

ORIGINAL RESEARCH ARTICLE

Hepato- and Immunoprotective Dynamics of Standardised *Andrographis paniculata* Extract in a Murine Polymicrobial Sepsis Model

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ABSTRACT

Background: Sepsis-associated liver injury drives mortality in polymicrobial sepsis yet lacks targeted adjunctive therapy. *Andrographis paniculata* (Burm. f.) Nees, known in Indonesia as sambiloto, yields andrographolide, a diterpene lactone that suppresses nuclear factor kappa B (NF-κB) and activates nuclear factor erythroid 2-related factor 2 (Nrf2) and haem oxygenase-1 (HO-1).

Objective: To evaluate standardised *A. paniculata* ethanolic extract (APE, 30% w/w andrographolide) in a murine caecal ligation and puncture (CLP) sepsis model.

Methods: Male BALB/c mice (n = 60) were randomised to Sham, CLP + vehicle, CLP + APE 100 mg/kg, CLP + APE 200 mg/kg, or CLP + imipenem/cilastatin 30 mg/kg (n = 12 per arm), treated at 1 h and 12 h post-CLP. Outcomes at 24 h were serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin; plasma tumour necrosis factor-α (TNF-α), interleukin (IL)-6 and IL-10; hepatic NF-κB, Nrf2, HO-1 and NOD-like receptor protein 3 (NLRP3); peritoneal bacterial load; and Suzuki histology, with survival followed to 72 h.

Results: APE 200 mg/kg reduced ALT by 58.4%, AST by 54.9%, TNF-α by 62.6% and IL-6 by 59.3%, and raised IL-10 2.3-fold (all p < 0.001). Hepatic p-NF-κB p65 fell 62%; nuclear Nrf2 and HO-1 rose 2.5- and 3.1-fold; peritoneal bacterial load fell 1.8 log₁₀; the Suzuki score fell from 8.4 to 3.2; and survival at 72 h rose from 25% to 67% (log-rank p < 0.001; hazard ratio 0.42).

Conclusion: Standardised APE attenuated hepatic injury and systemic hyperinflammation through dual NF-κB/Nrf2 modulation while preserving bacterial clearance, supporting its development as a complementary adjunct to antimicrobial therapy.

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

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1. Introduction

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection and currently accounts for an estimated 11 million deaths worldwide each year, approximately 19.7% of all global deaths.¹ In Indonesia and the wider Southeast Asian region the burden is particularly heavy: critical-care cohorts continue to report high case-fatality

among patients admitted with sepsis and septic shock, well in excess of high-income-country benchmarks, and the majority of cases arise from community-acquired pneumonia, urinary tract infection and intra-abdominal infections with secondary peritonitis.² Despite decades of refinement in bundle-based management articulated by the Surviving Sepsis Campaign, mortality has plateaued, and incremental gains from antimicrobial escalation have been largely

counterbalanced by the relentless global rise in antimicrobial resistance.^{3,4} Adjunctive therapies that modulate the host inflammatory response without compromising pathogen clearance therefore remain a pressing unmet need.

Among the organs injured during sepsis, the liver occupies a uniquely bidirectional role: it acts as the principal guardian against circulating pathogen-associated molecular patterns via Kupffer cells, yet it is also a prominent target of inflammatory injury, microcirculatory dysfunction, hepatocyte apoptosis and cholestasis.⁵ Sepsis-associated liver injury (SALI), classically defined by transaminitis, hyperbilirubinaemia and prolongation of the international normalised ratio, is observed in up to 60% of intensive-care-unit (ICU) sepsis admissions and is an independent predictor of 28-day mortality. At the molecular level SALI is driven by nuclear factor kappa B (NF- κ B) activation in hepatocytes and Kupffer cells, overproduction of tumour necrosis factor- α (TNF- α) and interleukin-6 (IL-6), activation of the NOD-like receptor protein 3 (NLRP3) inflammasome, depletion of hepatic glutathione (GSH), and loss of Kelch-like ECH-associated protein 1–nuclear factor erythroid 2-related factor 2 (Keap1–Nrf2) antioxidant signalling.⁶ Therapeutic approaches targeting these nodes simultaneously — suppressing NF- κ B while restoring Nrf2 and haem oxygenase-1 (HO-1) tone — are conceptually appealing but have struggled to yield clinically useful molecules.

Andrographis paniculata (Burm. f.) Nees (*Acanthaceae*), known in Indonesia as sambiloto, is one of the best-characterised Indonesian medicinal herbs, with an ethnomedicinal record extending across Java, Sumatra and Sulawesi for the management of fever, jaundice, hepatitis and infections.^{7,8} Monographs for Herba *Andrographidis* appear in the Indonesian Materia Medika, the Pharmacopoeia Indonesia and the 2017 Formularium Obat Herbal Asli Indonesia, with a minimum andrographolide content of 1% w/w typically required. The aerial parts yield a complex phytochemical matrix dominated by *ent-labdane* diterpene lactones — andrographolide, 14-deoxyandrographolide, neoandrographolide and 14-deoxy-11,12-didehydroandrographolide — accompanied by flavonoid glycosides and phenolic acids.⁹ Andrographolide, the principal bioactive compound, contains a reactive α,β -unsaturated- γ -butyrolactone moiety that covalently binds cysteine 62 of the NF- κ B p50 subunit, thereby suppressing transcription of TNF- α , IL-6 and inducible nitric oxide synthase; it also activates the Keap1–Nrf2 axis by cysteine modification, upregulating haem oxygenase-1

and NAD(P)H:quinone oxidoreductase-1 (NQO-1), and inhibits NLRP3 inflammasome assembly.^{10–12} Recent preclinical studies have demonstrated that andrographolide attenuates hepatic injury and the systemic cytokine storm in sepsis models, and that it enhances — rather than impairs — bacterial clearance in intra-abdominal (caecal ligation and puncture) sepsis.^{13,14}

Nevertheless, three evidence gaps persist. First, most published preclinical studies have used purified andrographolide rather than the standardised whole ethanolic extract used in clinical and traditional Indonesian practice, leaving the contribution of minor diterpenes, flavonoids and phenolic acids insufficiently characterised. Second, the integration of hepatic biochemistry, systemic cytokines, pathogen clearance and survival within a single framework compliant with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 guidelines is uncommon. Third, despite the importance of *A. paniculata* to the Indonesian herbal economy, Indonesian chemotypes remain under-represented in the international pharmacology literature, and validated chromatographic standardisation of andrographolide content is essential to anchor any formulation-development programme.¹⁵ Several reports have also raised the concern that excessive NF- κ B inhibition might compromise bacterial clearance; any candidate adjunct must therefore be evaluated in a polymicrobial infection model where both immunomodulation and pathogen control can be measured simultaneously.¹⁴

