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# Hepatoprotective Potential of *Schleichera oleosa* Leaf Extract Against Paracetamol-Induced Liver Injury in Rats

## Potensi Hepatoprotektif Ekstrak Daun *Schleichera oleosa* terhadap Kerusakan Hati akibat Paracetamol pada Tikus

**Zulham[1]*, Andi Ulfah Magefirah Rasyid[2], Asril Burhan[3]**

[1]*Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Health Sciences, Universitas Almarisah Madani, Makassar, 90245, Indonesia*
[2]*Department of Pharmaceutical Microbiology, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Makassar, Makassar, 90222, Indonesia*
[3]*Department of Pharmaceutical Biology, Faculty of Health Sciences, Universitas Almarisah Madani, Makassar, 90245, Indonesia*

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**ABSTRACT**

*Schleichera oleosa* L. is traditionally used in herbal medicine and has shown pharmacological activities such as antioxidant, anticancer, and antimicrobial effects. Its antioxidant capacity is attributed to polyphenols, which neutralize free radicals and contribute to liver protection. This study evaluated the hepatoprotective effect of *S. oleosa* leaf extract against paracetamol-induced liver injury in rats. Antioxidant activity was measured using the DPPH method, yielding an $IC_{50}$ of 11.43 µg/mL, indicating strong activity. Rats were administered 50, 100, or 150 mg/kg body weight of extract, followed by 1000 mg/kg paracetamol to induce liver damage. SGOT and SGPT levels were significantly reduced in all extract groups compared to the negative control, with the 100 mg/kg dose being the most effective. However, curcumin showed superior protection. These results suggest that *S. oleosa* has hepatoprotective potential, but further studies are needed to confirm its long-term safety and efficacy.

**Kata kunci:**
Antioksidan
Hepatoprotektif
Kerusakan hati
Obat tradisional
*Schleichera oleosa* L.

**ABSTRAK**

*Schleichera oleosa* L. dikenal secara tradisional memiliki aktivitas farmakologis seperti antioksidan, antikanker, dan antimikroba. Aktivitas antioksidannya dikaitkan dengan kandungan polifenol yang mampu menetralisir radikal bebas dan berperan dalam perlindungan hati. Penelitian ini mengevaluasi efek hepatoprotektif ekstrak daun *S. oleosa* terhadap kerusakan hati akibat paracetamol pada tikus. Aktivitas antioksidan diuji dengan metode DPPH dan menunjukkan nilai $IC_{50}$ sebesar 11,43 µg/mL. Tikus diberi ekstrak sebesar 50, 100, atau 150 mg/kg BB, diikuti induksi paracetamol 1000 mg/kg BB. Kadar SGOT dan SGPT menurun signifikan di semua kelompok perlakuan, dengan dosis 100 mg/kg menunjukkan efek terbaik. Namun, efeknya masih lebih rendah dibandingkan kurkumin. Hasil ini menunjukkan bahwa *S. oleosa* berpotensi sebagai agen hepatoprotektif, namun diperlukan studi lanjutan untuk menjamin keamanan dan efektivitas jangka panjangnya.

**Corresponding author:*
*Zulham (zulham.murtadha@gmail.com)*

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## 1. INTRODUCTION

The liver plays a critical role in detoxifying exogenous substances such as drugs and metabolic waste by converting them into inactive, excretable forms (Wahid et al., 2020). Liver injury can arise from various factors, including drug-induced hepatotoxicity, alcohol consumption, viral infections, autoimmune disorders, and hepatitis. Hepatoprotective agents are compounds that maintain liver function by protecting hepatocytes, promoting tissue regeneration, and restoring normal hepatic physiology.

