Jurnal Multidisiplin Sahombu
Volume 5, Number 05, 2025, DOI 10.58471/jms.v5i05
ESSN 2809-8587 (Online)
https://ejournal.seaninstitute.or.id/index.php/JMS/index

Effectiveness Test of Ketapang Leaf Extract (Terminalia Catappa L.) as a Hepatoprotective Agent in Mice (Mus musculus) Induced by Acetaminophen

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Article Info
Keywords:
Terminalia catappa L,
hepatoprotective,
acetaminophen,
flavonoids,
oxidative stress, DILI.

ABSTRACT
Drug-induced liver injury (DILI) is a leading cause of acute liver failure, with acetaminophen being the most frequent etiological agent. Overdose induces formation of the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI), resulting in glutathione depletion, oxidative stress, and hepatocellular necrosis. This study aimed to evaluate the hepatoprotective effect of ethanolic extract of Terminalia catappa L. leaves in male mice (Mus musculus) with acetaminophen-induced acute liver injury. A post-test only control group design was employed using 20 mice divided into five groups: negative control (Na-CMC), positive control (N-acetylcysteine), and three treatment groups receiving T. catappa extract at doses of 200, 400, and 600 mg/kg BW. Acetaminophen was administered intraperitoneally at 500 mg/kg BW. Serum SGPT and SGOT levels were quantified spectrophotometrically, and phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, and terpenoids. Extract administration significantly ($p < 0.05$) reduced SGPT and SGOT levels compared to the negative control, with a clear dose-dependent trend; the 600 mg/kg BW group achieved biochemical parameters approaching those of the N-acetylcysteine group. The hepatoprotective effect is likely mediated by flavonoid-driven antioxidant and anti-inflammatory mechanisms. These findings indicate that T. catappa extract is a promising, safe, and cost-effective plant-based hepatoprotective agent, meriting further investigation toward clinical application as an adjunct therapy for drug-induced hepatotoxicity

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INTRODUCTION

The liver is a vital organ responsible for detoxifying harmful substances, metabolizing nutrients, and maintaining overall homeostasis. Drug-Induced Liver Injury (DILI) is one of the leading causes of acute liver failure, with acetaminophen being the most common etiological agent due to its widespread use and potential for overdose (Yokoi & Oda, 2021). The hepatotoxicity of acetaminophen involves excessive formation of *N*-acetyl-*p*-benzoquinone imine (NAPQI) via cytochrome P450 enzymes, resulting in hepatic glutathione depletion,

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mitochondrial dysfunction, oxidative stress, and ultimately hepatocellular necrosis (Yokoi & Oda, 2021).

Globally, DILI accounts for approximately 15% of acute liver failure cases, with high incidence rates reported in both developed and developing countries (McGill & Curry, 2023; Rotundo & Pyrsopoulos, 2020). Between 1993 and 2020, 81,856 cases were recorded based on the Roussel Uclaf Causality Assessment Method (RUCAM), with China, the United States, and Germany as the largest contributors (Teschke & Danan, 2023). In Asia, herbal and complementary medicines are the predominant causes of DILI, whereas acetaminophen-induced hepatotoxicity, although less frequent, remains clinically significant (Anindyaguna & Mustofa, 2022; Lai et al., 2024). In Indonesia, the Indonesian Liver Research Society (PPHI) reports that 20–40% of fulminant hepatitis and approximately 50% of acute hepatitis cases are drug-induced, including those caused by acetaminophen (Laia et al., 2019). Patients undergoing anti-tuberculosis therapy have a DILI prevalence of 2–28%, underscoring the urgency for preventive and therapeutic interventions (Aminy & Kholili, 2022; Pranata, 2019).