The ethnopharmacological heritage of sambiloto in Indonesia is extensive, encompassing Javanese *jamu* formulations for febrile illness, Sundanese decoctions for jaundice and hepatitis, and Batak and Minangkabau preparations for typhoid fever and urinary infections; these indications overlap substantially with the microbial aetiologies that precipitate polymicrobial sepsis in Indonesian ICUs. In the modern Indonesian herbal-medicine industry sambiloto is one of the most prescribed monographic herbs, with phytopharmaca formulations licensed by the Indonesian National Agency of Drug and Food Control (BPOM) marketed for immune support, and randomised clinical evidence supports its short-term safety in acute infection.¹⁶

Against this backdrop, the novelty of the present study lies in four features: using a fully standardised Indonesian ethanolic APE with validated 30% andrographolide content; evaluating it in a CLP model that reproduces the polymicrobial insult and secondary peritonitis seen clinically; integrating hepatic biochemistry, histology, signalling pathways,

systemic cytokines, peritoneal bacterial load and survival to 72 h into a single framework; and benchmarking against imipenem/cilastatin to demonstrate an additive rather than antagonistic effect. The aim of this study was to determine the hepato- and immunoprotective dynamics of standardised APE in a murine polymicrobial sepsis model.

2. Methods

2.1. Study design and setting

A controlled *in vivo* preclinical experimental study was conducted at the Eureka Research Laboratory, Palembang, Indonesia, between February and November 2024, and reported in accordance with the ARRIVE 2.0 guidelines. Ethical approval and animal-welfare compliance are set out in full under Declarations.

2.2. Plant material and extract preparation

Aerial parts (Herba *Andrographidis*) of *A. paniculata* were collected at the flowering stage from a certified medicinal-plant farm in Karanganyar, Central Java (7°37'S, 110°57'E); voucher specimens were authenticated and deposited at the Eureka herbarium, Indonesia (voucher no. AP-2024-033). The dried, milled herb (1 kg) was macerated in 70% ethanol (v/v) for 72 h at 25 °C, filtered, concentrated in a rotary evaporator (45 °C, 50 mbar) and lyophilised (yield 12.8% w/w). Standardisation used high-performance liquid chromatography (HPLC) with ultraviolet detection at 225 nm on a C18 column (Phenomenex Luna, 150 × 4.6 mm, 5 µm) with acetonitrile–water (35:65, v/v) at 1.0 mL/min against andrographolide, 14-deoxyandrographolide and neoandrographolide standards (Sigma-Aldrich).

2.3. Animals and randomisation

Male BALB/c mice (8–10 weeks, 22–28 g) were acclimatised for seven days at 22 ± 2 °C, 55 ± 10% relative humidity and a 12-h light/dark cycle with *ad libitum* sterile food and water. They were then randomised to five arms by computer-generated block sequence (block size 5) by a data manager independent of outcome assessors: Sham; CLP + vehicle 10 mL/kg; CLP + APE 100 mg/kg; CLP + APE 200 mg/kg; and CLP + imipenem/cilastatin 30 mg/kg (n = 12 each). Allocation was concealed by coded cage labels and all outcome assessors were blinded until data lock.

2.4. Sample size

Sample size was determined *a priori* using G*Power 3.1.9.7 for a one-way analysis of variance (ANOVA; five groups, f = 0.55, α = 0.05, power = 0.90) based on the

hepatic-injury data of comparable andrographolide CLP studies,¹³ returning a minimum of 10 per arm; 12 per arm were enrolled to accommodate the anticipated 15% peri-operative mortality.

2.5. Sepsis induction

Polymicrobial sepsis with secondary peritonitis was induced by caecal ligation and puncture (CLP) following the standardised method of Rittirsch and colleagues.¹⁷ Under isoflurane anaesthesia, a 1 cm midline laparotomy was performed, the caecum ligated to 50% of its length with 4-0 silk below the ileocaecal valve and punctured once through-and-through with a 21-gauge needle. Sham animals underwent identical laparotomy without ligation or puncture. All mice received subcutaneous warmed saline and buprenorphine 0.05 mg/kg. Treatments were given intraperitoneally at 1 h and 12 h after CLP; APE was dissolved in 5% dimethyl sulfoxide (DMSO) in sterile saline (vehicle).

2.6. Outcome measurements

All 12 animals per arm entered survival follow-up to 72 h. At the protocol-specified 24-h timepoint, 6 animals per arm (a pre-randomised subset surviving to 24 h) were humanely killed for terminal sampling and treated as censored in the survival analysis. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin were measured on a Hitachi 7180 analyser; plasma TNF-α, IL-6 and IL-10 by enzyme-linked immunosorbent assay (ELISA; R&D Systems DuoSet) in duplicate. Liver tissue was processed for histology, for malondialdehyde (MDA), GSH and superoxide dismutase (SOD) assays, and for Western blotting of p-NF-κB p65, IκBα, nuclear Nrf2, HO-1 and NLRP3. Hepatic injury was scored (Suzuki, 0–12) by a blinded pathologist. Peritoneal lavage was plated on tryptic soy agar for colony-forming-unit (CFU) enumeration. The remaining 6 animals per arm were followed to 72 h with 4-hourly welfare scoring; humane endpoints were a clinical-severity score ≥ 4 or ≥ 20% weight loss.

2.7. Statistical analysis

Continuous variables are mean ± standard deviation (SD). Between-group comparisons used one-way ANOVA with Tukey's honestly significant difference (HSD) test, or the Kruskal–Wallis test with Dunn correction. Effect sizes are Hedges' g with bootstrap 95% confidence intervals (CI). Survival was analysed by Kaplan–Meier (log-rank) and Cox proportional-hazards models. Multiplicity used gatekeeping on the primary endpoint (ALT) with hierarchical testing; exploratory outcomes used the Benjamini–Hochberg

false-discovery rate (FDR). Analyses used R 4.3.1, SPSS 28 and GraphPad Prism 9.5; two-sided $\alpha = 0.05$.

3. Results

3.1. Baseline characteristics and feasibility

All 60 animals completed randomisation, surgery and treatment with no first-hour mortality. Baseline body

mass, age, biochemistry and cytokines were balanced across the five arms (Table 1; $p > 0.05$). By 24 h, body-mass loss was $11.8 \pm 2.4\%$ in CLP + vehicle and $5.4 \pm 1.5\%$ in APE 200 mg/kg, consistent with mid-grade CLP.¹⁷

Table 1. Baseline characteristics of the experimental cohort (n = 60 mice).

