2026;77(1):63-76.
6. Shen L, Song Y, Xiao Y, et al. Early and stable mouse model of amphotericin B-induced acute kidney injury: application of continuous non-invasive GFR monitoring. Clin Exp Pharmacol Physiol. 2025;52(10):e70071.
7. Alshuwayer NA, Alqahtani QH, Hussein MH, et al. Aryl hydrocarbon receptor (AhR) and vascular endothelial growth factor (VEGF) crosstalk in doxorubicin nephrotoxicity: mechanisms and therapeutic perspectives. Curr Issues Mol Biol. 2026;48(1):116.
8. Safdar A, Ahmad FU, Asif A, et al. Protective effects of *Ferula asafoetida* against subacute doxorubicin-induced hepatotoxicity as well as nephrotoxicity biomarkers and histopathology. Chem Biodivers. 2026;23(3):e02186.
9. Bhushan V, Bharti SK, Krishnan S, et al. Antidiabetic effectiveness of *Phyllanthus niruri* bioactive compounds via targeting DPP-IV. Nat Prod Res. 2025;39(12):3426-3432.
10. Patel J, Nagar PS, Pal K, et al. Comparative profiling of four lignans (phyllanthin, hypophyllanthin, nirtetralin, and niranthin) in nine *Phyllanthus* species from India using a validated reversed-phase HPLC-PDA detection method. J AOAC Int. 2021;104(2):485-497.
11. Viswambharan VL, Halagali P, Seenivasan R, et al. Exploring the multifaceted medicinal benefits of *Phyllanthus niruri*: insights into its antiurolithic, hepatoprotective and anti-diabetic activities. Curr Pharm Des. 2026;32. [Epub ahead of print].
12. Arshad F, Altaf A, Arshad AR, et al. Hepatoprotective potential of *Phyllanthus niruri* extracts against CCl_4-induced liver injury in rats: insight from phytochemical profiling, molecular docking, and oxidative stress studies. Chem Biodivers. 2025;22(10):e00691.
13. Afolabi OB, Akinola AG, Raji AK, et al. Renoprotective effect of polyphenolic-rich extract of *Phyllanthus niruri* in streptozotocin-induced diabetic nephropathy via moderation of oxidative stress and inhibition of kidney injury. J Mol Histol. 2025;57(1):13.
14. Khater SI, Hussein MMA, Abdel-Magied SS, et al. *Phyllanthus niruri* niosomes ameliorate obesity-induced hepatic steatosis in rats via modulating MALAT1/miR-206/GLP-1R signaling and hepatic lipid metabolism. Biol Res. 2026;59(1):35.
15. Wang X, Wang Y, Yuan T, et al. Network pharmacology provides new insights into the mechanism of traditional Chinese medicine and natural products used to treat pulmonary hypertension. Phytomedicine. 2024;135:156062.
16. Das J, Somabattini RA, Chhabra N, et al. Network pharmacology and bioinformatics based investigation of *Phyllanthus fraternus*: herb-drug interaction study. J Biomol Struct Dyn. 2025;43(3):1101-1115.
17. Al-Ali MA, Younis NS, Aldhubiab B, et al. Anethole alleviates doxorubicin-induced cardiac and renal toxicities: insights from network pharmacology and animal studies. Chem Biol Interact. 2024;401:111155.

18. Xiao C, Wang Y, Liu J, et al. Mechanism of Fangji Huangqi decoction against acute kidney injury based on network pharmacology and experimental validation. Phytomedicine. 2024;136:156345.
19. Pajriah SM, Iswantini D, Purwaningsih H, et al. Formulation of Blumea balsamifera, Anredera cordifolia, and *Phyllanthus niruri* extracts as potential anti-inflammatory agents. Curr Issues Mol Biol. 2026;48(4):405.
20. Abdel-Naby DH, El-Sheikh MM, Abd El-Rahman SS, et al. GSK-3β/Notch-1 activation promotes radiation-induced renal damage: the role of gallic acid in mitigation of nephrotoxicity. Environ Toxicol. 2024;39(11):4871-4883.
21. Yufang W, Mingfang L, Nan H, et al. Quercetin-targeted AKT1 regulates the Raf/MEK/ERK signaling pathway to protect against doxorubicin-induced nephropathy in mice. Tissue Cell. 2023;85:102229.
22. Seker U, Kavak DE, Dokumaci FZ, et al. The nephroprotective effect of quercetin in cyclophosphamide-induced renal toxicity might be associated with MAPK/ERK and NF-κB signal modulation activity. Drug Chem Toxicol. 2024;47(6):1165-1174.
23. Mohsen AM, Wagdi MA, Salama A. Rutin loaded bilosomes for enhancing the oral activity and nephroprotective effects of rutin in potassium dichromate induced acute nephrotoxicity in rats. Sci Rep. 2024;14(1):23799.