Among the organs, the liver is particularly vulnerable to oxidative stress due to its central metabolic role. Reactive oxygen species (ROS)—a type of free radical—can disrupt cellular homeostasis and are implicated in the pathogenesis of numerous conditions, including hepatic dysfunction, inflammation, cardiovascular disease, diabetes, cancer, neurodegenerative disorders, immune impairments, renal dysfunction, and aging (Mbaoji & Amuche, 2020; Salem et al., 2018; Sen et al., 2010). Antioxidants combat oxidative stress by neutralizing free radicals through a synergistic defense system that protects cellular structures (Balasaheb & Pal, 2015). Consequently, antioxidant activity is considered a fundamental mechanism underlying hepatoprotective effects (Mbaoji & Amuche, 2020).

Phenolic compounds, particularly flavonoids, represent one of the principal classes of natural antioxidants due to their potent free radical scavenging capabilities (Singh & Rajbir, 2011). Flavonoids exert antioxidant effects by directly neutralizing ROS, inhibiting ROS production, and stimulating endogenous antioxidant enzyme activity. These mechanisms involve the inhibition of enzymes such as xanthine oxidase and NADPH oxidase, as well as metal ion chelation that prevents redox-mediated radical formation (Akhlaghi & Bandy, 2009; Atmani et al., 2009). Numerous studies have confirmed that flavonoids—such as quercetin, rutin, apigenin, catechin, venoruton, and naringenin—possess hepatoprotective, anti-inflammatory, antiviral, cardioprotective, and anticancer properties (Kumar & Pandey, 2013; Muthukrishnan & Sivakkumar, 2018). Additionally, flavonoids and anthraquinones have shown protective effects against drug-induced liver damage in experimental models (Situmeang et al., 2018). Although many plants are rich in phenolic compounds, the specific chemical constituents and pharmacological potential of numerous species remain insufficiently characterized. One such underexplored plant is *Schleichera oleosa* (Lour.) Oken.

*S. oleosa* is distributed across South and Southeast Asia, including India, Nepal, Malaysia, and Indonesia, and is recognized for its traditional medicinal applications (Tapas et al., 2008). Phytochemical analyses have identified several secondary metabolites in its leaves—such as flavonoids, alkaloids, tannins, phenolics, and steroids—that may account for its bioactivity (Chaerunisa et al., 2018; Tapas et al., 2008). Studies evaluating antioxidant activity in various fractions of *S. oleosa* leaf extract have revealed that the ethyl acetate fraction exhibits the strongest antioxidant properties, likely due to its high flavonoid content (Tapas et al., 2008). Given the critical role of antioxidant mechanisms in hepatoprotection, investigating the hepatoprotective potential of *S. oleosa* is both relevant and necessary.

This study aimed to evaluate the antioxidant capacity and hepatoprotective effects of *S. oleosa* leaf extract. Antioxidant activity was assessed via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and hepatoprotective effects were investigated in rats subjected to paracetamol-induced hepatotoxicity (1000 mg/kg body weight), a widely accepted model of acute liver injury. The extract was administered as a post-treatment intervention, while curcumin served as the positive control due to its well-established hepatoprotective profile. The findings of this research are expected to support the development of plant-based therapeutic agents for liver protection.

## 2. METHODS

### 2.1. Plant materials

*S. oleosa* leaves were collected from South Sulawesi, Indonesia. Taxonomic identification was confirmed at the Research Center for Biology, National Research and Innovation Agency (BRIN), Bogor, Indonesia, with reference number 726/IPH.1.01/If.07/VII/2020.

The dried powdered leaves (simplicia) were extracted using the maceration method with 70% ethanol, following the procedure described by Zhang et al. (2018). Initially, the powder was pre-wetted and immersed in solvent, then stored in a dark environment at room temperature for 72 hours with intermittent stirring. After filtration, the residue underwent a second maceration with the same solvent. Combined filtrates were concentrated using a rotary evaporator at 60 °C and 75 rpm, yielding a viscous extract, which was stored at 4 °C.