Characteristic	Sham	CLP + vehicle	APE 100	APE 200	Imipenem
Body mass (g)	25.1 $\pm$ 1.6	25.4 $\pm$ 1.7	24.8 $\pm$ 1.8	25.2 $\pm$ 1.5	25.0 $\pm$ 1.6
Age (weeks)	9.1 $\pm$ 0.5	9.0 $\pm$ 0.6	9.2 $\pm$ 0.4	9.1 $\pm$ 0.5	9.0 $\pm$ 0.5
Baseline ALT (U/L)	42.3 $\pm$ 5.1	41.8 $\pm$ 5.6	41.6 $\pm$ 5.4	42.1 $\pm$ 5.8	41.4 $\pm$ 5.2
Baseline AST (U/L)	82.5 $\pm$ 8.4	83.1 $\pm$ 8.7	82.8 $\pm$ 8.5	82.4 $\pm$ 8.9	82.1 $\pm$ 8.3
Baseline TNF- α (pg/mL)	18.2 $\pm$ 4.0	18.6 $\pm$ 4.2	18.4 $\pm$ 3.9	18.3 $\pm$ 4.1	18.5 $\pm$ 4.0
Baseline hepatic GSH (nmol/mg)	14.1 $\pm$ 1.5	14.3 $\pm$ 1.4	14.0 $\pm$ 1.6	14.2 $\pm$ 1.5	14.1 $\pm$ 1.4
Successful operation, n (%)	12 (100)	12 (100)	12 (100)	12 (100)	12 (100)
Weight loss at 24 h (%)	2.1 $\pm$ 0.8	11.8 $\pm$ 2.4	7.6 $\pm$ 1.9	5.4 $\pm$ 1.5	5.1 $\pm$ 1.4

Means $\pm$ SD; categorical values are n (%). No significant between-arm differences in the baseline rows ($p > 0.05$ by ANOVA or χ^2); the final row is a 24-h outcome and differs between arms. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GSH, glutathione.

3.2. Phytochemical standardisation

HPLC quantification of the extract batch used throughout the study yielded andrographolide $30.4 \pm$

0.8% w/w, 14-deoxyandrographolide $6.2 \pm 0.3\%$ and neoandrographolide $3.1 \pm 0.2\%$ (Table 2). These values exceed the minimum 1% andrographolide specified in the Indonesian Materia Medika.

Table 2. Phytochemical profile of the standardised *Andrographis paniculata* ethanolic extract (APE).

Constituent	Content (% w/w)
Andrographolide	30.4 $\pm$ 0.8
14-Deoxyandrographolide	6.2 $\pm$ 0.3
Neoandrographolide	3.1 $\pm$ 0.2

Single-batch assay values determined by HPLC with ultraviolet detection at 225 nm; means $\pm$ SD of triplicate injections.

3.3. Hepatic biochemistry

CLP + vehicle produced marked transaminitis and hyperbilirubinaemia at 24 h (ALT 248.7 ± 28.4 U/L, AST 398.6 ± 39.2 U/L, bilirubin 2.14 ± 0.32 mg/dL), a six- to seven-fold rise over Sham (Figure 1A–B; Table

3). APE 100 mg/kg reduced ALT to 148.6 ± 18.2 U/L (40.2%, $p = 0.003$) and APE 200 mg/kg to 103.5 ± 14.1 U/L (58.4%, 95% CI 51.2–65.6; $p < 0.001$); AST fell in parallel by 54.9% (Hedges' $g = -6.47$). The APE 200 versus imipenem difference was not significant ($p = 0.42$).

Table 3. Primary outcomes at 24 h post-CLP.

Variable	Sham	CLP + vehicle	APE 100	APE 200	Imipenem
Serum ALT (U/L)	41.2 $\pm$ 5.8	248.7 $\pm$ 28.4	148.6 $\pm$ 18.2**	103.5 $\pm$ 14.1***§	96.8 $\pm$ 13.6***
Serum AST (U/L)	82.4 $\pm$ 9.1	398.6 $\pm$ 39.2	248.1 $\pm$ 24.8**	179.8 $\pm$ 20.3***§	168.4 $\pm$ 19.1***
Total bilirubin (mg/dL)	0.31 $\pm$ 0.08	2.14 $\pm$ 0.32	1.32 $\pm$ 0.24*	0.86 $\pm$ 0.18***§	0.78 $\pm$ 0.16***
Plasma TNF- α (pg/mL)	18.4 $\pm$ 4.1	412.8 $\pm$ 41.6	256.1 $\pm$ 26.4**	154.2 $\pm$ 18.7***§	138.6 $\pm$ 16.8***
Plasma IL-6 (pg/mL)	22.1 $\pm$ 4.8	385.2 $\pm$ 36.4	241.8 $\pm$ 24.1**	156.9 $\pm$ 18.4***§	142.3 $\pm$ 17.2***
Plasma IL-10 (pg/mL)	48.6 $\pm$ 7.1	82.4 $\pm$ 11.6	146.8 $\pm$ 15.4**	189.5 $\pm$ 17.8***	112.3 $\pm$ 12.9**
Hepatic MDA (nmol/mg)	1.21 $\pm$ 0.18	4.86 $\pm$ 0.51	3.02 $\pm$ 0.34**	2.12 $\pm$ 0.26***	2.28 $\pm$ 0.28***
Hepatic GSH (nmol/mg)	14.2 $\pm$ 1.5	4.82 $\pm$ 0.71	8.94 $\pm$ 1.12**	11.38 $\pm$ 1.24***	9.18 $\pm$ 1.08**
Hepatic SOD (U/mg)	26.4 $\pm$ 2.8	10.1 $\pm$ 1.6	17.6 $\pm$ 1.9**	21.8 $\pm$ 2.1***	18.4 $\pm$ 2.0**

Variable	Sham	CLP + vehicle	APE 100	APE 200	Imipenem
Peritoneal bacterial load (log ₁₀ CFU/mL)	< 1.0	7.82 ± 0.41	6.98 ± 0.46**	6.02 ± 0.42***	4.74 ± 0.38***
Suzuki injury score (0–12)	0.3 ± 0.5	8.4 ± 1.2	5.6 ± 1.1**	3.2 ± 0.9***§	3.6 ± 1.0***

Means ± SD, n = 6 per arm (24-h cohort). *p < 0.05, **p < 0.01, ***p < 0.001 versus CLP + vehicle (ANOVA, Tukey's HSD); §non-significant versus imipenem. MDA, malondialdehyde; SOD, superoxide dismutase; CFU, colony-forming units.

