

Protective Role of *Caesalpinia bonduc* Leaf Extract Against Ampicillin-Triggered Hepatotoxicity in Rats: A Review

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ABSTRACT

*Ampicillin, a widely used β -lactam antibiotic, is generally safe but can infrequently produce drug-induced liver injury (DILI) ranging from mild transaminitis to clinically important hepatocellular or cholestatic damage. Botanicals with antioxidant, anti-inflammatory and membrane-stabilizing properties are promising as hepatoprotective agents. *Caesalpinia bonduc* (family Fabaceae) is a medicinal plant traditionally used for several ailments; phytochemical studies report flavonoids, tannins, saponins, alkaloids and terpenoids — constituents associated with antioxidative and hepatoprotective actions. Several experimental studies show *C. bonduc* extracts (seed and leaf, various solvents) reduce elevated serum transaminases and restore antioxidant enzyme levels in chemically induced hepatic injury models (CCl₄, paracetamol, ethanol, gentamicin). However, a focused evaluation of *C. bonduc* against antibiotic-triggered hepatotoxicity — specifically ampicillin — is lacking. This review synthesizes available phytochemical and pharmacological evidence, summarizes mechanisms by which ampicillin may cause hepatic injury, and proposes a rigorous rat model experimental protocol (dosing, controls, biomarkers, histopathology, statistical analysis) to assess hepatoprotection by *C. bonduc* leaf extract. We identify key experimental gaps and recommend standardized extraction, phytochemical fingerprinting (HPLC/MS), dose-response testing, and mechanistic assays (oxidative stress, inflammatory cytokines, apoptosis markers). The review aims to guide translational preclinical work that could support future clinical evaluation of *C. bonduc* as an adjuvant hepatoprotective agent during antibiotic therapy.*

Keywords: - *Caesalpinia bonduc*, hepatoprotective, ampicillin, drug-induced liver injury, rats, oxidative stress.

1. INTRODUCTION

Drug-induced liver injury (DILI) is a major cause of acute liver failure and remains a significant challenge in clinical practice and drug development, often leading to drug withdrawal and heightened regulatory oversight (1). DILI can result from intrinsic mechanisms, which are dose-dependent and predictable, or from idiosyncratic reactions, which are unpredictable and influenced by host susceptibility.

The pathogenesis frequently involves oxidative stress, where excessive reactive oxygen species (ROS) damage cellular lipids, proteins, and DNA, impair mitochondrial function, and trigger apoptotic or necrotic cell death (2, 3). In some cases, immune-mediated injury exacerbates hepatocellular damage, while certain drugs interfere with bile acid transport, causing cholestasis (4).

Antibiotics are among the most frequently implicated drug classes in DILI, reflecting their widespread use and diverse hepatotoxic mechanisms, including reactive metabolite formation, mitochondrial injury, and transporter inhibition. While amoxicillin–clavulanate is the most common cause of antibiotic-related cholestatic DILI, ampicillin-induced hepatotoxicity is relatively rare but documented, often presenting as hepatocellular or cholestatic injury with elevated aminotransferases and, in some cases, jaundice. Ampicillin-related DILI is generally idiosyncratic and may involve hypersensitivity-mediated immune mechanisms rather than direct toxicity. Animal models are valuable for exploring the mechanisms of ampicillin hepatotoxicity and testing hepatoprotective interventions, as they allow the evaluation of biochemical, histological, and molecular endpoints. Increasingly, plant-derived antioxidants and anti-inflammatory agents are being investigated for hepatoprotection in experimental settings, with several studies using models such as carbon tetrachloride (CCl₄), paracetamol, and gentamicin-induced injury. *Caesalpinia bonduc* (syn. *Caesalpinia bonducella*), a medicinal plant traditionally used in Ayurveda and other systems, has attracted attention for its potential hepatoprotective properties. Phytochemical analyses show that its seeds and leaves contain flavonoids, phenolics, fatty acids, and terpenoids with strong antioxidant and anti-inflammatory activity (5).

