© 2025 Journal of Pharmacy & Pharmacognosy Research, 13 (4), 1232-1244, 2025
ISSN 0719-4250
https://jppres.com

DOI: https://doi.org/10.56499/jppres24.2242_13.4.1232

*Original Article*

# Spray-dried *Centella asiatica* ethanol extract preserves liver functions in Gal/LPS-induced hepatitis rats through anti-inflammatory and antioxidant properties

[Extracto etanólico de *Centella asiática* secado por aspersión preserva las funciones hepáticas en ratas con hepatitis inducida por Gal/LPS a través de propiedades antiinflamatorias y antioxidantes]

**Tri Yuliani[1*], Indah D. Dewijanti[1], Marissa Angelina[1], Sofa Fajriah[1], Rizna T. Dewi[1], Gian Primahana[1], Ahmad Randy[1], Salsabila Fitriani[2], Yulvian Sani[3]**

[1]Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN) Komplek Cibinong Science Center – BRIN. Jln. Raya Bogor Km 46, Cibinong 16911, Bogor, Jawa Barat, Indonesia.
[2]Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Riau, Pekanbaru, Riau 28293, Indonesia.
[3]Research Centre for Veterinary Science, National Research and Innovation Agency (BRIN) Komplek Cibinong Science Center – BRIN. Jln. Raya Bogor Km 46, Cibinong 16911, Bogor, Jawa Barat, Indonesia.
*E-mail: triy014@brin.go.id

## Abstract

*Context*: The hepatoprotective activity of *Centella asiatica* is very well described. However, it is often administered to the animals before prominent damage to the liver occurs.

*Aims*: To evaluate the hepatoprotective activity of spray-dried *Centella asiatica* ethanol extract (CSD) administered to the animals after prominent liver damage.

*Methods*: Sprague-Dawley rats (n = 56) were divided into 7 groups, i.e., normal control, negative control, positive control, comparative control, low dose of CSD, medium dose of CSD, and high dose of CSD. A combination of Gal 300 mg/kg and LPS 10 µg/kg was used to induce acute liver injury/hepatitis. Methylprednisolone (50 mg/kg; i.p) was used as a positive control (given at 2 hours after injury) and comparative control (given at 8 hours after injury). Low dose of CSD (113 mg/kg; p.o), medium dose of CSD (450 mg/kg; p.o), and high dose of CSD (900 mg/kg; p.o) were given at 8 hours after injury.

*Results*: Serum alanine transaminase (ALT) was dramatically increased, indicating prominent liver damage six hours after the injury. CSD (450 mg/kg; p.o) administered 8 hours after the injury reduced the gamma-glutamyl transferase (GGT) and the total bilirubin in serum by *ca.* 40%-50% compared to the hepatitis group. Although the reduction of GGT was not significant, it appeared almost similar to methylprednisolone. Interestingly, histopathological findings revealed that there was significant regeneration of liver epithelial cells and reduction of cell infiltration with CSD administration. CSD also reversed the changes in both serum superoxide dismutase level and liver interleukin-6 expression.

*Conclusions*: These data indicate that CSD could alleviate acute hepatitis progression *in vivo*, possibly through its antioxidant and anti-inflammatory properties.

*Keywords*: *Centella asiatica*; galactosamine; inflammation; lipopolysaccharide; liver; superoxide dismutase.

## Resumen

*Contexto*: La actividad hepatoprotectora de la *Centella asiática* está bien descrita. Sin embargo, a menudo se administra a los animales antes de que se produzca un daño hepático significativo.

*Objetivos*: Evaluar la actividad hepatoprotectora del extracto etanólico de *Centella asiática* (CSD) secado por aspersión administrado a los animales después de un daño hepático significativo.

*Métodos:* Ratas Sprague-Dawley (n = 56) se dividieron en 7 grupos: control normal, control negativo, control positivo, control comparativo, dosis baja de CSD, dosis media de CSD y dosis alta de CSD. Se utilizó una combinación de Gal 300 mg/kg y LPS 10 µg/kg para inducir daño hepático agudo/hepatitis. Se utilizó metilprednisolona (50 mg/kg; i.p.) como control positivo (administrado 2 horas después de la lesión) y control comparativo (administrado 8 horas después de la lesión). Se administraron dosis bajas de CSD (113 mg/kg; p.o.), dosis medias de CSD (450 mg/kg; p.o.) y dosis altas de CSD (900 mg/kg; p.o.) 8 horas después de la lesión.

*Resultados*: La alanina transaminasa (ALT) sérica aumentó drásticamente, lo que indica un daño hepático significativo seis horas después de la lesión. La CSD (450 mg/kg; p.o.) administrada 8 horas después de la lesión redujo la gamma-glutamil transferasa (GGT) y la bilirrubina total sérica en aproximadamente un 40%-50% en comparación con el grupo con hepatitis. Aunque la reducción de GGT no fue significativa, pareció casi similar a la de la metilprednisolona. Curiosamente, los hallazgos histopatológicos revelaron una regeneración significativa de las células epiteliales hepáticas y una reducción de la infiltración celular con la administración de CSD. El CSD también revirtió los cambios en los niveles séricos de superóxido dismutasa y en la expresión hepática de interleucina-6.

*Conclusiones*: Estos datos indican que el CSD podría aliviar la progresión de la hepatitis aguda *in vivo*, posiblemente gracias a sus propiedades antioxidantes y antiinflamatorias.

*Palabras Clave*: *Centella asiática*; galactosamina; hígado; inflamación; lipopolisacárido; superóxido dismutasa.

ARTICLE INFO
Received: October 15, 2024.
Accepted: March 10, 2025.
Available Online: April 30, 2025.

AUTHOR INFO
ORCID: 0000-0002-7271-6580 (TY) 0000-0002-9597-1090 (RTD)
0000-0003-4650-7192 (IDD) 0000-0001-8176-3859 (GP)
0000-0003-4367-522X (MA) 0000-0003-3250-1216 (AR)
0000-0002-1758-2102 (SF) 0000-0003-0861-4624 (YS)

**Abbreviations:** ALP: alkaline phosphatase; ALT: alanine transaminase; AST: aspartate aminotransferase; CA: *Centella asiatica*; CSD: *Centella asiatica* ethanol extract; Gal: D-galactosamine; GGT: gamma-glutamyl transferase; HE: hematoxylin-eosin; IL-1β: interleukin-1β; IL-6: interleukin-6; LC-ESIHRMS: Liquid Chromatography–Electrospray Ionization High-Resolution Mass Spectrometry; LPS: lipopolysaccharide; MPT2: methylprednisolone; MPT8: comparative control group that received methylprednisolone eight hours after liver injury induction; qRT-PCR: quantitative real-time PCR; SOD: superoxide dismutase; TNF-α: tumor necrosis factor-α.