3.4. Systemic cytokine dynamics

Plasma TNF-α rose to 412.8 ± 41.6 pg/mL, a 22-fold increase over Sham (Figure 1C; Table 3), and IL-6 to 385.2 ± 36.4 pg/mL, a 17-fold increase (Table 3). APE

200 mg/kg reduced TNF-α by 62.6% and IL-6 by 59.3% (both p < 0.001; Hedges' g = -7.40 and -7.31). IL-10 rose from 82.4 ± 11.6 to 189.5 ± 17.8 pg/mL (2.3-fold, p < 0.001; Figure 1D), whereas imipenem produced a smaller IL-10 elevation (112.3 ± 12.9 pg/mL).

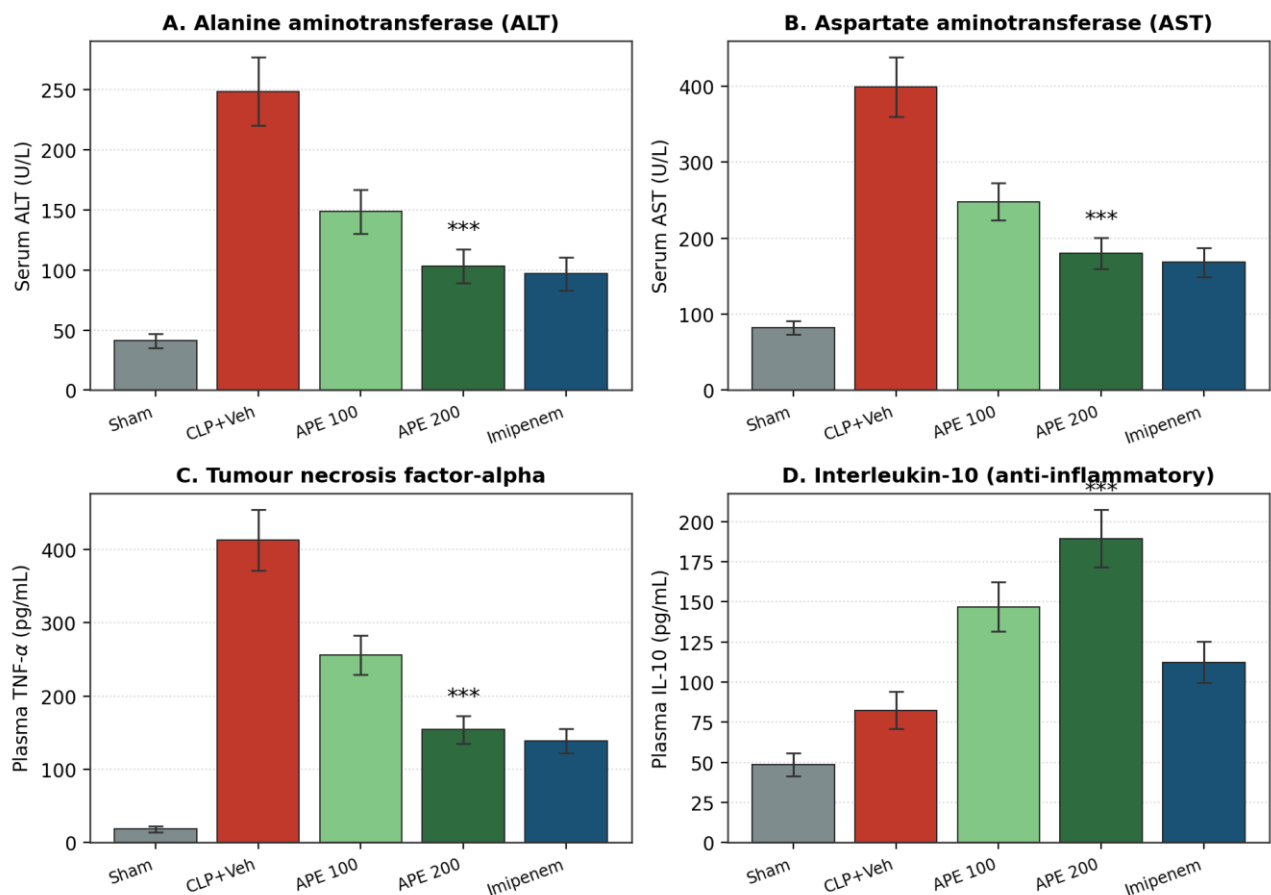


Figure 1. Hepatic and cytokine biomarker changes at 24 h after CLP (n = 6 per arm; means ± SD). (A) serum ALT, (B) serum AST, (C) plasma TNF-α and (D) plasma IL-10. *p < 0.001 versus CLP + vehicle (one-way ANOVA, Tukey's HSD).

3.5. Hepatic oxidative stress

Hepatic MDA rose four-fold in CLP + vehicle and fell by 56.4% at APE 200 mg/kg (p < 0.001), while GSH and SOD were partially restored, by 2.4- and 2.2-fold respectively (Table 3).

3.6. Hepatic signalling pathways

Western-blot analysis demonstrated the dual NF-κB/Nrf2 mechanism directly (Table 4; Figure 2).

Hepatic p-NF-κB p65 fell to 0.38 ± 0.08-fold of CLP + vehicle (62% reduction, p < 0.001) while IκBα was restored to 1.85 ± 0.21-fold. Nuclear Nrf2 rose 2.5-fold and HO-1 3.1-fold (both p < 0.001). NLRP3 and cleaved caspase-1 fell to 0.52 and 0.48 of the reference. Imipenem produced only marginal p-NF-κB p65 suppression (0.74 ± 0.12-fold).

Table 4. Hepatic signalling outcomes, inflammasome activity and survival.

Outcome	Sham	CLP + vehicle	APE 200	Imipenem	p-value
Hepatic p-NF-κB p65 (fold)	0.31 ± 0.06	1.00 ± 0.14	0.38 ± 0.08***	0.74 ± 0.12***	< 0.001
Hepatic IκBα (fold)	1.82 ± 0.18	1.00 ± 0.12	1.85 ± 0.21***	1.15 ± 0.16	< 0.001
Nuclear Nrf2 (fold)	1.42 ± 0.16	1.00 ± 0.12	2.46 ± 0.28***	1.28 ± 0.16	< 0.001
Hepatic HO-1 (fold)	1.21 ± 0.14	1.00 ± 0.12	3.12 ± 0.32***	1.41 ± 0.18	< 0.001
Hepatic NLRP3 (fold)	0.38 ± 0.07	1.00 ± 0.13	0.52 ± 0.10***	0.88 ± 0.12	< 0.001
Cleaved caspase-1 (fold)	0.41 ± 0.08	1.00 ± 0.14	0.48 ± 0.09***	0.82 ± 0.12	< 0.001
72-h survival (Kaplan-Meier, %)	100	25	67§	75	< 0.001
Hazard ratio for death (95% CI)	—	1.00 (ref)	0.42 (0.21–0.84)§	0.31 (0.14–0.69)	< 0.001
Mean survival time (h)	72.0 ± 0.0	34.8 ± 17.2	60.4 ± 14.8§	64.8 ± 12.6	< 0.001

Protein densitometry is fold change relative to CLP + vehicle (= 1.00). Means ± SD. ***p < 0.001 versus CLP + vehicle; §non-significant versus imipenem. HR, hazard ratio versus CLP + vehicle (Cox model).