### 2.2. Experimental Animals

Thirty healthy male Wistar rats (2–3 months old, 220–260 g) were used. The animals were procured from the School of Life Sciences and Technology, Institut Teknologi Bandung (ITB), Indonesia. During a one-week acclimatization period, rats were maintained under standard laboratory conditions (12 h light/dark cycle) with ad libitum access to food and water. Only rats with stable or increasing body weight were included. Ethical approval for the study was granted by the Research Ethics Committee of Universitas Padjadjaran (Approval No. 498/UN6.KEP/EC/2020).

### 2.3. Experimental Procedures

#### 2.3.1. Phytochemical Screening

Preliminary phytochemical screening was performed using standard protocols to identify secondary metabolites, including phenolics, flavonoids, alkaloids, steroids, terpenoids, tannins, and saponins (Thilagavathi et al., 2015).

#### 2.3.2. Quantitative Analysis of Bioactive Compounds

Total phenolic and flavonoid contents were quantified due to their reported antioxidant and hepatoprotective potential (Muthukrishnan & Sivakkumar, 2018). For phenolic content, 10 mg of the extract was dissolved in 5 mL ethanol. A 0.025 mL aliquot was reacted with 1 mL of 7.5% Folin–Ciocalteu reagent and 1 mL sodium carbonate, and diluted to 5 mL with distilled water. After 15 minutes of incubation at room temperature, absorbance was measured at 656 nm. Results were expressed in mg gallic acid equivalent per gram of extract (Blainski et al., 2013).

Flavonoid content was determined using the aluminum chloride colorimetric method. A 0.75 mL sample was mixed with 0.1 mL of 1 M sodium acetate and 0.1 mL aluminum chloride, diluted to 5 mL with ethanol, and incubated at room temperature for 30 minutes. Absorbance was measured at 442 nm. Results were expressed in mg quercetin equivalent per gram of extract (Da Silva et al., 2015).

#### 2.3.3. Antioxidant Activity

Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method, as described by Marinova and Batchvarov (2011). Extracts were prepared in ethanol at concentrations of 5, 10, 15, 20, and 25 ppm. Each dilution was mixed with 1.5 mL of 160 ppm DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 516 nm. Ascorbic acid served as the reference standard. Antioxidant activity was calculated as:

$$\%\ Inhibition\ = \frac{Absorbance\ Control - Absorbance\ Sample}{Absorbance\ Control}\ x\ 100\%$$

The percentage inhibition at each concentration was evaluated using linear regression, with the equation $y = A + Bx$, where $x$ denoted the concentration (g/mL) and $y$ represented the percentage inhibition (%). Antioxidant activity was reported as the 50% Inhibition Concentration ($IC_{50}$), defined as the sample concentration required to reduce DPPH radicals by 50%. The $IC_{50}$ value was calculated by substituting $y = 50$ into the regression equation and solving for $x$.

#### 2.3.4. Hepatoprotective Activity

Rats were randomly divided into six groups (n = 5 per group):

1. Normal control
2. Negative control (paracetamol 1000 mg/kg BW)
3. Positive control (curcumin 100 mg/kg BW)
4. Treatment group 1 (extract 50 mg/kg BW)
5. Treatment group 2 (extract 100 mg/kg BW)
6. Treatment group 3 (extract 150 mg/kg BW)

After a one-week acclimatization period, all groups received their respective treatments for seven consecutive days. Normal and negative control groups received 2% gum arabic solution. Hepatotoxicity was induced by administering paracetamol (1000 mg/kg BW) orally on days 8 and 9, except for the normal control group. On day 10, rats were anesthetized, and blood samples were collected via the orbital sinus. Serum was obtained by centrifugation at 3000 rpm for 15 minutes and analyzed for SGOT and SGPT levels using the IFCC-recommended kinetic enzymatic method with a UV spectrophotometer at 340 nm (Chaerunisa et al., 2018; Zulham et al., 2023).

#### 2.3.5. Histopathological Analysis

Liver samples were rinsed with 0.9% NaCl and fixed in 10% formalin. After dehydration and embedding in paraffin, tissues were sectioned at 6–8 μm using a microtome and stained with hematoxylin and eosin. Microscopic analysis evaluated central vein condition, necrosis, hydropic changes, fatty degeneration, hemorrhage, sinusoid condition, and inflammatory cell infiltration (Chaerunisa et al., 2018; Zulham et al., 2023).