## INTRODUCTION

Acute hepatitis is a common health problem worldwide (Asrani et al., 2019). Although most hepatitis cases are caused by viral infection, inflammatory response plays a crucial role in the pathogenesis of the disease (Tilg et al., 2006). The inflammatory response that occurs during acute hepatitis has been modeled in laboratory animals to study the pathogenesis of the disease as well as in search for alternative therapy. One of the animal models commonly used in hepatitis research is the D-galactosamine (Gal) and lipopolysaccharide (LPS) model. This model, despite being unable to resemble all aspects of human disease perfectly, possesses several characteristics that resemble human acute hepatitis, particularly the inflammatory response (Jaeschke, 2000). Gal is a particular hepatotoxic substance that amplifies the harmful effects of LPS (Ben Ari, 2012). The stimulation caused by LPS can initiate the Toll-like receptor four signaling pathway, leading to the activation of NF-κB. This process causes the release of pro-inflammatory cytokines, which are critical for Gal/LPS-induced acute hepatitis progression (Kim et al., 2010). Thus, decreasing inflammation may serve as a preventive measure against the initiation of acute liver failure.

*Centella asiatica* (CA), commonly known as gotu kola or pegagan in Indonesia, is a medicinal herb used traditionally to treat several diseases, such as colicky abdominal pain and skin disorders (Brinkhaus et al., 2000). Its scientific evidence has been reviewed recently for gastrointestinal diseases, immune diseases, endocrine diseases, cardiovascular diseases, respiratory diseases, gynecological diseases, neurological diseases, and skin diseases. The mechanism behind its bioactivities includes anti-inflammatory, anti-oxidative stress, anti-apoptosis, and improvement in mitochondrial function. The principal bioactive components reported from this herb are triterpenes, specifically asiaticoside (the marker compound), asiatic acid, madecassoside, and madecassic acid (Sun et al., 2020).

Like many other medicinal herbs, CA has been reported to have hepatoprotective activities (Lu et al., 2021; Park et al., 2021). However, in the design of the studies, it was often administered before prominent damage to the liver occurred, except in one study by Vidyaniati et al. (2009). In their study, multiple doses of its methanolic extract for three days (200 mg/kg) administered after prominent liver damage induced by carbon tetrachloride were able to ameliorate liver injury, reflected by a decrease of both alanine transaminase (ALT) activity and necrotic area (Vidyaniati et al., 2009).

The present study aims to investigate the potency of CA to alleviate the progression of liver injury in Gal/LPS-induced hepatitis rat model and elucidate the underlying mechanism, particularly the anti-inflammatory and antioxidant activities. It should be noted that CA was administered after significant liver damage was observed. For this purpose, a standardized CA extract was used. The extract was obtained through a spray-drying process of 70% ethanol extract with maltodextrin as the filler. This process resulted in a spray-dried CA ethanol extract (CSD) that met the Indonesian Herbal Pharmacopeia (IHP) standard. The spray-dry process encapsulates the extract and results in a homogenous extract that is protected from environmental conditions, i.e., light, temperature, humidity, and interactions with other substances. Maltodextrin was used due to its gel and film-forming properties, suitability as a binder, and solubility in cold water (Jafari et al., 2023). The ratio of maltodextrin: CA extract used in this study is 1:1. The result of the present study could provide scientific evidence of the traditional use of CA for colicky abdominal pain that is perhaps related to acute hepatitis. Both colicky abdominal pain and hepatitis involve inflammatory response. Therefore, the anti-inflammatory property of CA was also investigated in the present study.

## MATERIAL AND METHODS

### Preparation of spray-dried *Centella asiatica* ethanol extract (CSD)

*Centella asiatica* leaves (CA leaves) were obtained from the Indonesian Spice and Medicinal Crops Research Institute (Sukabumi, Indonesia, 6°46'29.9"S 106°48'22.9"E) and identified at the Herbarium Bogoriense Research Center for Biology-National Research and Innovation Agency (BRIN) (Bogor, Indonesia) (voucher number CA01). The CA leaves were dried at 50°C with air circulation, sorted, and ground. Extraction was performed through maceration and percolation using 70% ethanol at room temperature. The extract was then concentrated at 50°C to attain a total soluble solids value of 10%. Maltodextrin filler was introduced to the concentrated extract at a ratio

of 1:1 and stirred for 2 hours at 600 rpm. The resulting mixture was processed in a spray dryer (Closed Cycle Spray Dryer; model: CL-8, Ohkawara Kakohki, Japan) with a feed flow rate of 500 mL/h and an inlet temperature of 150°C (Krishnaiah et al., 2015). The resulting spray-dried CSD was collected, weighed, packed into a sealed container, and stored in a dry place. A non-specific analysis as well as asiaticoside content in CSD was performed according to Indonesian Herbal Pharmacopoeia (Ministry of Health, Republic Indonesia, 2008).

### Identification of chemical compounds in *Centella asiatica* ethanol extract

Chemical profiling of the crude extract of CSD was conducted using Liquid Chromatography–Electrospray Ionization High-Resolution Mass Spectrometry (LC-ESIHRMS) on Waters Xevo-G2 XS QToF (Waters, Milford, Massachusetts, USA) equipped with Waters BEH C18 column (2.1 × 50 mm, 1.7 µm) as a stationary phase and the mobile phases consisted of acetonitrile (B) and Milli-Q water (A) supplemented with 0.1% formic acid. Gradient elution from 5% B in 1 min, followed by increasing to 100% B in 10 minutes, holding at 100% B for 3 min, and finally bringing back to the initial gradient for 3 min, with a total run of 17 min and a flow rate of 0.3 mL/min. Each run was compared to a blank sample, and the injection volume was 1 µL. The UNIFI software version 1.5 was used to process data and tentatively determine molecular formulae by performing isotope abundance analysis and reporting the best-fitting empirical formula. Waters built-in library version 1.8 and Dictionary of Natural Products, released version 29.2, were used as database searches. The results from the databases searched were reviewed for compounds identified from the analyzed genera with molecular formulas or masses corresponding to the LC-HRMS data. Any matches were investigated by comparing the literature and the experimental data. Tentative compound assignments were made when matches were identified. The MS conditions were as follows: column temperature 40°C, mass range: 100–1200 Da, cone voltage 30 V, capillary 2kV, source temperature 120°C, desolvation temperature 500°C, cone gas flow 50 L/h, desolvation gas flow 1000 L/h, collision energy (ramp: 10–40 eV). Leucine enkephaline was used as an internal mass correction, infused every 10 s during the whole run (Swandiny et al., 2023).

### Animals

The animal experiment protocol was approved by the ethical committee of the National Research and Innovation Agency (76/Klirens/VIII/2021). Male Sprague-Dawley rats (250-350 g; 8-10 weeks) were obtained from the animal laboratory of the National Agency for Drug and Food Control of Indonesia. The animals were fed on a standard chow diet and water freely. They were housed under normal laboratory conditions (23 ± 2°C, 12 h light–dark cycle) with continuous access to food and water.

### Chemicals

D-(+)-Galactosamine hydrochloride (Gal) was purchased from Tokyo Chemical Industry (Japan; G0007). Lipopolysaccharides (LPS) (*E.Coli* O111:B4) were purchased from Sigma (Germany; L2630). Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and total bilirubin estimation kits were purchased from ELITech Clinical Systems (France). Methylprednisolone sodium succinate (MP; Dexa Medica) was purchased from a local pharmacy.