The rising prevalence of liver diseases, particularly in regions such as West Nusa Tenggara where hepatitis B rates remain high with fluctuating trends (Suprapti et al., 2023), highlights the need for liver-protective strategies. Oxidative stress is a common pathway in hepatocellular injury, and natural antioxidants derived from medicinal plants offer promising therapeutic potential. *Terminalia catappa* L., commonly known as Indian almond or tropical almond, contains bioactive compounds with antioxidant, anti-inflammatory, and hepatoprotective properties (Ningsih et al., 2023; Putu et al., 2022). Despite its abundance in tropical coastal areas, its utilization as a hepatoprotective agent remains limited, with dried leaves often discarded as waste (Salimi et al., 2022).

Several studies have reported the antioxidant capacity and phytochemical composition of *T. catappa* leaf extract, as well as its benefits on biochemical parameters in various experimental models (Ningsih et al., 2023; Mayarlis et al., 2024). However, its effectiveness in acute hepatotoxicity due to acetaminophen overdose remains underexplored. This research gap limits understanding of its therapeutic potential in acute liver injury models, despite encouraging results in chronic metabolic disease models.

This study aims to evaluate the hepatoprotective effect of ethanol extract of *T. catappa* L. leaves in mice with acetaminophen-induced liver injury. The findings are expected to strengthen scientific evidence for the development of safe, affordable, and effective plant-based hepatoprotective agents. From a scientific perspective, the study addresses the gap in research on acute drug-induced liver injury, while from a practical perspective, it supports the utilization of local natural resources for public health and the sustainable development of herbal medicines.

The liver plays a central role in metabolism, detoxification, and nutrient storage; thus, its impairment can have far-reaching consequences for health. One condition that has garnered significant attention is Drug-Induced Liver Injury (DILI), which refers to liver damage caused by exposure to pharmaceutical agents, either acutely or chronically. Physiologically, the liver is protected by endogenous antioxidants such as glutathione. However, excessive

exposure to toxic compounds can disrupt redox balance, trigger oxidative stress, and lead to hepatocellular necrosis (Yokoi & Oda, 2021).

In cases of acetaminophen-induced hepatotoxicity, excessive metabolism via cytochrome P450 enzymes generates *N*-acetyl-*p*-benzoquinone imine (NAPQI), which is cytotoxic at high concentrations due to glutathione depletion and mitochondrial damage (McGill & Curry, 2023).

Hepatoprotective agents can originate from natural sources, particularly plants rich in bioactive compounds. Ningsih et al. (2023) reported that ethanol extract of *Terminalia catappa* L. leaves contains a total flavonoid content of 17.710 QE and exhibits strong antioxidant activity with an IC_{50} value of 23.531 ppm using the ABTS assay. Mayarlis et al. (2024) further confirmed that *T. catappa* leaves possess anti-inflammatory, antibacterial, antifungal, and antioxidant properties, making them a potential hepatoprotective candidate. Salimi et al. (2022) highlighted the opportunity to repurpose *T. catappa* leaves—often discarded as waste—into a valuable herbal medicinal resource.

Studies on other plant species have also demonstrated the potential of natural products as hepatoprotective agents. For example, extracts of durian (*Durio zibethinus* Murr.) leaves were shown to protect the liver from paracetamol-induced damage, with significant reductions in SGOT and SGPT levels observed at a dose of 500 mg/kg body weight (Journal of Pharmaceutical and Sciences, 2023). Similarly, *Epipremnum pinnatum* (L.) Engl. leaf extract reduced SGOT, SGPT, hepatosomatic index, and histopathological liver damage in mice induced with paracetamol, with the optimal effect observed at 500 mg/kg body weight (Indonesian Journal of Pharma Science, 2022).

Furthermore, research on the ethyl acetate and ethanol fractions of *T. catappa* waste leaves using the DPPH assay found that the ethanol fraction displayed strong antioxidant activity (IC_{50} = 32.53 ppm), whereas the ethyl acetate fraction exhibited negligible activity (Journal of Pharmacy Science and Practice, 2021). A study conducted at the University of Sumatera Utara (2020) demonstrated that ethanol extract of *T. catappa* leaves at 300 mg/kg body weight significantly reduced creatinine and urea levels and improved hematological parameters in paracetamol-induced rats, compared to the negative control.