Experimental studies have demonstrated its efficacy in mitigating CCl₄-induced hepatotoxicity by lowering serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels, and restoring hepatic architecture (5). Similarly, methanolic leaf extracts have been shown to prevent gentamicin-induced liver injury, reduce oxidative stress, and preserve tissue integrity (6). The mechanistic overlap between oxidative stress, inflammation, and hepatotoxic pathways in both chemical and antibiotic-induced models provides a rationale for testing *C. bonduc* against ampicillin-induced liver injury. A well-designed preclinical study could involve adult Wistar rats allocated into control, ampicillin-only, pre-treatment, post-treatment, and plant-only groups. Key outcome measures would include liver function markers, oxidative stress indices, antioxidant enzyme activities, inflammatory cytokine levels, apoptotic markers, and histopathological changes. Such a study would provide insights into the efficacy, safety, and mechanism of *C. bonduc* in preventing antibiotic-related liver damage, potentially supporting its development as a phytotherapeutic adjunct in clinical settings.

2. BOTANICAL PROFILE AND TRADITIONAL USES OF CAESALPINIA BONDUC

Caesalpinia bonduc (syn. *Caesalpinia bonducella*), commonly referred to as fever nut or bonduc nut, is a sturdy, spiny climber—or liana—within the Fabaceae family that thrives across tropical and subtropical regions worldwide, including India, Sri Lanka, Southeast Asia, and various Pacific and African coastlines (7, 8). The plant sports bipinnate leaves approximately 30–60 cm in length, yellow flowers arranged in supra-axillary or terminal racemes, and smooth, glossy greyish seeds—typically one or two per spiky pod—that are buoyant enough to disperse via ocean currents (7 – 9).

Traditional medicinal systems, including Ayurveda and folk medicine, employ diverse plant parts—roots, bark, seeds, and leaves—for an extensive range of therapeutic purposes, which underscores its ethnomedical importance and justifies its modern experimental exploration. Specifically, *C. bonduc* is used as an antipyretic, febrifuge, tonic, and anthelmintic; roasted seed powder has served as a quinine substitute, while root bark decoctions are utilized to alleviate fever, intestinal parasites, cough, tumors, menstrual disorders, and to assist in placental expulsion after childbirth (7, 8, 10). The leaves and their extracts are employed externally and internally, addressing skin ailments, liver complaints, elephantiasis, smallpox, toothache, and even ascites (8, 10). Other practices include using seed sprouts for tumor treatment, seed oil for convulsions and cosmetic applications, and external applications for inflammatory swellings and hydrocele—often combining seeds or leaves with castor oil or honey (8, 11, 12).

Complementing its traditional repertoire, pharmacognostic and phytochemical analyses reveal a rich profile of bioactive constituents and diagnostic traits. Mehra (2016) (9) described the seeds as green-grey to bluish-grey, round, smooth, and shiny, with distinctive anatomical features such as palisade cells and resin-containing parenchyma; physico-chemical parameters (e.g., ash values, extractive yields) and preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, triterpenoids, saponins, steroids, tannins, glycosides, and carbohydrates. Additionally, GC–MS and related profiling efforts have identified cassane diterpenoids (e.g., caesalpinins, bonducellpins), furanoditerpenes, fatty acids (palmitic, stearic, oleic, linoleic), phytosterols (e.g., β -sitosterol), homoisoflavones, amino acids, and neutral saponins (12, 13).

The diverse phytoconstituents align well with the plant's reputed antioxidant, hepatoprotective, anti-inflammatory, and membrane-stabilizing effects—functional attributes which underpin its traditional use in treating fever, inflammation, and liver disorders, and support its selection for experimental hepatoprotective studies.