### Induction of acute liver injury

The 56 healthy male Sprague-Dawley rats were divided into seven groups (n = 8 per group). The groups consist of 4 controls and 3 test substances (CSD). The control groups were normal, negative control (acute liver injury/hepatitis; Gal (300 mg/kg; i.p) followed immediately by LPS (10 µg/kg; i.p); Gal/LPS), positive control (acute liver injury/hepatitis, treated with methylprednisolone (50 mg/kg; i.p; once) at 2 hours after liver injury induction; Gal/LPS + MPT2), and comparative control (acute liver injury/hepatitis, treated with methylprednisolone (50 mg/kg; i.p; once) at 8 hours after liver injury induction; Gal/LPS + MPT8). The test substance groups received low-, medium-, and high-doses of CSD suspended in water orally once at 8 hours after liver injury induction, i.e., 113 mg/kg (as Gal/LPS + low CSD T8), 450 mg/kg (as Gal/LPS + medium CSD T8), 900 mg/kg (as Gal/LPS + high CSD T8). The doses for CSD were equivalent to CA ethanol extract of 50 mg/kg, 200 mg/kg, and 400 mg/kg and were chosen because of previous studies (Choi et al., 2016; Park et al., 2021; Vidyaniati et al., 2009). Of note, the chosen dose was also tested *in vivo* using a mouse model of paracetamol overdose (unpublished). The methylprednisolone dose was chosen based on Higuchi et al. (2007).

Acute liver injury/hepatitis was induced by Gal/LPS injection after overnight fasting (12 hours) with free access to water (Wang et al., 2014). A pelleted diet was given 2 hours after the liver injury induction. Six hours after induction of liver injury, the blood samples were collected from the orbital sinus under isoflurane anesthesia (5%). A freshly prepared serum was used for ALT estimation spectrophotometrically using the Microlab 300 clinical system

**Table 1.** Primers used in qRT-PCR experiments.

| Primer | Nucleotide sequence (5' – 3') |
|---|---|
| Rats GAPDH Forward | TCCCAGCTTAGGTTCATCAGG |
| Rats GAPDH Reverse | CCAATACGGCCAAATCCGTTC |
| Rats TNF Forward | CCCACGTCGTAGCAAACCA |
| Rats TNF Reverse | CTTTGAGATCCATGCCGTTGG |
| Rats IL6 Forward | GACTGGGGATGTCTGTAGCTC |
| Rats IL6 Reverse | CAACTGGATGGAAGTCTCTTGC |
| Rats IL1B Forward | GCCACCTTTTGACAGTGATGAG |
| Rats IL1B Reverse | AGGTTTGGAAGCAGCCCTT |

(ELITech Group, France). Twenty-four hours after the induction of liver injury, all animals were sacrificed by decapitation under isoflurane anesthesia (5%), and blood was collected. The right lobe of the liver was separated and placed in 10% buffered neutral formalin for histology study (hematoxylin-eosin staining). The left lobe of the liver was sampled and kept at -80°C for mRNA study (IL-6, IL-1β, and TNF-α).

### Measurement of blood hepatic enzyme biomarkers

The enzyme activities of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and gamma globulin transferase (GGT), as well as total bilirubin level, were analyzed using the Microlab 300 clinical system (ELITech Group, France) from a serum sample. Superoxide dismutase (SOD) level was also determined from the serum sample (Elabscience®).

### Histopathological liver assay

The right lobe of the liver was collected and immediately fixed in 10% buffered neutral formalin. The samples were then dehydrated in graded ethanol (50% to 100%) series and cleared in xylene before embedding in paraffin. The sections were prepared and stained with hematoxylin and eosin (HE) dyes (Humason, 1979).

### Gene expression analysis

Gene expression was analyzed using quantitative real-time PCR (qRT-PCR) (Nolan et al., 2006). Total RNA was isolated from liver specimens using an easy-red total RNA extraction kit (Intron Biotechnology, Korea), and the RNA quantity was determined by spectrophotometry at 260 nm. The cDNA synthesis and the PCR step were conducted in a one-step reverse transcription PCR. Briefly, 2 µg of total RNA was used for the one-step reverse-transcription PCR using QuantiNova SYBR Green RT-PCR Kit (Qiagen, Germany). The mRNA level of genes related to inflammation was detected using specific oligonucleotide primers for rats interleukin-6 (IL-6), interleukin 1-beta (IL-1β), and TNF-α (Table 1). The one-step reaction was performed using an AriaMX real-time PCR system (Agilent, USA) with temperature programming according to the QuantiNova SYBR Green RT-PCR kit recommendation.

Relative gene expression was quantified using a comparative Ct values method ($2^{-\Delta\Delta Ct}$) to compare the treated group with the normal untreated group (Livak and Schmittgen, 2001). Rat GAPDH primers were used as an internal control for data normalization.

### Statistical analysis

The GraphPad Prism 5 (San Diego, CA) was used to analyze the data using one-way analysis of variance (One-way ANOVA) followed by Tukey's multiple comparison tests to compare means between groups. The calculated values were presented as mean ± SEM. A p-value of less than 0.05 was considered statistically significant.

## RESULTS

### Chemical constituents in *Centella asiatica* ethanol extract

According to the survey of Dictionary Database online (https://dnp.chemnetbase.com, accessed on October 2024), 57 compounds have been isolated from *Centella asiatica*, which belong to the group of isoprenoids (sesquiterpenes, plant sterols, pentacyclic triterpenoids, and saponins) and phenylpropanoid derivatives (eugenol derivatives, caffeoylquinic acids, and flavonoids) (Arifin et al., 2011). Analysis of the crude extract of CSD by using Liquid Chromatography – Electrospray Ionization High-Resolution Mass Spectrometry tentatively identified eleven compounds. These tentatively identified compounds include asiaticoside, flavonoids, and caffeoylquinic acids (Fig. 1, Table 2).

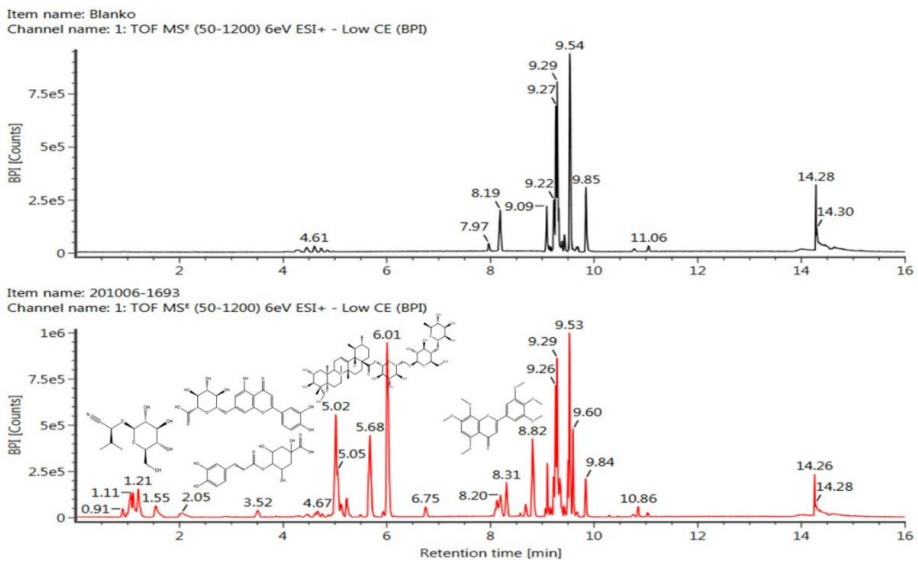


**Figure 1.** Chemical profiling of crude CSD extract was conducted using liquid chromatography–electrospray ionization with high-resolution mass spectrometry (LC-ESIHRMS).