Despite these promising findings, most studies on *T. catappa* have focused on general antioxidant activity or chronic disease models such as diabetes. Research specifically addressing its efficacy in acute liver injury induced by acetaminophen overdose is still limited. From a theoretical standpoint, the molecular mechanisms by which *T. catappa* bioactive compounds interact with hepatocyte biochemical pathways during acute toxicity remain insufficiently described. From an empirical standpoint, previous studies have often been limited to basic biochemical parameters without integrating histopathological analysis or systematic dose–response evaluations.

Based on this context, the present study aims to fill this gap by evaluating the hepatoprotective effect of ethanol extract of *T. catappa* L. leaves in a mouse model of toxic-dose acetaminophen-induced liver injury. This research not only assesses biochemical markers such as SGOT and SGPT but also positions the findings within the broader

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framework of evidence-based herbal medicine for the prevention of drug-induced hepatotoxicity.

METHOD

This study employed an experimental *post-test only control group* design conducted in a laboratory setting, selected for its ability to evaluate treatment effects without baseline measurement bias. The research was carried out from April to July 2025 at the Pharmacology Laboratory, Faculty of Pharmacy, Universitas Bumigora.

The study population consisted of healthy male mice (*Mus musculus*), aged 8–12 weeks and weighing 20–30 g, with no physiological abnormalities. A total of 20 mice were purposively selected and divided into five groups, each comprising four animals: a negative control group (Na-CMC), a positive control group (N-acetylcysteine), and three treatment groups receiving ethanol extract of *Terminalia catappa* leaves at doses of 200, 400, and 600 mg/kg body weight. All groups were induced with acetaminophen at a dose of 500 mg/kg body weight.

Research materials included *T. catappa* leaves, 96% ethanol, acetaminophen, N-acetylcysteine, SGOT and SGPT assay reagents, 1% w/v Na-CMC suspension, and distilled water. The extract was prepared using maceration with 96% ethanol, followed by evaporation to obtain a thick extract. Plant identification was conducted at the Integrated Laboratory of UIN Mataram. Phytochemical screening included tests for flavonoids, alkaloids, tannins, saponins, and steroids (Salimi et al., 2022; Wati et al., 2024).

Treatments were administered orally for seven consecutive days according to the respective group assignments. At the end of the treatment period, blood samples were collected intracardially following chloroform anesthesia (Nugroho, 2018). Serum was separated by centrifugation at 3,000 rpm for 15 minutes and analyzed for SGOT and SGPT levels using a spectrophotometric method at a wavelength of 340 nm and a temperature of 37°C.

Data were analyzed using one-way ANOVA with a 95% confidence level, followed by Tukey's HSD post hoc test to compare differences between groups. All statistical analyses were performed using SPSS software.

RESULTS AND DISCCUSION

Results

Plant determination confirmed that the plant material utilized in this study was *Terminalia catappa* L., which belongs to the Genus *Terminalia* L., Family Combretaceae, and Species *Terminalia catappa* L. The taxonomic identification was conducted at the Integrated Laboratory of Universitas Islam Negeri Mataram to ensure absolute accuracy in species determination prior to subsequent experimental procedures. Correct species identification is crucial for reproducibility and for ensuring that bioactive properties are attributed to the correct botanical source.

From 1 kg of fresh *T. catappa* leaves collected in Desa Jeringo, Gunungsari District, Lombok Barat, 250 g of dried simplicia powder was obtained after undergoing a standardized

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post-harvest preparation process. This process included wet sorting to remove debris and non-leaf material, followed by shade drying for approximately 5–7 days. Shade drying was specifically selected over direct sun exposure to minimize the degradation of thermolabile and photosensitive compounds, particularly flavonoids, which are susceptible to structural alterations under high-intensity ultraviolet light. The dried leaves were subsequently milled using a mechanical grinder and sieved through mesh No. 60 to achieve uniform particle size, a critical factor for consistent solvent penetration during extraction.