### 2.4. Statistical Analysis

All data were analyzed using one-way ANOVA followed by Least Significant Difference (LSD) post hoc tests using SPSS version 24. Statistical significance was accepted at $p < 0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1. Phytochemical Analysis

Phytochemical screening of *S. oleosa* leaf extract confirmed the presence of phenolics, flavonoids, alkaloids, steroids, and tannins. Among these, phenolic and flavonoid compounds are considered to be the key bioactives responsible for antioxidant and hepatoprotective activity (Muthukrishnan & Sivakkumar, 2018).

### 3.2. Quantitative Analysis of Major Components

Quantitative evaluation revealed total phenolic content of 42.96 mg gallic acid equivalent (GAE)/g extract and total flavonoid content of 1.52 mg quercetin equivalent (QE)/g extract. This supports the high presence of antioxidant constituents in S. oleosa leaves (Blainski et al., 2013; Da Silva et al., 2015).

### 3.3. Antioxidant Activity

The DPPH assay indicated that the extract had strong antioxidant activity, with an $IC_{50}$ value of 11.43 μg/mL, although it was less potent than vitamin C ($IC_{50}$: 3.54 μg/mL). This scavenging ability is closely linked to its phenolic and flavonoid contents, which can donate electrons to neutralize free radicals (Stagos, 2020; Kumar et al., 2014; Singh & Rajbir, 2011).

### 3.4. Hepatoprotective Activity

#### 3.4.1. Serum Biochemical Analysis

Serum SGOT and SGPT levels were significantly elevated in the negative control group (NEC) following paracetamol induction, confirming hepatotoxicity. In contrast, rats treated with *S. oleosa* extract at doses of 50, 100, and 150 mg/kg body weight exhibited a significant reduction in these liver enzyme levels ($p < 0.05$), as shown in **Figure 1**.

The most prominent hepatoprotective effect was observed in the 100 mg/kg BW group (ELSE-2), which closely approached the effect of the positive control group (curcumin 100 mg/kg).

Interestingly, the 150 mg/kg BW dose (ELSE-3) resulted in slightly lower protection than ELSE-2, indicating a non-linear dose-response effect, possibly due to biological saturation or metabolic feedback.