CSD: Spray-dried *Centella asiatica* ethanol extract; BPI: Base peak intensity; CE: Collision energy.

**Table 2.** Chemical constituents present in the crude extract of CSD (tentatively assigned).

| No. | Compound | Mol. formula | *m/z* (theoretical) | *m/z* (experimental) | Δ*m* (mDa) | $t_R$ (min) | Fragments (reference) | Relative percentage (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | Heterodendrin | $C_{11}H_{19}NO_6$ | 261.1212 $[M+H]^+$ | 262.1287 | 0.2 | 1.21 | - | 6.87 |
| 2 | 4-O-Caffeoylquinic acid | $C_{16}H_{18}O_9$ | 378.0832 $[M+Na]^+$ | 377.0833 | 1.0 | 3.51 | 377 (Kilinç, 2021) | 0.41 |
| 3 | Quercetin-3-O-β-D-glucuronide | $C_{21}H_{18}O_{13}$ | 478.0747 $[M+H]^+$ | 479.0825 | 0.5 | 4.67 | 479, 301 (Cioffi et al., 2023) | 3.87 |
| 4 | Luteolin-7-O-glucuronide | $C_{21}H_{18}O_{12}$ | 462.0798 $[M+H]^+$ | 463.0870 | 0.1 | 5.02 | 463, 337, 287 (Kilinç, 2021) | 32.45 |
| 5 | 5-Hydroxy-6,4'-dimethoxy-flavone-7-O-β-D-glucopyranoside | $C_{23}H_{24}O_{11}$ | 476.1318 $[M+Na]^+$ | 499.1234 | 2.4 | 5.04 | - | 22.32 |
| 6 | 3β-Hydroxy-11α,12α-epoxyolean-28-13β-olide | $C_{30}H_{46}O_5$ | 486.3345 $[M+H]^+$ | 487.3417 | 0.1 | 5.68 | - | 12.45 |
| 7 | Asiaticoside | $C_{48}H_{78}O_{19}$ | 958.5137 $[M+H]^+$ | 981.5010 | 1.9 | 6.02 | 959, 797, 635, 453 (Gupta et al., 2018) | 10.56 |
| 8 | 5,7,2',5'-Tetrahydroxy-flavone | $C_{15}H_{10}O_6$ | 286.0477 $[M+H]^+$ | 287.0552 | 0.2 | 6.76 | - | 4.08 |
| 9 | 3',4',5',5,7,8-Hexame-thoxy flavone | $C_{21}H_{22}O_8$ | 402.1314 $[M+H]^+$ | 403.1379 | 0.8 | 8.03 | - | 1.53 |
| 10 | 3,5,6,7,8,3',4'-Heptemethoxyflavone | $C_{22}H_{24}O_9$ | 432.1420 $[M+H]^+$ | 433.1488 | 0.5 | 8.58 | - | 2.31 |
| 11 | 3β,6β,23-Trihydroxy-urs-12-en-28-oic acid | $C_{30}H_{48}O_5$ | 488.3501 $[M+Na]^+$ | 511.3392 | 0.2 | 8.82 | - | 3.14 |

**Table 3.** Evaluation of spray-dried CA ethanol extract (CSD) according to Indonesian Herbal Pharmacopoeia (IHP).

| Parameter | IHP | CSD |
|---|---|---|
| Asiaticoside | >0.90% | 3% |
| Loss on drying | < 10% | 1.52±0.1 |
| Total Ash (%) | < 16.6% | 12.30±1.48 |
| Acid-insoluble ash (%) | < 2.3% | 1.37±0.47 |
| Heavy metal determination* | | |
| Mercury (Hg) | Not detected | Not detected |
| Lead (Pb) | Not detected | Not detected |
| Cadmium (Cd) | Not detected | Not detected |
| Arsenic (As | Not detected | Not detected |
| Microbial contamination | | |
| Test for *Escherichia coli*/g | Absent | Absent |
| Test for *Staphylococcus aureus*/g | Absent | Absent |
| Test for *Salmonella spp*/g | Absent | Absent |
| Test for *Pseudomonas aeruginosa*/g | Absent | Absent |
| Test for *Escherichia coli*/g | Absent | Absent |

*Limit of detection (mg/kg): Pb ≤ 10, Cd ≤ 0.3, Hg ≤ 0.5, As ≤ 5.

### Evaluation of spray-dried *Centella asiatica* ethanol extract (CSD) according to Indonesian Herbal Pharmacopoeia (IHP)

The extraction process and solvent selection are primary concerns in obtaining active compounds from natural materials for therapeutic purposes. Essential considerations include solubility, selectivity, safety, and cost. Typically, phytochemical studies use ethanol and methanol as universal solvents due to their high solvation capabilities and volatile properties, facilitating easy separation from the extracted compounds (Okselni et al., 2023). Ethanol at a concentration of 70% is an effective solvent for extracting hydrophilic (water-soluble) and lipophilic (fat-soluble) compounds from natural materials. It also helps to maintain compound stability throughout the extraction process and enhances efficiency in the industrial-scale extraction process. The spray-drying process of CA extract was chosen to preserve the homogeneity and stability of the active compounds within the extract and to assist in formulating CA extract into standardized herbal medicine. The spray-dried CA ethanol extract (CSD) used in the present study met the standards established by the Indonesian Herbal Pharmacopoeia (Ministry of Health, Republic of Indonesia, 2008; Table 3).

### The effects of spray-dried *Centella asiatica* ethanol extract (CSD) on blood hepatic enzyme biomarkers

Blood hepatic enzyme tests usually include measurement of the activities of ALT, AST, ALP, and GGT, as well as total bilirubin levels. ALT and AST in serum reflect liver injury, while GGT reflects liver synthesis functions. The total bilirubin level reflects liver excretory function (Lala et al., 2023). In this study, six biomarkers were applied to determine the hepatocyte injury.

The hepatoprotective effect of spray-dried *Centella asiatica* ethanol extract (CSD) after prominent liver damage was investigated using the Gal/LPS model. Liver damage was detected at 6 hours after the liver injury induction by a dramatic increase of serum ALT activity (>8 times; Fig. 2A. ALT normal 56 ± 3 IU/L, Gal/LPS 456 ± 64 IU/L, low CSD 423 ± 62 IU/L, medium CSD 553 ± 75 IU/L, high CSD 400 ± 63 IU/L, MPT8 562 ± 45 IU/L, and MPT2 454 ± 90 IU/L; $p<0.05$ compared to normal). Even the positive control group, i.e., the MPT2 groups that were given methylprednisolone at two hours after liver injury induction, showed an almost similar increase in ALT activity. After the ALT activity determination, four groups received different treatments, i.e., CSD low, medium, high, and methylprednisolone, while the rest of the three groups (i.e., normal, negative, and MPT2) did not receive anything at this point, i.e., eight hours after liver injury induction.

An interesting result was observed 24 hours after the liver injury induction, i.e., after sacrifice. Dramatic increases of serum liver injury markers were observed in the negative control group (Fig. 2B-F), ALT normal 61 ± 3 IU/L vs. Gal/LPS 13823 ± 1525 IU/L, p<0.05; AST normal 137 ± 6 IU/L vs. Gal/LPS 11874 ± 1980 IU/L, p<0.05; ALP normal 452 ± 30 IU/L vs. Gal/LPS 999 ± 40 IU/L, p<0.05; and total bilirubin normal 0.4 ± 0.3 IU/L vs. Gal/LPS 10 ± 1 IU/L p<0.05).