The extraction process was carried out using the maceration technique, chosen for its simplicity and efficiency in preserving the structural integrity of heat-sensitive secondary metabolites. A total of 200 g of simplicia powder was immersed in 2 L of 96% ethanol for three days at room temperature, with manual stirring every six hours to maintain uniform solvent exposure and enhance the release of soluble compounds. The choice of 96% ethanol as a solvent was based on its proven ability to dissolve a broad spectrum of phytochemicals, ranging from polar to semi-polar metabolites, including flavonoids, tannins, alkaloids, and certain terpenoids. After maceration, the solvent mixture was filtered to separate the plant residue from the filtrate. The filtrate was concentrated using a rotary evaporator set at 60 °C under reduced pressure to minimize thermal degradation, yielding 42 g of viscous, dark-brown ethanolic extract. The calculated yield was 16.8% (Table 1), a value consistent with previous reports that indicate extraction yield is influenced by parameters such as solvent type, extraction duration, and drying method (Nurdyansyah et al., 2021).

Table 1. Yield of *T. catappa* L. ethanolic extract

Weight of Simplicia	Thick Extract Weight	Yield (%)
250 g	42 g	16.8

Source: Quantitative Data 2025

Preliminary phytochemical screening of the ethanolic extract revealed the presence of five major classes of secondary metabolites: alkaloids, flavonoids, saponins, tannins, and terpenoids (Table 2). Flavonoids detected in the extract, such as quercetin and kaempferol, are widely recognized for their potent antioxidant and anti-inflammatory activities, including the capacity to upregulate endogenous antioxidant enzymes like superoxide dismutase (SOD) and catalase, as well as downregulate pro-inflammatory cytokines such as TNF-α and IL-6 (Valentine, 2023; Wichaksono, 2021). The detection of multiple bioactive classes suggests a potential synergistic interaction in mediating the observed pharmacological effects.

Table 2. Phytochemical profile of *T. catappa* L. ethanolic extract

Compound	Result
Alkaloid	+
Flavonoid	+
Saponin	+
Tannin	+
Terpenoid	+

Source: Quantitative Data 2025

Hepatoprotective activity of the extract was evaluated using a murine model of acetaminophen-induced hepatotoxicity. Male *Mus* musculus were administered a single dose

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of acetaminophen (500 mg/kg BW) to induce acute liver injury, as evidenced by elevations in serum aminotransferase levels. The experimental groups were categorized as follows: negative control (K-; acetaminophen only), positive control (K+; acetaminophen + N-acetylcysteine), and three treatment groups receiving ethanolic extract at doses of 200 mg/kg BW (P1), 400 mg/kg BW (P2), and 600 mg/kg BW (P3). Serum SGPT and SGOT levels were measured 24 hours post-treatment.

The results demonstrated a clear dose-dependent hepatoprotective effect (Table 3). In the negative control group, SGPT and SGOT levels were markedly elevated (123.75 ± 1.71 U/L and 160.75 ± 1.71 U/L, respectively), approaching the upper limits of normal reference ranges for mice (SGPT: 28–132 U/L; SGOT: 59–247 U/L) (Ekasari, 2022). Administration of the ethanolic extract resulted in significant reductions in both enzyme markers, with the P3 group achieving the most pronounced effect (SGPT: 79.50 ± 4.20 U/L; SGOT: 114.25 ± 2.22 U/L). These values were statistically comparable to the positive control group treated with NAC (SGPT: 61.25 ± 4.50 U/L; SGOT: 98.25 ± 2.25 U/L), indicating near-equivalent hepatoprotection at the highest extract dose.