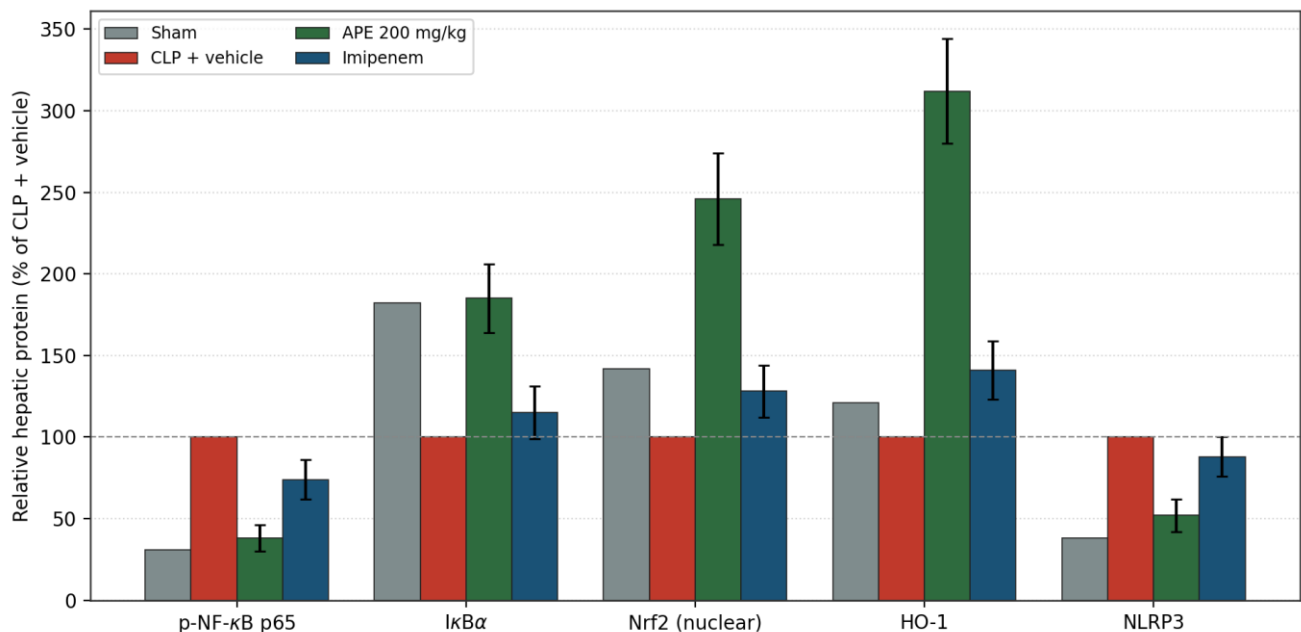


Figure 2. Hepatic NF-κB, Nrf2, HO-1 and NLRP3 signalling by Western-blot densitometry, expressed as a percentage of the CLP + vehicle reference (dashed line, 100%) and equivalent to the fold changes in Table 4. *A. paniculata* extract suppressed p-NF-κB p65 and NLRP3 while elevating IκBα, nuclear Nrf2 and HO-1.

3.7. Peritoneal bacterial burden

Peritoneal lavage at 24 h yielded $7.82 \pm 0.41 \log_{10}$ CFU/mL in CLP + vehicle (Table 3). APE 100 and 200 mg/kg achieved 0.84 and 1.80 $\log_{10}$ reductions (both $p \leq 0.001$); imipenem achieved 3.08 $\log_{10}$. APE did not increase bacterial load at either dose.

3.8. Histological hepatic injury

The blinded Suzuki score rose from 0.3 ± 0.5 (Sham) to 8.4 ± 1.2 (CLP + vehicle); APE 200 mg/kg reduced it to

3.2 ± 0.9 (61.9%, $p < 0.001$) and imipenem to 3.6 ± 1.0 (Table 3).

3.9. Effect-size synthesis

Figure 3 summarises the standardised mean differences (APE 200 versus CLP + vehicle) across ten outcomes plus the mortality hazard ratio. Effect sizes consistently exceeded a Hedges' g of $|4.0|$, with all 95% CIs excluding zero. The hazard ratio of 0.42 (95% CI 0.21–0.84) corresponds to a 58% reduction in the instantaneous risk of death over 72 h.