Administered two hours after liver injury induction as a positive control, methylprednisolone (MPT2) showed significant protection compared to the negative control in all biomarkers except for the AST activity, in which a slight reduction occurred. Meanwhile, in the comparative control group (MPT8) that received methylprednisolone eight hours after liver injury induction, less hepatoprotection observed in which only total bilirubin was reduced significantly compared to the negative control (Fig. 2B-F), ALT Gal/LPS 13823 ± 1525 IU/L vs. MPT2 5729 ± 1949 IU/L, p<0.05, vs. MPT8 10681 ± 1612 IU/L, p>0.05; AST Gal/LPS 11874 ± 1980 IU/L vs. MPT2 8109 ± 2294 IU/L, p<0.05, vs. MPT8 12606 ± 1528 IU/L p>0.05; ALP Gal/LPS 999 ± 40 IU/L vs. MPT2 777 ± 74 IU/L, p<0.05, vs. MPT8 969 ± 56 IU/L p>0.05; total bilirubin Gal/LPS 10 ± 1 vs. MPT2 0.7± 0.3 mg/dL p<0.05, vs. MPT8 1 ± 0.2 mg/dL p<0.05). This data indicated that the effect of methylprednisolone was time-dependent in which earlier treatment showed better liver protection.

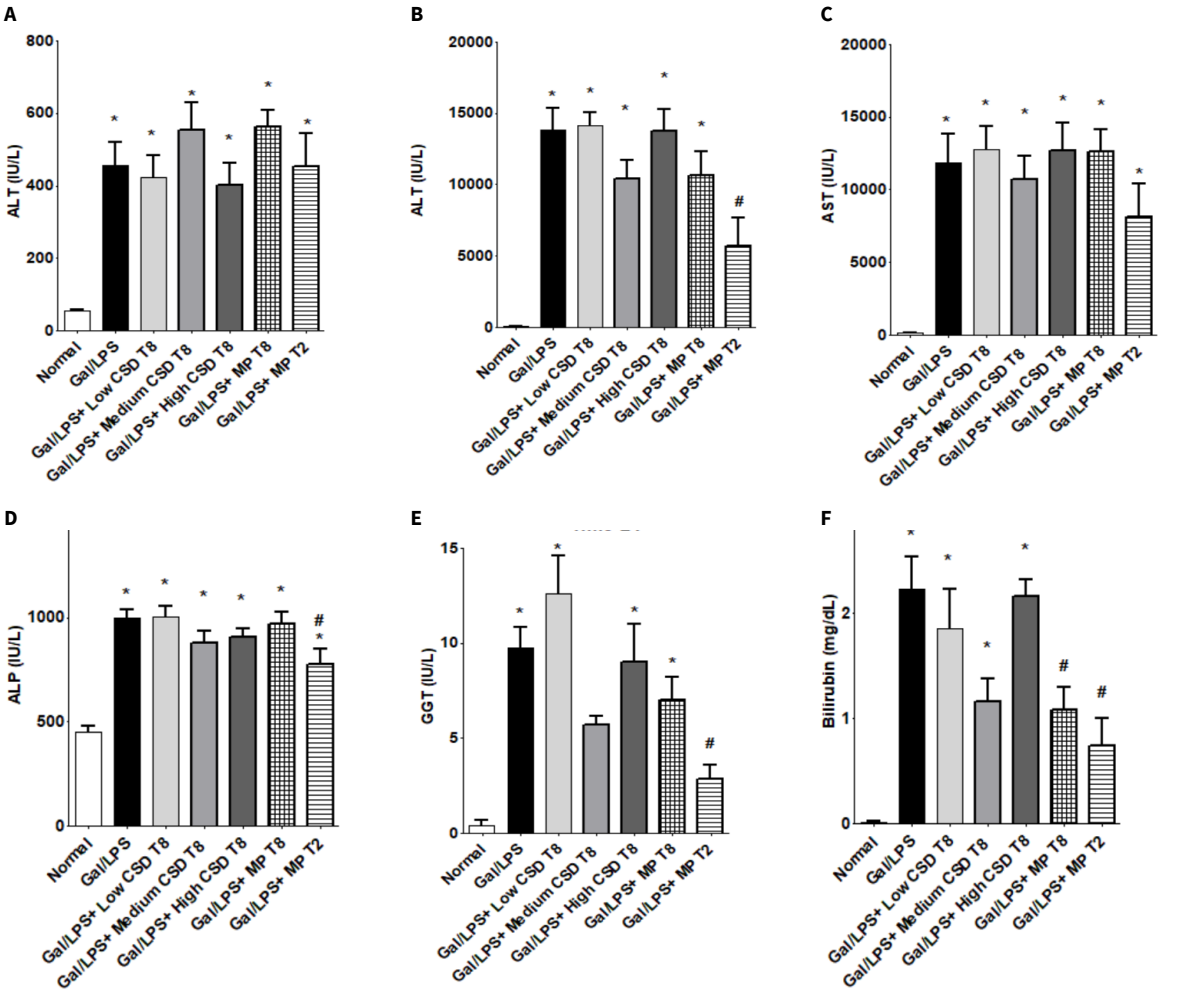


**Figure 2.** Blood hepatic enzyme biomarkers.

**(A)** Serum ALT activity six hours after liver injury induction. **(B-F)** Activities of ALT, AST, ALP, and GGT, as well as total bilirubin level, respectively, in serum 24 hours after liver injury induction.

Data are expressed as means ± SEM (n = 8) and were analyzed using one-way ANOVA. *Significantly different from normal group (p<0.05). #Significantly different from Gal + LPS (p<0.05). Gal/LPS: D-(+)-galactosamine hydrochloride (300 mg/kg; i.p) followed immediately by lipopolysaccharides (10 µg/kg; i.p); CSD: spray-dried *Centella asiatica* ethanol extract; Low CSD: CSD 113 mg/kg; p.o.; Medium CSD: CSD 450 mg/kg; p.o.; High CSD: CSD 900 mg/kg; p.o.; MP: methylprednisolone (50 mg/kg; i.p.); T2: 2 hours after Gal/LPS injection; T8: 8 hours after Gal/LPS injection; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase.