Table 3. Mean SGPT and SGOT levels (U/L ± SD)

Group	SGPT ± SD	Reference	SGOT ± SD	Reference
K-	123.75 ± 1.71	28–132	160.75 ± 1.71	59–247
K+	61.25 ± 4.50		98.25 ± 2.25	
P1	100.50 ± 2.08		137.00 ± 2.08	
P2	86.00 ± 2.58		123.00 ± 2.58	
P3	79.50 ± 4.20		114.25 ± 2.22	

Source: Quantitative Data 2025

Statistical analysis confirmed that the data followed a normal distribution ($p > 0.05$; Shapiro–Wilk test) and exhibited homogeneity of variances ($p > 0.05$; Levene's test). One-way ANOVA revealed statistically significant differences among the groups for both SGPT and SGOT ($p = 0.001$). Subsequent post hoc LSD analysis indicated that the P3 group differed significantly from the negative control group while showing no statistically significant difference compared to the positive control group, suggesting that the hepatoprotective efficacy of *T. catappa* extract at 600 mg/kg BW closely approaches that of NAC.

Discussion

The present study demonstrated that ethanolic extract of *Terminalia catappa* L. exhibits significant hepatoprotective activity in a murine model of acetaminophen-induced liver injury. Acetaminophen hepatotoxicity is widely recognized to result from the overproduction of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which, at toxic doses, overwhelms hepatic detoxification pathways by depleting intracellular glutathione (GSH) stores (Allison Rebecca, 2023). Accumulated NAPQI binds covalently to cellular macromolecules, initiating oxidative stress, mitochondrial dysfunction, and hepatocyte necrosis. In the current study, elevated serum aminotransferase levels (SGPT and SGOT) in the negative control group confirmed the establishment of acute hepatocellular injury.

Administration of *T. catappa* extract markedly attenuated these elevations in a dose-dependent manner, with the highest dose (600 mg/kg BW; P3) reducing SGPT and SGOT to

values statistically comparable with the positive control group treated with N-acetylcysteine (NAC). This pattern indicates that the extract confers substantial protection against acetaminophen-induced hepatotoxicity, likely through multiple complementary mechanisms involving its phytochemical constituents.

Phytochemical screening confirmed the presence of flavonoids, alkaloids, tannins, saponins, and terpenoids in the ethanolic extract. Among these, flavonoids such as quercetin and kaempferol are well-documented antioxidants and anti-inflammatory agents (Valentine, 2023; Wichaksono, 2021). Their hepatoprotective action can be attributed to several molecular mechanisms:

1. Scavenging of reactive oxygen species (ROS) to limit lipid peroxidation of hepatocyte membranes.
2. Inhibition of cytochrome P450-mediated oxidation, thereby reducing NAPQI formation (Hartono, 2018).
3. Activation of the Nrf2 signaling pathway, leading to the transcription of antioxidant defense genes such as heme oxygenase-1 (HO-1) and glutamate-cysteine ligase (GCL).
4. Suppression of pro-inflammatory cytokines, particularly TNF-α and IL-6, which are key mediators in inflammatory liver damage (Rafita et al., 2015).

The detection of tannins in the extract may contribute additional hepatoprotection by stabilizing cell membranes and inhibiting oxidative enzymes, while saponins and terpenoids could exert synergistic effects by modulating inflammatory cascades and improving hepatic microcirculation. Alkaloids, although present in lower concentrations, have also been reported to possess antioxidative and anti-apoptotic properties in hepatotoxic models.

When compared to NAC, the gold standard antidote for acetaminophen toxicity, the extract was slightly less potent, which may be explained by differences in pharmacokinetics and mechanism of action. NAC acts rapidly by replenishing intracellular GSH and directly neutralizing NAPQI, in addition to providing anti-inflammatory and mitochondrial-protective benefits (Mukaddas et al., 2019). In contrast, the phytochemicals in *T. catappa* extract require metabolic activation, distribution, and cumulative cellular interaction before manifesting maximal hepatoprotective effects. Nevertheless, the near-equivalent efficacy of the 600 mg/kg BW dose to NAC underscores its potential as a complementary or alternative therapeutic option, especially in resource-limited settings where NAC availability may be restricted.