3. PHYTOCHEMISTRY RELEVANT TO HEPATOPROTECTION

Caesalpinia bonduc is rich in multiple classes of phytochemicals notably associated with antioxidant and hepatoprotective activity, including flavonoids (such as kaempferol and quercetin derivatives), tannins, saponins, triterpenoids, alkaloids, and general phenolic compounds (14, 15). Quantitative analyses indicate that its methanolic seed extract contains approximately 0.50 mg quercetin equivalents per gram and 0.55 mg gallic acid equivalents per gram, underscoring its substantial antioxidant container (14). Furthermore, bioassay-guided isolation efforts have identified quercetin-3-methyl ether and kaempferol glycosides, as well as kaempferol-3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranoside from the ethyl acetate fraction, with potent antioxidant activity (14, 16).

These flavonoids and phenolics are well established in the literature to scavenge reactive oxygen species (ROS), up-regulate endogenous antioxidant defenses such as superoxide dismutase (SOD), catalase, and glutathione (GSH), and inhibit lipid peroxidation (as measured by malondialdehyde, MDA) (17, 18). Experimental hepatoprotective studies also support the functional role of kaempferol: in CCl₄-induced liver damage models in rats, kaempferol administration ameliorated histological abnormalities and serum markers of injury, reduced ROS levels and lipid peroxidation, and enhanced GSH levels and Nrf2/heme oxygenase-1 signaling, implicating the AMPK/Nrf2 and MAPK/NF- κ B pathways (19).

Docking and network pharmacology studies further reveal that quercetin and kaempferol have strong binding interactions with peroxisome proliferator-activated receptor (PPAR) pathways—particularly PPAR α , which plays a key role in lipid metabolism and hepatoprotection (20, 21).

Given the complex chemical makeup and the documented bioactivities, establishing a robust phytochemical fingerprint—identifying and quantifying key flavonoids and phenolics—is essential for preclinical reproducibility, enabling consistent dosing and mechanistic interpretation. Thus, the presence of these multiple bioactive phytoconstituents in *C. bonduc* seeds and leaves provides a strong basis for its exploration as a hepatoprotective agent, particularly in oxidative or toxin-induced liver injury models.

4. MECHANISMS OF ANTIBIOTIC-INDUCED HEPATOTOXICITY WITH EMPHASIS ON AMPICILLIN

Antibiotic-induced liver injury (DILI) can arise through several interrelated mechanisms, including direct hepatocellular toxicity, immune-mediated injury through hapten formation, impairment of bile flow leading to cholestasis, and antibiotic-induced alterations in gut microbiome that heighten susceptibility to liver damage (22).

Although ampicillin infrequently causes hepatotoxicity, when it does, the injury often manifests in cholestatic or mixed patterns and may involve hypersensitivity-mediated immune mechanisms (23, 24). In such cases, drugs or their reactive metabolites bind covalently to hepatic proteins, forming haptens that elicit a T-cell-mediated immune response via CD4⁺ and CD8⁺ lymphocytes, stimulating cytokines like TNF- α and IFN- γ , which drive hepatocyte apoptosis and inflammation (25).

Cholestatic liver injury typically reflects interference with bile secretion mechanisms—either through inhibition of bile acid transporters such as BSEP, MRP2–4, and MDR3, or through disruption of canalicular structure and tight-junction integrity (26, 27). In parallel, antibiotics may induce dysbiosis by depleting beneficial gut bacteria (e.g., *Bifidobacterium*, *Lactobacillus*), resulting in altered bile acid metabolism, increased gut-derived inflammatory mediators, and heightened liver inflammatory response (23).

Animal models of antibiotic-induced hepatotoxicity commonly employ biochemical, oxidative, inflammatory, and histopathological measures to characterize injury and explore mechanisms. These include serum elevations in ALT, AST, ALP, and bilirubin; oxidative stress markers such as increased lipid peroxidation (measured via malondialdehyde, MDA) and depletion of endogenous antioxidants including glutathione (GSH), superoxide dismutase (SOD), and catalase; upregulation of inflammatory cytokines like TNF- α and interleukin-6 (IL-6); and histological changes including hepatocyte necrosis, inflammatory infiltration, and cholestasis (24, 25, 28).