For CSD groups that received either a low-, medium-, or high-dose of CSD eight hours after liver injury induction, a bell-shaped dose-response was observed, in which the medium dose showed the best liver protection in all biomarkers. In Fig. 2B, although the level of ALT was not significantly different compared to negative control, medium dose of CSD could reduce ALT activity by *ca.* 25%, an almost similar level to the comparative control (Gal/LPS 13823 ± 1525 IU/L; low CSD 14162 ± 907 IU/L, p>0.05; medium CSD 10400 ± 1316 IU/L, p>0.05; high CSD 13788 ± 1500 IU/L, p>0.05; MPT8 10681 ± 1612 IU/L, p>0.05). Although the levels of AST, ALP, and GGT were not significantly different compared to negative control in Fig 2C-E, a medium dose of CSD appeared to show better protection based on the activities of AST, ALP, and GGT than comparative control (MPT8) (Fig 2C) AST Gal/LPS 11874 ± 1980 IU/L; low CSD 12783 ± 1602 IU/L, p>0.05; medium CSD 10720 ± 1565 IU/L, p>0.05; high CSD 12681 ± 1891 IU/L, p>0.05; MPT8 12606 ± 1528 IU/L, p>0.05. Fig 2D shows that ALP Gal/LPS 999 ± 40 IU/L; low CSD 1003 ± 50 IU/L, p>0.05; medium CSD 880 ± 52 IU/L, p>0.05; high CSD 909 ± 35 IU/L, p>0.05; MPT8 969 ± 56 IU/L, p>0.05. In Fig 2E also is exhibited that GGT Gal/LPS 10 ± 1 IU/L; low CSD 13 ± 2 IU/L, p>0.05; medium CSD 6 ± 0.4 IU/L, p>0.05; high CSD 9 ± 2 IU/L, p>0.05; MPT8 7 ± 1.2 IU/L, p>0.05). An almost similar effect of a medium dose of CSD to comparative control (MPT8) was also observed at the total bilirubin level. Although only significant in MPT8, both of them could reduce total bilirubin level to *ca.* 50% (Fig. 2F, total bilirubin Gal/LPS 2 ± 0.3 mg/dL; low CSD 2 ± 0.4 mg/dL, p>0.05; medium CSD 1 ± 0.2 mg/dL, p>0.05; high CSD 2 ± 0.2 mg/dL, p>0.05; MPT8 1 ± 0.2 mg/dL, p<0.05).

**Spray-dried *Centella asiatica* ethanol extract (CSD) inhibits histopathological liver injury**

An interesting result was also observed 24 hours after the liver injury induction on liver histology stained with hematoxylin-eosin (Fig. 3A-H). A significant increase in liver injury was observed in the negative control group (Gal/LPS). Administered two hours after liver injury induction as a positive control (MPT2), methylprednisolone showed significant protection compared to the negative control (Gal/LPS). The effect of methylprednisolone was time-dependent, in which earlier treatment showed better liver protection. Meanwhile, for CSD, a dose-dependent effect was observed, in which both medium and high doses of CSD could ameliorate liver injury significantly (Fig. 3H; histology score normal 1.8 ± 0.3; Gal/LPS 3.9 ± 0.3, p<0.05; low CSD 2.6 ± 0.7, p>0.05; medium CSD 1.3 ± 0.3, p<0.05; high CSD 0.9 ± 0.3, p<0.05; MPT8 2 ± 0.5, p>0.05; and MPT2 1.1 ± 0.4, p<0.05).

**The effects of spray-dried *Centella asiatica* ethanol extract (CSD) on inflammatory factors of liver tissue**

The *in vivo* anti-inflammatory effect of medium doses of CSD was determined by analyzing the liver mRNA levels of inflammatory markers such as IL-6, TNF-α, and IL-1β by qRT-PCR. The CSD appeared to inhibit the inflammatory response in the rat model induced by Gal/LPS. As shown in Table 4, Gal/LPS treatment induced inflammation demonstrated by the increased mRNA levels of IL-6 (p<0.05; compared to normal). The inhibition of inflammatory response by CSD was particularly demonstrated by significantly lower levels of IL-6 mRNA (p<0.05; compared to Gal/LPS). The effect of CSD on inflammatory markers was comparable with methylprednisolone (p<0.05), an anti-inflammatory drug used as a positive control in this experiment. However, unlike the IL-6, post-treatment with CSD did not inhibit the production of TNF-α and IL-1b in the Gal/LPS model (p>0.05; compared to Gal/LPS).

**The effects of spray-dried *Centella asiatica* ethanol extract (CSD) on systemic oxidative stress status**

An interesting result was also noted 24 hours after the liver injury induction on serum SOD level (Fig. 4), a marker of oxidative stress. A significant increase of SOD level was found in the negative control group (normal 0.15 ± 0.01 ng/mL vs. Gal/LPS 0.24 ± 0.02 ng/mL; p<0.05). Methylprednisolone given either at two hours (MPT2 0.18 ± 0.01 ng/mL, p<0.05) or 8 hours (MPT8 0.18 ± 0.01 ng/mL; p<0.05) after liver injury induction reduced SOD level significantly compared to the negative control. Although the SOD levels were not significantly different, the medium dose of CSD could reduce SOD levels almost similar to methylprednisolone treatment, i.e., reduced by *ca.* 20% (both MPT2 and MPT8) compared to the negative control (CSD 0.19 ± 0.01 ng/mL; p>0.05).