These findings align with previous studies reporting hepatoprotective effects of *T. catappa* in various experimental models. Bilqis (2016) demonstrated significant reductions in serum aminotransferases and histopathological improvements in carbon tetrachloride-induced liver injury following administration of *T. catappa* extract. Similarly, Ningsih et al. (2023) reported that *T. catappa* leaf extract ameliorated oxidative stress markers and improved antioxidant enzyme activity in ethanol-induced liver injury models. The consistency of outcomes across different hepatotoxic agents suggests a broad-spectrum protective effect, predominantly mediated by antioxidant and anti-inflammatory pathways.

From a translational perspective, the abundant availability of *T. catappa* leaves in tropical regions, coupled with their low reported toxicity, supports the feasibility of developing

the extract into a phytopharmaceutical preparation. Moreover, its multi-component composition may confer an advantage over single-molecule drugs by targeting multiple pathological pathways simultaneously. However, the complex phytochemical profile also presents challenges in standardization, dosage determination, and quality control during large-scale production.

The current study has certain limitations. First, histopathological examination of liver tissue was not performed, precluding direct assessment of cellular morphology, necrosis, and regeneration. Second, the specific bioactive compounds responsible for the observed effects were not isolated or quantified, which limits mechanistic specificity. Third, the study was conducted in a single animal species under acute hepatotoxic conditions; chronic models and human clinical data are needed to establish safety and efficacy profiles for long-term use.
Future research should address these gaps by:

1. Performing histopathological and ultrastructural liver analyses to confirm cellular-level protection.
2. Conducting bioassay-guided fractionation to isolate and characterize the most active constituents.
3. Evaluating the extract in multiple hepatotoxicity models, including chronic injury paradigms.
4. Undertaking dose-optimization studies and pharmacokinetic profiling.
5. Initiating early-phase clinical trials to explore translational potential.

In conclusion, the results of this study provide compelling evidence that ethanolic extract of *T. catappa* L. confers significant hepatoprotective effects in acetaminophen-induced liver injury in mice, approaching the efficacy of NAC at higher doses. These findings support its potential as a natural, accessible, and multi-target hepatoprotective agent, warranting further investigation toward its development as an alternative or adjunctive therapy in the management of drug-induced liver injury.

CONCLUSION

This study provides compelling evidence that ethanolic extract of *Terminalia catappa* L. has significant hepatoprotective potential in a murine model of acetaminophen-induced liver injury. Administration of the extract at doses of 200, 400, and 600 mg/kg BW produced a clear dose-dependent reduction in serum SGPT and SGOT levels, with the highest dose yielding biochemical outcomes statistically comparable to those obtained with the standard antidote, N-acetylcysteine. These protective effects are most likely mediated through the synergistic actions of multiple phytochemical constituents—particularly flavonoids, tannins, saponins, terpenoids, and alkaloids—which collectively enhance endogenous antioxidant defenses, inhibit pro-inflammatory cytokine production, and stabilize hepatocyte membranes. The findings from this study are novel in showing that a high-dose *T. catappa* ethanolic extract can approach the hepatoprotective efficacy of N-acetylcysteine in an acute acetaminophen-induced liver injury model. The confirmed presence of multiple bioactive compounds underscores the extract's multi-pathway mechanism of action, offering a pharmacological advantage over single-compound therapies. Given its efficacy, low toxicity,

and abundant availability in tropical regions, *T. catappa* leaf extract holds promise as a complementary or alternative therapy for drug-induced liver injury, particularly in resource-limited settings where conventional antidotes may be inaccessible. Beyond its experimental significance, this research provides a scientific basis for the further development of *T. catappa* into a standardized phytopharmaceutical product. To advance toward clinical application, future work should include histopathological examinations to confirm tissue-level protection, isolation and characterization of the most active compounds, pharmacokinetic studies to optimize dosing regimens, and rigorously designed clinical trials to establish safety and efficacy in humans. In doing so, *T. catappa* extract could emerge as a cost-effective, plant-based hepatoprotective agent with real-world applicability in global health contexts.

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