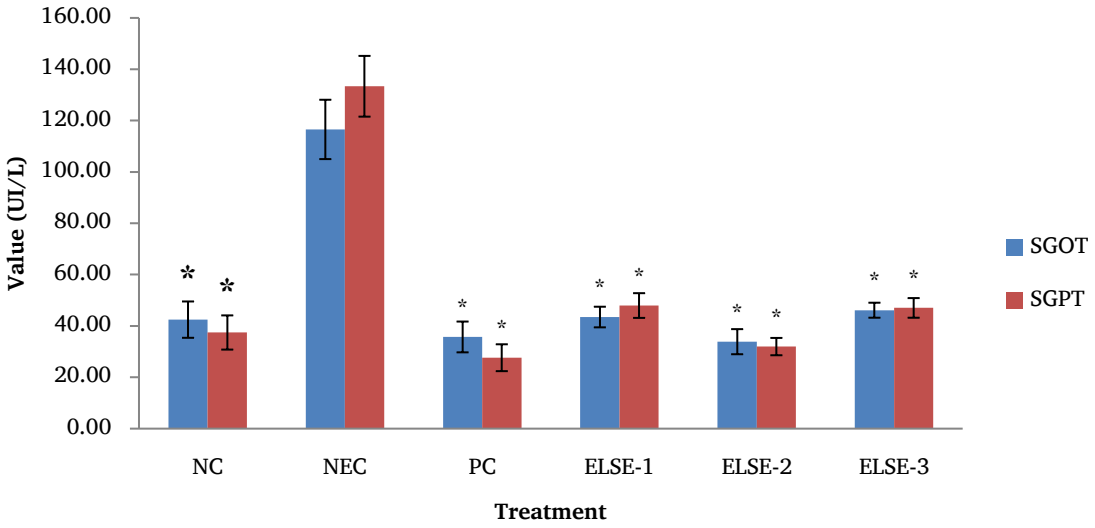


**Figure 1**. Effect of *S. oleosa* Leaf Extract on Serum SGOT and SGPT Levels in Rats. *, the graph shows that all extract-treated groups significantly decreased enzyme levels compared to the NEC group. ELSE-2 (100 mg/kg) showed the strongest hepatoprotective effect, approaching that of curcumin. **NC**: Normal control; **NEC**: Paracetamol only; **PC**: Curcumin; **ELSE-1 to ELSE-3**: Extract doses 50, 100, 150 mg/kg respectively.

### 3.4.2. Histopathological Observations

Histological evaluation further supported the biochemical findings. As shown in **Figure 2**, liver sections from the NEC group displayed extensive pathological alterations, including central vein dilation, hepatocyte necrosis, fatty degeneration, and inflammatory cell infiltration. These findings were consistent with oxidative liver injury induced by paracetamol (Mirza, 2022; Rotundo & Pyrsopoulos, 2020).

Conversely, the PC and extract-treated groups, especially ELSE-2, demonstrated preserved hepatic architecture with minimal histological damage, closely resembling the normal control. The histological features are summarized in **Table 1**.