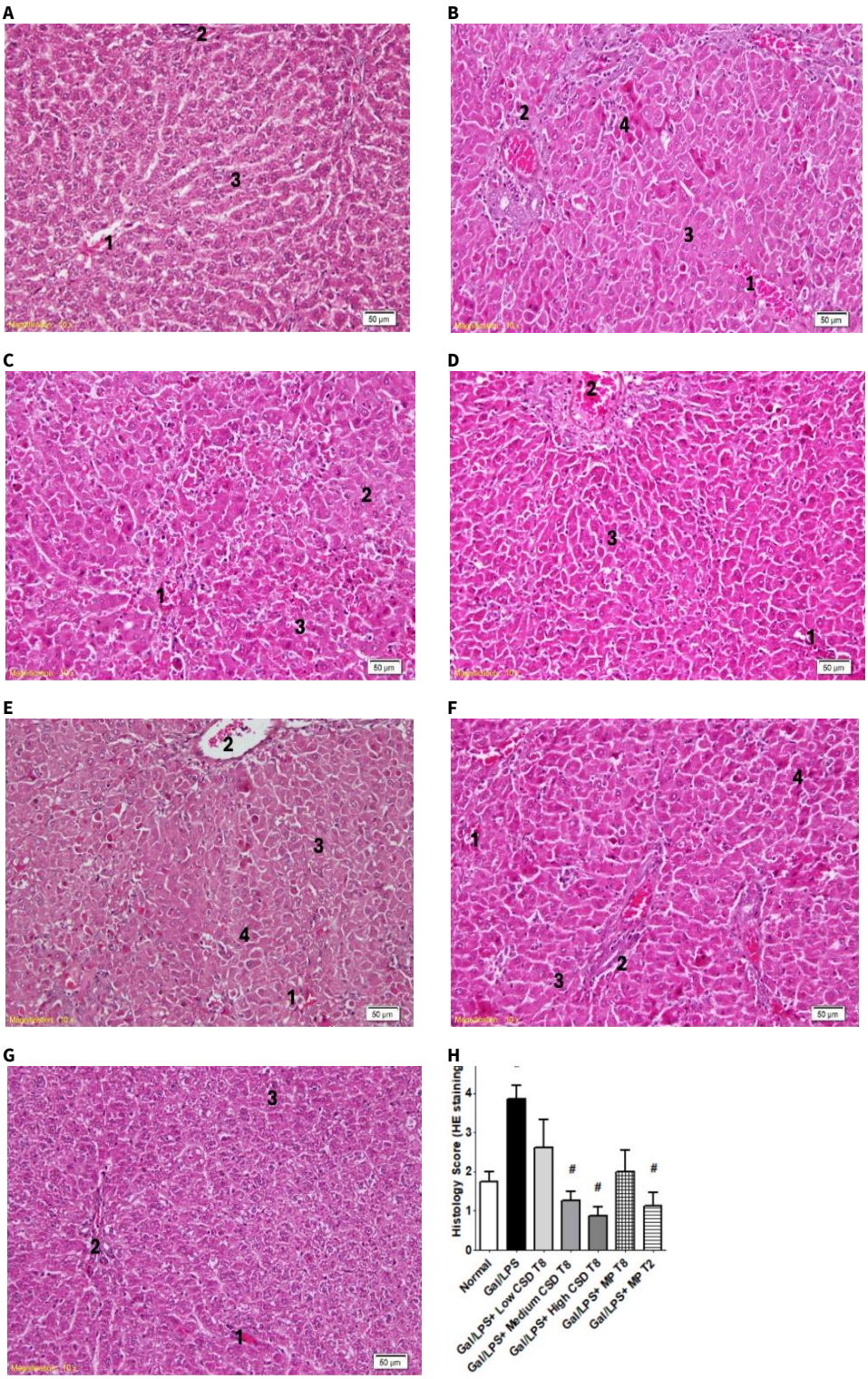


**Figure 3**. Representative photomicrographs of liver tissue of rats.

**(A)** Normal group. No pathological changes in the liver. No specific abnormality: 1. Vena centralis, 2. Portal tract, and 3. Epithelial cells of the liver. **(B)** Gal/LPS group. The liver shows hepatic necrosis and choledictis: 1. Vena centralis, 2. Necrosis and infiltration of mononuclear cells (lymphocytes and macrophages) in the portal tract, 3. Liver epithelial cells, and 4. Regeneration of hepatic epithelial cells. **(C)** Low dose of CSD with Gal/LPS. The liver shows hepatic regeneration: 1. Vena centralis, 2. Liver epithelial cells, and 3. Regeneration of liver epithelial cells (basophilic). **(D)** Medium dose of CSD with Gal/LPS. The liver shows hepatic regeneration: 1. Vena centralis, 2. Portal tract, 3. Liver epithelial cells, and 4. Regeneration of liver epithelial cells (basophilic). **(E)** High dose of CSD with Gal/LPS; hepatic. The liver shows hepatic regeneration: 1. Vena centralis, 2. Portal tract, 3. Liver epithelial cells, and 4. Regeneration of liver epithelial cells (basophilic). **(F)** Methylprednisolone (MPT8) with Gal/LPS. The liver appears to have no specific pathological changes, no specific abnormality: 1. Vena centralis, 2. Portal tract, and 3. Epithelial cells of the liver. **(G)** Methylprednisolone (MPT8) with Gal/LPS. The liver showed no specific pathological changes, no specific abnormality: Vena centralis, 2. Portal tract, and 3. Epithelial cells of the liver. **(H)** Histopathological changes in liver tissue were quantified by lesion scores according to the severity of the lesion, at 24 h.

Data are expressed as means ± SEM (n = 8) and were analyzed using one-way ANOVA. *Significantly different from Gal + LPS ($p < 0.05$), #Significantly different from normal ($p < 0.05$). Gal/LPS: D-(+)-galactosamine hydrochloride (300 mg/kg; i.p) followed immediately by lipopolysaccharides (10 µg/kg; i.p); CSD: spray-dried *Centella asiatica* ethanol extract; Low CSD: CSD 113 mg/kg; p.o.; Medium CSD: CSD 450 mg/kg; p.o.; High CSD: CSD 900 mg/kg; p.o.; MP: methylprednisolone (50 mg/kg; i.p); T2: 2 hours after Gal/LPS injection; T8: 8 hours after Gal/LPS injection; HE: hematoxylin and eosin. HE staining, magnification 200×.

## DISCUSSION

The present study aims to test CSD potency in a hepatitis model. To attain more relevant results for clinical settings, we decided that two things should be met: (1) the test substance should be administered after the liver injury occurs (2) a standard therapy that is administered after the liver injury occurs (or expected to occurs) should be able to reduce hepatitis-like symptom in the chosen model. We found that the Gal/LPS-induced hepatitis rat model met these two criteria. A standard therapy (methylprednisolone) that was given two hours after liver injury could alleviate hepatitis-like symptoms in this model (Higuchi et al., 2007).

**Table 4.** The effects of CSD on mRNA levels of inflammatory markers.

| Biomarker | Normal | Gal/LPS | Gal/LPS + Medium CSD T8 | Gal/LPS + MP T2 |
|---|---|---|---|---|
| IL-6 | 1.661 ± 0.762 | 29.51 ± 9.893* | 2.647 ± 0.146# | 1.602 ± 0.683# |
| TNF-α | 1.175 ± 0.188 | 3.877 ± 1.315 | 1.662 ± 0.213 | 2.324 ± 0.569 |
| IL-1β | 1.045 ± 0.187 | 3.792 ± 0.187 | 2.250 ± 0.187 | 1.967 ± 0.187 |

The activity of CSD to attenuate inflammation was through the regulation of key inflammation factors. The total RNA was extracted from liver tissue, and the liver mRNA levels of IL-6, TNF-α, and IL-1β were analyzed using a one-step qRT-PCR using specific primers. Levels of mRNA were expressed as relative mRNA expression levels, calculated using a comparative Ct value (2-ΔΔCt), normalized with GAPDH mRNA levels, and compared to the normal group. Data presented as mean ± SEM. *p<0.05 compared to normal; #p<0.05 compared to Gal + LPS and were analyzed using one-way ANOVA. Gal/LPS: D-(+)-galactosamine hydrochloride (300 mg/kg; i.p) followed immediately by lipopolysaccharides (10 µg/kg; i.p); CSD: spray-dried *Centella asiatica* ethanol extract; Medium CSD: CSD 450 mg/kg; p.o.; MP: methylprednisolone (50 mg/kg; i.p); T2: 2 hours after Gal/LPS injection.

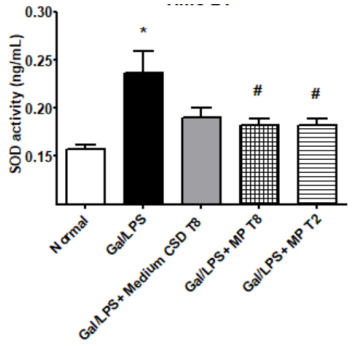


**Figure 4.** Superoxide dismutase (SOD) level in serum collected 24 hours after Gal/LPS injection.

Data presented as mean ± SEM. *p<0.05 compared to normal. #p<0.05 compared to Gal + LPS. Gal/LPS: D-(+)-galactosamine hydrochloride (300 mg/kg; i.p) followed immediately by lipopolysaccharides (10 µg/kg; i.p); CSD: spray-dried *Centella asiatica* ethanol extract; Medium CSD: CSD 450 mg/kg; p.o.; MP: methylprednisolone (50 mg/kg; i.p); T2: 2 hours after Gal/LPS injection; T8: 8 hours after Gal/LPS injection.