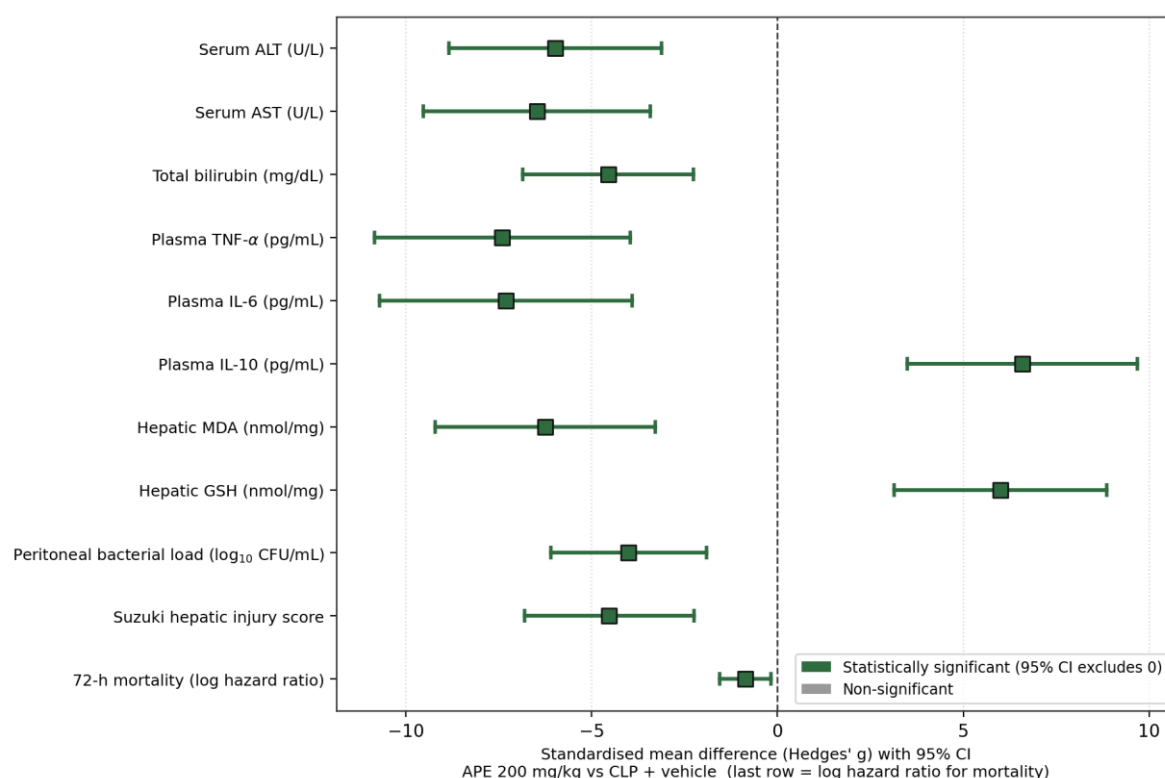


Figure 3. Forest plot of standardised mean differences (Hedges' g, 95% CI) for APE 200 mg/kg versus CLP + vehicle across ten outcomes; the final row shows the log hazard ratio for mortality at 72 h. Green markers denote statistically significant effects (95% CI excludes 0).

3.10. Survival analysis

Kaplan–Meier curves are shown in Figure 4. Survival at 72 h was 100% (Sham), 25% (CLP + vehicle), 58% (APE 100 mg/kg), 67% (APE 200 mg/kg) and 75% (imipenem; Table 4).

The log-rank test gave $p < 0.001$; Cox hazard ratios were 0.42 (0.21–0.84) for APE 200 and 0.31 (0.14–0.69) for imipenem, the APE 200 versus imipenem contrast being non-significant ($p = 0.14$). Schoenfeld residuals confirmed proportional hazards.

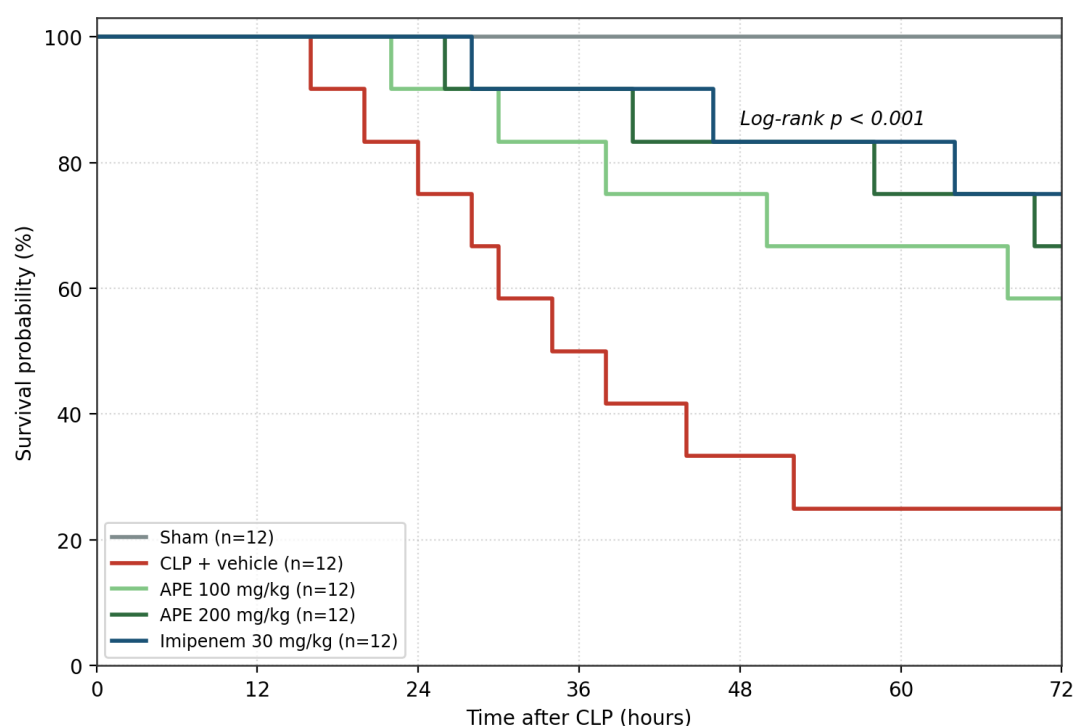


Figure 4. Kaplan–Meier survival over 72 h after CLP for the five arms ($n = 12$ per arm). Survival at 72 h was 100% (Sham), 25% (CLP + vehicle), 58% (APE 100 mg/kg), 67% (APE 200 mg/kg) and 75% (imipenem); log-rank $p < 0.001$.

4. Discussion

4.1. Hepatoprotection and convergence with antimicrobial therapy

The absence of a significant difference between APE 200 mg/kg and imipenem on the hepatic-biochemistry endpoints indicates convergent hepatic benefit achieved through different mechanisms, consistent with the hepatoprotection reported for andrographolide in sepsis-induced acute liver injury.¹³ Where imipenem acts by removing the microbial stimulus, APE acts on the host response itself, and the two therefore address complementary arms of the same pathophysiology.

4.2. Cytokine modulation and the IL-10 dissociation

The magnitude of TNF- α and IL-6 suppression achieved with APE 200 mg/kg is comparable to the cytokine suppression reported for andrographolide in lipopolysaccharide (LPS)-stimulated macrophages.¹⁸ The contrast between the 2.3-fold IL-10 rise under APE and the smaller elevation under imipenem is a clear dissociation between antimicrobial and immunomodulatory protection, consistent with the PPAR γ -linked promotion of IL-10 by andrographolide.⁹

4.3. Oxidative stress and the Keap1–Nrf2 axis

The phytochemical basis of the oxidative-stress findings is cysteine-directed modification of Keap1 by the α,β -unsaturated lactone of andrographolide, releasing Nrf2 to upregulate HO-1, NQO-1 and glutamate–cysteine ligase.^{11,12} The same Nrf2/HO-1 axis underlies the antioxidant protection of other natural products in sepsis-induced liver injury,¹⁹ and the magnitude of MDA reduction parallels the antioxidant rescue reported for andrographolide in sepsis-associated acute kidney injury.²⁰

4.4. Dual NF- κ B/Nrf2 signalling and inflammasome suppression

The concurrent fall in NLRP3 and cleaved caspase-1 is consistent with andrographolide-mediated NLRP3 suppression in sepsis-associated acute lung injury and in andrographolide cardioprotection.^{21,22} The marginal p-NF- κ B p65 suppression under imipenem indicates that its hepatic benefit is mediated mainly by pathogen-load reduction, whereas APE engages the transcriptional programme directly.