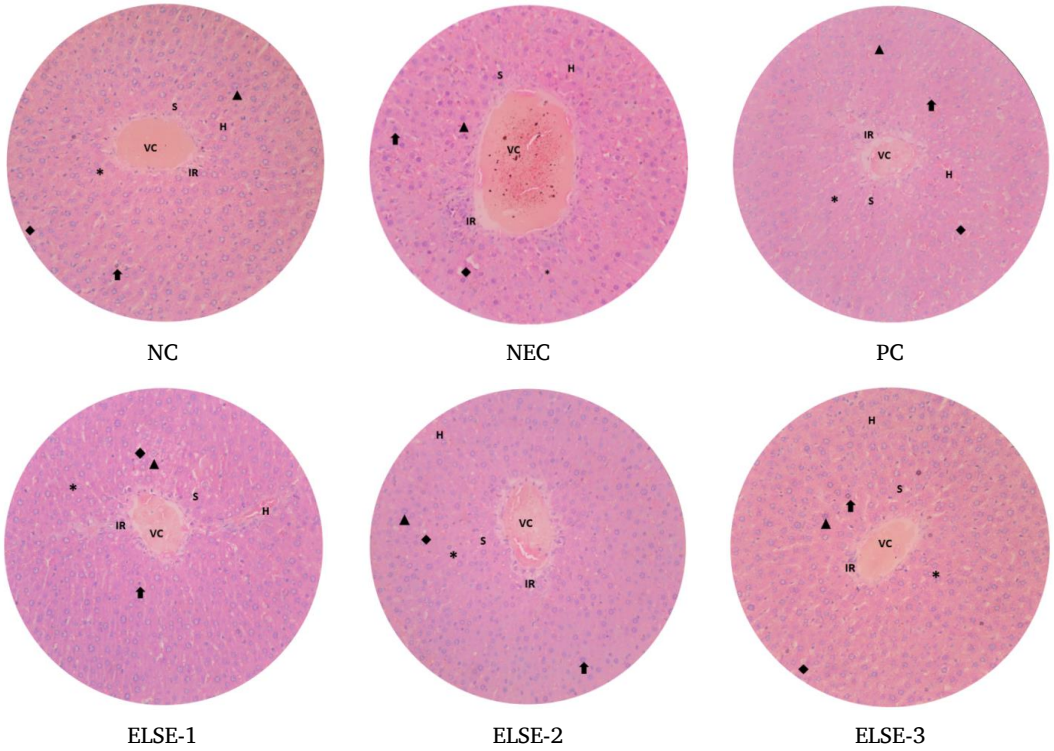


**Figure 2.** Histological Liver Sections of Rats After Treatment (H&E staining, ×400). VC: Central vein, S: Sinusoids, **IR**: Inflammatory cell infiltration, **H**: Bleeding ✶: Normal cells, ⬆: Necrosis, ▲: Hydropic degeneration, ◆: Fatty degeneration. **NC**: Normal control; **NEC**: Paracetamol only; **PC**: Curcumin; **ELSE-1 to ELSE-3**: Extract doses 50, 100, 150 mg/kg respectively.

**Table 1.** Quantitative and Qualitative Histopathological Analysis of Rat Liver Tissues

| Group | Number of Cells Observed/1000 Cells | | | | Cell Observation | | | |
|---|---|---|---|---|---|---|---|---|
| | Normal | Necrosis | Hydropic Degeneration | Degeneration Fatty | Central Vein | Sinusoids | Bleeding | Inflammatory Cell Infiltration |
| NC | 852 | 66 | 49 | 33 | Normal | Normal | Local | Local |
| NEC | 792 | 104 | 63 | 41 | Lesions | Widen | Local | Spread |
| PC | 836 | 81 | 51 | 32 | Normal | Normal | Local | Local |
| ELSE-1 | 824 | 82 | 58 | 36 | Normal | Normal | Local | Local |
| ELSE-2 | 822 | 85 | 58 | 35 | Normal | Normal | Local | Local |
| ELSE-3 | 810 | 93 | 60 | 37 | Normal | Normal | Local | Local |

Note: NC (Normal control); NEC (Negative control); PC (Positive control); ELSE-1 (S. oleosa leaf extract, dose 50 mg/kg); ELSE-2 (S. oleosa leaf extract, dose 100 mg/kg); ELSE-3 (S. oleosa leaf extract, dose 150 mg/kg).

The hepatoprotective effect of *S. oleosa* is likely attributed to its antioxidant capacity. Paracetamol is metabolized into NAPQI, a reactive intermediate that depletes glutathione and induces oxidative stress and hepatocellular damage (Mirza, 2022; Rotundo & Pyrsopoulos, 2020). Flavonoids and phenolics in the extract may neutralize these radicals and support enzymatic defenses, thereby preventing liver injury (Vona et al., 2021; Khan et al., 2019).

## 4. CONCLUSION

The findings of this study demonstrate that *S. oleosa* leaf extract exhibits strong antioxidant activity and confers significant hepatoprotective effects against paracetamol-induced liver injury in rats. These protective effects are likely attributed to the presence of phenolic and flavonoid compounds known for their free radical scavenging properties. The 100 mg/kg BW dose showed the most pronounced efficacy, both biochemically and histologically, comparable to the positive control (curcumin). These results highlight the potential of *S. oleosa* as a promising candidate for the development of hepatoprotective herbal therapeutics. However, further studies are warranted to elucidate its mechanisms of action, evaluate long-term safety, and validate its efficacy in clinical settings before progressing toward pharmaceutical formulation.

## AUTHOR CONTRIBUTIONS

Z. was responsible for project design and preparation, resource provision, and manuscript revision, and conducted the research, including extraction, phytochemical screening of extracts, hepatoprotective activity assays, data analysis, and drafting of the manuscript. A.U.M. performed the determination of total phenolic and flavonoid contents, antioxidant activity assays, and contributed to manuscript drafting. A.B. assisted in extraction, phytochemical screening of extracts, and analysis of research data. All authors have read and approved the final version of the manuscript for publication.

## INSTITUTIONAL REVIEW BOARD STATEMENT

Ethical approval for the study was granted by the Research Ethics Committee of Universitas Padjadjaran (Approval No. 498/UN6.KEP/EC/2020).

## INFORMED CONSENT STATEMENT

This study did not involve human subjects.

## DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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