Collectively, these endpoints provide a comprehensive evaluation of antibiotic-induced liver injury, capturing direct cellular damage, immune-driven mechanisms, cholestatic alterations, and microbiome-mediated susceptibility, thereby informing the design, monitoring, and interpretation of experimental hepatotoxicity studies.

5. SUMMARY OF EXPERIMENTAL EVIDENCE FOR HEPATOPROTECTIVE ACTIVITY OF *C. BONDUC*

Multiple experimental models report hepatoprotective effects of *C. bonduc* extracts:

- Seed kernel and ethanolic extracts decreased serum ALT, AST, ALP and bilirubin and improved histology in CCl₄ and paracetamol models; efficacy compared favorably with silymarin in some studies.
- Methanolic leaf extract protected against gentamicin-induced hepatotoxicity and nephrotoxicity, restoring liver enzymes and antioxidant parameters.
- In silico docking and biochemical screening studies suggest flavonoids in *C. bonduc* could

modulate PPAR α and antioxidant pathways.

Taken together, the body of evidence supports antioxidative, anti-inflammatory, and membrane-protective effects — mechanisms plausibly beneficial in antibiotic-triggered liver injury. Nonetheless, direct studies against ampicillin-induced hepatotoxicity are not abundant, representing a gap (29 – 33).

6. CRITICAL GAPS AND RATIONALE FOR TESTING *C. BONDUC* AGAINST AMPICILLIN HEPATOTOXICITY

Gaps include:

- Most hepatoprotection data for *C. bonduc* come from paracetamol, CCl₄, ethanol or gentamicin models — which differ mechanistically from antibiotic-induced DILI.
- Standardization of extract (solvent, part used, dose) and phytochemical fingerprinting is often missing.
- Mechanistic assays (cytokines, apoptosis markers, bile acid transporters) are limited.

Rationale: Given *C. bonduc*'s antioxidant and anti-inflammatory constituents, testing in an ampicillin model will assess its broadness of hepatoprotection and potential for clinical adjuvant use during antibiotic therapy (29 – 34).

7. PROPOSED PRECLINICAL EXPERIMENTAL DESIGN (DETAILED PROTOCOL) 35 - 39

7.1 Objectives

- Primary: To evaluate whether standardized *C. bonduc* leaf extract prevents or attenuates biochemical and histological liver injury induced by ampicillin in Wistar rats.
- Secondary: To elucidate mechanisms (oxidative stress, inflammatory cytokines, apoptosis).

7.2 Materials & methods (outline)

- Animals: Adult male Wistar rats (180–220 g), n = 6–8 per group (power calculation suggested; see statistical section).
- Extract preparation: Fresh leaves → air-dry → powdered → maceration or Soxhlet extraction with 70% ethanol (or methanol/water) → evaporate to dryness → store at 4°C. Standardize to a marker (e.g., total flavonoid content; HPLC profile for quercetin/kaempferol).
- Doses: Pilot acute toxicity (OECD 423 or 425) to select safe doses. Test doses e.g., 125, 250, 500 mg/kg p.o. based on prior studies. Compare with positive control silymarin (100 mg/kg).

- Ampicillin hepatotoxicity induction: Literature shows repeated high dose ampicillin or ampicillin/clavulanate regimens can induce liver injury in rodents. Example: ampicillin 200–400 mg/kg i.p./p.o. for 7–14 days (adapt dose after literature validation). Include vehicle control.

7.3 Experimental groups (example)

- Group I: Normal control (vehicle).
- Group II: Ampicillin only (hepatotoxic dose).
- Group III: Ampicillin + *C. bonduc* low dose (125 mg/kg).
- Group IV: Ampicillin + *C. bonduc* mid dose (250 mg/kg).
- Group V: Ampicillin + *C. bonduc* high dose (500 mg/kg).
- Group VI: Ampicillin + Silymarin (100 mg/kg) — positive control.
- (Optional) Group VII: *C. bonduc* only high dose.