4.5. Immunomodulation without loss of pathogen control

APE did not increase bacterial load at either dose, confirming that its immunomodulation remained

compatible with pathogen clearance — in agreement with the observation that andrographolide enhances bacterial clearance in intra-abdominal sepsis.¹⁴ This addresses directly the concern that excessive NF- κ B inhibition might compromise host defence, and it is the principal safety argument for developing APE as an adjunct rather than a substitute.

4.6. Phytochemical–mechanistic integration

The 62% reduction in p-NF- κ B p65 and the 2.5-fold rise in nuclear Nrf2 are quantitatively consistent with the hepatoprotective signalling of andrographolide in sepsis-induced acute liver injury,¹³ and the IL-10 elevation aligns with its macrophage-directed anti-inflammatory action.¹⁸ The whole extract likely acts through a phytochemical triad: andrographolide drives NF- κ B inhibition and Nrf2 activation by direct cysteine modification, 14-deoxyandrographolide adds antioxidant activity, and neoandrographolide augments immunomodulation through PPAR γ . This co-action explains why 200 mg/kg of extract, delivering approximately 60 mg/kg andrographolide, produced effects similar to higher nominal doses of the pure compound.

4.7. Comparison with published research

Andrographolide ameliorates sepsis-induced acute liver injury and activates Nrf2-dependent antioxidant signalling;¹³ the present study observed similar transaminase reductions (58% ALT, 55% AST) using the whole extract. Andrographolide attenuates intra-abdominal sepsis while enhancing bacterial clearance;¹⁴ the reduced peritoneal bacterial load and Suzuki necrosis observed here (from 8.4 to 3.2) are consistent. Andrographolide also inhibits ferroptosis via Nrf2 in sepsis-associated acute kidney injury;²⁰ the present hepatic MDA reduction (from 4.86 to 2.12 nmol/mg) and GSH restoration indicate that the same axis operates in the liver. Linking NLRP3 suppression to concomitant NF- κ B inhibition and Nrf2 activation within one organ lends the mechanism greater explanatory power than single-pathway cardiac or pulmonary studies.^{21,22}

4.8. Indonesian and Asian herbal context

Sambiloto is listed in the Pharmacopoeia Indonesia, the Indonesian Materia Medika and the Formularium Obat Herbal Asli Indonesia 2017 (andrographolide $\geq$ 1% w/w; commercial standardised products now target 30%). Beyond Indonesia, Thailand includes sambiloto in its National List of Essential Medicines for acute respiratory infection, and randomised clinical evidence and meta-analysis support its short-term safety,¹⁶ positioning APE as a candidate for

phytopharmaca development under BPOM Regulation No. 32/2019.

4.9. Safety considerations

APE 200 mg/kg produced no macroscopic or microscopic nephro-, spleno- or cardiotoxicity in the 24-h panel, and body-mass recovery paralleled imipenem. Published pharmacology and clinical-safety reviews document acceptable bioavailability and short-term safety of standardised *A. paniculata* and andrographolide.^{9,16} Nonetheless, andrographolide inhibits CYP1A2 and CYP2C9, so interactions with anticoagulants and P-glycoprotein substrates common in ICU settings must be addressed prospectively.

4.10. Translational pathway

A phase Ib tolerability and pharmacokinetic trial in healthy Indonesian volunteers (human-equivalent dose approximately 16 mg/kg, or 900–1200 mg twice daily) is feasible given the existing safety literature.^{9,16} This would be followed by a phase IIa randomised trial of APE as an add-on to standard antimicrobial therapy in adult intra-abdominal sepsis, using ALT trajectory, cytokine kinetics and the Sequential Organ Failure Assessment (SOFA) score as surrogate endpoints and 28-day mortality as a secondary endpoint, with drug-interaction monitoring. Registration would follow the Indonesian *Obat Herbal Terstandar to Fitofarmaka* progression under BPOM Regulation No. 32/2019.

4.11. Strengths and limitations

Strengths include a fully standardised HPLC-validated extract, an ARRIVE-2.0-compliant pre-registered blinded design, and integration of biochemical, immunological, signalling, microbiological and survival endpoints. Limitations are the 72 h window, which cannot capture late persistent inflammation, immunosuppression and catabolism syndrome (PICS); the abdominal-only source of the CLP model; and reliance on Western-blot densitometry that should be corroborated by conditional-knockout approaches.

5. Conclusion

A standardised Indonesian *A. paniculata* ethanolic extract (30% andrographolide) protected the liver and modulated the systemic inflammatory response in murine polymicrobial sepsis. APE 200 mg/kg reduced ALT by 58.4%, AST by 54.9%, TNF- α by 62.6% and IL-6 by 59.3%, raised IL-10 2.3-fold, restored hepatic glutathione, suppressed NF- κ B p65 phosphorylation by 62%, upregulated Nrf2 2.5-fold and HO-1 3.1-fold, attenuated the NLRP3 inflammasome and improved survival at 72 h from 25% to 67% (log-rank $p < 0.001$), without impairing pathogen clearance. The mechanism

that best fits the data is the dual action of andrographolide on NF- κ B, through covalent inhibition by the α,β -unsaturated lactone, and on Keap1-Nrf2, through cysteine modification, augmented by 14-deoxyandrographolide and neoandrographolide. These findings support standardised APE as a credible complementary adjunct to antimicrobial therapy in sepsis-associated liver injury; a phase II randomised trial with integrated biochemical, immunological and safety monitoring is the recommended next step.

Declarations

Use of Generative Artificial Intelligence. The authors declare that no generative artificial intelligence tool was used in the conception, design, conduct, analysis or interpretation of this study, nor to generate any data, figure or reference. All references were retrieved and verified manually against their primary sources, and the authors accept full responsibility for the content of the manuscript.

Author Contributions. S.A. conceived and designed the study, supervised the animal experiments and the extract standardisation, performed the statistical analysis, and drafted the manuscript. S. contributed to the plant authentication, extract preparation and interpretation of the phytochemical data, and critically revised the manuscript. K.J. supervised the chromatographic standardisation and the biochemical assays, and revised the manuscript. D.H.M. contributed to the study design, the histopathological and survival analyses, and the critical revision of the manuscript. All authors have reviewed and approved the final version of the manuscript.

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Conflict of Interest. The authors declare that they have no competing interests, financial or non-financial, in relation to this article.

Ethical Approval. Ethical approval was obtained from the CMHC Research Center Ethics Committee (Approval No. 2024/204). All procedures complied with the Guide for the Care and Use of Laboratory Animals, 8th edition, and the Indonesian Basic Principles for Animal Research (2018). Reporting follows the ARRIVE 2.0 guidelines.

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

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References

- Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. *Lancet*. 2020;395(10219):200–211. doi:10.1016/S0140-6736(19)32989-7
- Rusmawatiningsy D, Oktaria V, Pudjiadi AH, Makrufardi F, van Woensel JBM. Clinical characteristics and outcome of critically ill children referred to a tertiary hospital in Indonesia: a prospective observational study. *BMC Pediatr*. 2024;24(1):478. doi:10.1186/s12887-024-04940-7
- Evans L, Rhodes A, Alhazzani W, et al. Surviving Sepsis Campaign: international guidelines for management of sepsis and septic shock 2021. *Intensive Care Med*. 2021;47(11):1181–1247. doi:10.1007/s00134-021-06506-y
- Naghavi M, Vollset SE, Ikuta KS, et al. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. *Lancet*. 2024;404(10459):1199–1226. doi:10.1016/S0140-6736(24)01867-1
- Gao Y, Tian X, Zhang X, et al. The roles of tissue-resident macrophages in sepsis-associated organ dysfunction. *Heliyon*. 2023;9(11):e21391. doi:10.1016/j.heliyon.2023.e21391
- Huo L, Liu C, Yuan Y, Liu X, Cao Q. Pharmacological inhibition of ferroptosis as a therapeutic target for sepsis-associated organ damage. *Eur J Med Chem*. 2023;257:115438. doi:10.1016/j.ejmech.2023.115438
- Kumar S, Singh B, Bajpai V. *Andrographis paniculata* (Burm.f.) Nees: traditional uses, phytochemistry, pharmacological properties and quality control/quality assurance. *J Ethnopharmacol*. 2021;275:114054. doi:10.1016/j.jep.2021.114054
- Hossain S, Urbi Z, Karuniawati H, et al. *Andrographis paniculata* (Burm. f.) Wall. ex Nees: an updated review of phytochemistry, antimicrobial pharmacology, and clinical safety and efficacy. *Life (Basel)*. 2021;11(4):348. doi:10.3390/life11040348
- Zeng B, Wei A, Zhou Q, et al. Andrographolide: a review of its pharmacology, pharmacokinetics, toxicity and clinical trials and pharmaceutical researches. *Phytother Res*. 2022;36(1):336–364. doi:10.1002/ptr.7324
- Jain P, Sudandiradoss C. Andrographolide-based potential anti-inflammatory transcription inhibitors against nuclear factor NF- κ B p50 subunit: an integrated molecular and quantum-mechanical approach. *3 Biotech*. 2023;13(1):15. doi:10.1007/s13205-022-03431-9
- Agrawal P, Nair MS. An insight into the pharmacological and analytical potential of andrographolide. *Fundam Clin Pharmacol*. 2022;36(4):586–600. doi:10.1111/fcp.12757
- Prasertsuksri P, Kraokaew P, Pranweerapaiboon K, Sobhon P, Chaithirayanon K. Neuroprotection of andrographolide against neurotoxin MPP⁺-induced apoptosis in SH-SY5Y cells via activating mitophagy, autophagy, and antioxidant activities. *Int J Mol Sci*. 2023;24(10):8528. doi:10.3390/ijms24108528
- He J, Huang Z, Zou R. Andrographolide ameliorates sepsis-induced acute liver injury by attenuating endoplasmic reticulum stress through the FKBP1A-mediated NOTCH1/AK2 pathway. *Cell Biol Toxicol*. 2025;41(1):56. doi:10.1007/s10565-025-10007-9
- Yu L, Liu Y, Cao C, et al. Andrographolide attenuates inflammation due to intra-abdominal sepsis by enhancing bacterial clearance in mice. *J Inflamm Res*. 2023;16:4413–4423. doi:10.2147/JIR.S422342
- Jana SN, Banerjee S, Biswas S, Karati D, Pal TK. Quantification and standardization of andrographolide in *Andrographis paniculata* samples by validated RP-HPLC and HPTLC methods. *J Chromatogr Sci*. 2023;61(6):514–521. doi:10.1093/chromsci/bmad004
- Worakunphanich W, Thavorncharoensap M, Youngkong S, Thadanipon K, Thakkinstian A. Safety of *Andrographis paniculata*: a systematic review and meta-analysis. *Pharmacoepidemiol Drug Saf*. 2021;30(6):727–739. doi:10.1002/pds.5190
- Rittirsch D, Huber-Lang MS, Flierl MA, Ward PA. Immunodesign of experimental sepsis by cecal ligation and puncture. *Nat Protoc*. 2009;4(1):31–36. doi:10.1038/nprot.2008.214
- Low M, Suresh H, Zhou X, et al. The wide-spectrum anti-inflammatory activity of andrographolide in comparison to NSAIDs: a promising therapeutic compound against the cytokine storm. *PLoS One*. 2024;19(7):e0299965. doi:10.1371/journal.pone.0299965
- Dai JM, Guo WN, Tan YZ, et al. Wogonin alleviates liver injury in sepsis through Nrf2-mediated NF- κ B signalling suppression. *J Cell Mol Med*. 2021;25(12):5782–5798. doi:10.1111/jcmm.16604
- Zhang Y, Zeng Y, Huang M, et al. Andrographolide attenuates sepsis-induced acute kidney injury by inhibiting ferroptosis through the Nrf2/FSP1 pathway. *Free Radic Res*. 2024;58(3):156–169. doi:10.1080/10715762.2024.2330413
- Qin Y, Li W, Liu J, et al. Andrographolide ameliorates sepsis-induced acute lung injury by promoting autophagy in alveolar macrophages via the RAGE/PI3K/AKT/mTOR pathway. *Int Immunopharmacol*. 2024;139:112719. doi:10.1016/j.intimp.2024.112719
- Liu S, Liu J, Su N, et al. An integrated network-pharmacology and RNA-seq approach for exploring the protective effect of andrographolide in doxorubicin-induced cardiotoxicity. *Cardiovasc Drugs Ther*. 2025;39(3):517–531. doi:10.1007/s10557-024-07555-3