7.4 Treatment schedule

Pre-treatment design (prophylactic): *C. bonduc* daily for 7 days before starting ampicillin, continue both for total 14 days. Therapeutic design: start extract after 3–7 days of ampicillin (to model rescue). Choose based on intended clinical translation.

7.5 Endpoints and assays

Biochemical:

- Serum ALT, AST, ALP, total & direct bilirubin, GGT, total protein, albumin.

Oxidative stress:

- Hepatic MDA (lipid peroxidation), reduced glutathione (GSH), SOD, catalase.

Inflammation & apoptosis:

- TNF- α , IL-6 (ELISA), caspase-3, Bax/Bcl-2 (Western blot or RT-PCR).

Histopathology:

- H&E staining (necrosis, inflammation, steatosis, cholestasis), Sirius Red (fibrosis if long term).
Semi-quantitative scoring.

Immunohistochemistry:

- Markers of apoptosis (caspase-3), oxidative damage (4-HNE), bile transporter expression (BSEP, MRP2) if cholestasis suspected.

Molecular:

- qPCR for Nrf2 pathway genes, inflammatory NF- κ B components.

7.6 Statistical analysis

Use n determined by power calculation (e.g., to detect 30% change in ALT with $\alpha=0.05$, power=80%).

Data: mean $\pm$ SD. One-way ANOVA with Tukey's post hoc test (or Kruskal-Wallis if non-parametric). $P < 0.05$ considered significant. Provide effect sizes and confidence intervals.

8. EXPECTED OUTCOMES & INTERPRETATION

If *C. bonduc* is hepatoprotective vs. ampicillin:

- Lower serum ALT/AST/ALP and bilirubin vs. ampicillin group.
- Reduced MDA and restored GSH/SOD/catalase in liver tissue.
- Lower inflammatory cytokines (TNF- α , IL-6) and decreased apoptotic markers.
- Improved histological scores (less necrosis, inflammation).

Mechanisms likely include antioxidant activity (scavenging ROS, up-regulating Nrf2), anti-inflammatory effects (modulating NF- κ B), stabilization of hepatocyte membranes, and possible modulation of bile acid transporters (if cholestasis is involved). These anticipated mechanisms align with prior observations in non-antibiotic model (40 – 43).

9. LIMITATIONS AND CONSIDERATIONS

- Model selection: Antibiotic DILI can be idiosyncratic and immune-mediated; most rodent models mimic direct toxicity. Findings may not fully translate to human idiosyncrasy. Use of concurrent immune-sensitization models may be necessary for certain mechanistic claims.
- Standardization of extract and batch-to-batch variability must be controlled (HPLC fingerprint, marker quantification).
- Safety and herb–drug interactions: test for pharmacokinetic interactions (e.g., interference with antibiotic efficacy or hepatic metabolism).
- Appropriate ethical approvals and humane endpoints (44 – 48).

10. FUTURE DIRECTIONS AND TRANSLATIONAL POTENTIAL

If preclinical efficacy is validated, next steps include pharmacokinetic profiling, toxicity (sub-chronic) studies, testing for interference with antibiotic antibacterial activity, and eventual proof-of-concept clinical trials in populations at risk for antibiotic-associated liver injury. Identification of active compounds and possible development of standardized phytopharmaceuticals are recommended (50 – 55).

11. CONCLUSION

Preclinical evidence supports *C. bonduc*'s hepatoprotective properties in multiple chemically induced liver injury models, mediated largely through antioxidant and anti-inflammatory mechanisms. A targeted, well-controlled rat study using validated ampicillin hepatotoxicity protocols, standardized leaf extract, and a comprehensive panel of biochemical and molecular endpoints is warranted to test whether *C. bonduc* can prevent or attenuate ampicillin-triggered liver injury. Robust standardization and mechanistic work are essential for translation.

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