The Gal/LPS model is a well-known model for simulating the formation of acute liver failure in humans (Park et al., 2012). Gal can consume uridine monophosphate in hepatocytes, resulting in the depletion of nucleic acid and damage to hepatocyte structure and function, finally causing the apoptosis of cells (Tsutsui et al., 1997). LPS is a cell wall component of Gram-negative bacteria. High concentrations of LPS overcome the detoxification process and cause apparent liver damage (Jirillo et al., 2002). LPS combination models with Gal for acute liver failure help to demonstrate blood chemical profiles and metabolic changes. In this experiment, the concentration of Gal (300 mg/kg; i.p), followed immediately by LPS (10 µg/kg; i.p), was sufficient to induce acute liver failure and led to consistent results within the experimental groups. Of note, the standard therapy, methylprednisolone, as a positive control, could ameliorate the liver injury time-dependently and was in line with Higuchi et al. (2007), who reported a 50% ALT reduction compared to a 59% ALT reduction in the present study.

The present study found that CSD could alleviate acute liver injury induced by Gal/LPS, as shown by around a 50% reduction of GGT activity and total bilirubin level in serum. Although insignificant, these changes were almost similar to methylprednisolone. These indicate that the significant damage to liver cells was not followed by rapid deterioration of liver function. The damage to liver cells was reflected in other blood hepatic enzyme biomarkers (ALT, AST, ALP) that were still high after CSD treatment. These findings are in contrast to other studies that tend to show a similar trend of CA effects in all the blood hepatic enzyme biomarkers, i.e., ALT, AST, ALP, GGT, total bilirubin (Choi et al., 2016; Hong et al., 2021; Park et al., 2021).

Meanwhile, histological examination revealed that there was regeneration of liver epithelial cells and reduction of cell infiltration by CSD. These results indicated that the protective effects of CSD against Gal/LPS-induced liver injury in rats might aid in reducing tissue damage and inflammation at the microscopic level. Recent evidence has demonstrated the great potential of asiatic acid in CSD in ameliorating acute liver injury (Pang et al., 2020), consistent with the current findings. Histological lesions might improve, but the blood hepatic enzyme biomarkers might persist if a significant portion of the liver is still compromised. The liver can adapt, enabling the healthy areas to function adequately and maintain liver function markers near normal levels despite significant damage. Thus, the histological improvements might suggest healing in previously damaged areas, even though they may not directly impact the functional reserve.

In the present study, we also investigate the mechanism behind CSD hepatoprotective activity. Amelioration in IL-6 expression and SOD level were observed after CSD treatment. These indicated that the hepatoprotective effect of CSD is mediated by IL-6 and the antioxidant properties of CSD. Similar to our study, the anti-inflammatory and antioxidant activity of CA was also reported in the dimethylnitrosamine-induced liver toxicity model (Choi et al., 2016). In their study, post-treatment of CA ethanol extract at multiple doses of 200 mg/kg reduced serum IL-6 protein levels and increased SOD activity. The decreased SOD level after CSD treatment in our study seems the opposite of their study, which reported an increase of SOD activity after CA treatment. It may be caused by different study designs, ie our study was an acute response to the toxin (24 hours) while their study is a later response to the toxin (five days) leading to increased SOD levels in our negative control group but decreased SOD activities in their negative control group. Nevertheless, similarity exists, i.e., the SOD changes in both study designs were both restored by CA. In other studies, an increase of serum SOD levels in acute hepatitis was associated with disease severity (Tian et al., 2018; 2022). Therefore, both IL-6 data and SOD data in the present study imply a less severe hepatitis in CSD treated group.

Silymarin, a complex mixture of compounds obtained from *Silybum marianum* that is commonly used as a reference drug in hepatitis studies, was not used as the positive control in the present study because it has never been administered to the animals after the liver injury occurs (or is expected to occur). Furthermore, two studies reported a better hepatoprotective activity of CA than silymarin: (1) post-treatment of CA ethanol extract at multiple doses of 200 mg/kg in dimethylnitrosamine-induced liver toxicity model showed better hepatoprotection than silymarin 200 mg/kg based on histology score, blood hepatic enzyme tests, liver antioxidant markers (MDA, SOD, GPX, CAT), and serum inflammatory markers (Choi et al., 2016), (2) pre-treatment of standardized CA ethanol extract at multiple doses of 200 mg/kg in Gal/LPS induced hepatitis mice showed better apoptotic marker than silymarin despite similar hepatoprotective activity to silymarin based on HE staining and blood hepatic enzyme tests (ALT, AST) (Hong et al., 2021).

The hepatoprotective activity of CA is not without controversy. Jorge and Jorge (2005) reported hepatotoxicity cases related to CA. However, their study is not strong enough to completely exclude CA from being promoted as an alternative therapy for liver disease: (1) The study involved only three patients, (2) It was a chronic consumption of CA (20-60 days), (3) all of them use CA to lose weight, therefore, their diet may lead them to a condition that makes them more prone to hepatoxicity, and (4) the hepatotoxicity is seems related to CA, but, (a) in case 1, patient was an elderly (female, 61 years old, 30 days consumption) (b) in case 2 (female, 52 years, 21 days) despite only 21 days consumption, the patient has a history of six months CA consumption last year (c) in case 3 (female, 49 years old, 60 days consumption) there was no re-challenge and even if it was positive after re-chalenge, case 3 consumed CA for quite a long time. Besides some limitations in their study, however, it provides data that CA should be consumed under certain conditions, i.e., not being consumed for a long time. Therefore, the present study tried to explore the single-dose potency of CA as a hepatoprotector.

Our study is not without limitations. Several limitations of the present study are as follows: (1) a five days survival rate study was not performed to compare the effect of single vs. multiple doses of CA, (2) the specific mechanism of action of CA was not tested, i.e., using specific agonist/antagonist or enzyme inhibitor *in vivo*, therefore, the specific target of CA (enzyme or receptor) is unknown, (3) time-dependent effect of CA was not investigated, (4) more specific stain for a more specific marker of cell injury was not used, (5) liver oxidative status was not measured, (6) marker of oxidation products, such as isoprostane, was not measured, (7) protein level inflammatory markers were not measured, (8) the SOD level and inflammatory markers were not measured in all groups, but only selected group.

Despite all the limitations of the study, the abovementioned pharmacological data, together with CSD evaluation according to the Indonesian Herbal Pharmacopoeia, could be used to provide scientific evidence to support the traditional use of CA for colicky abdominal pain. In other words, this study promotes CA as a hepatoprotector candidate for Standardized Herbal Medicine in Indonesia.

## CONCLUSION

This study demonstrates the hepatoprotective potency of CSD administered after a profound liver injury. The dramatic acute liver injury caused by Gal/LPS injection could be alleviated by CSD, possibly through its antioxidant and anti-inflammatory properties. Although significant damage to the liver cells still occurred in the CSD group, it seemed that liver function was not profoundly impaired. Liver function, as reflected by both serum GGT and total bilirubin, seemed to be protected from the Gal/LPS toxicity. In contrast, some signs of liver injury were noted, reflected by ALT,

AST, and ALP. Histologically, CSD could promote the regeneration of liver epithelial cells and the reduction of cell infiltration. CSD could restore the oxidative stress imbalance reflected by SOD level as well as some degree of inflammation as reflected by IL-6 expression.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## ACKNOWLEDGMENTS

The study was supported by internal funds of BRIN (B-653/III/PL.04/3/2021) to Dr. Sofa Fajriah. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## REFERENCES

Ariffin F, Heong Chew S, Bhupinder K, Karim AA, Huda N (2011) Antioxidant capacity and phenolic composition of fermented *Centella asiatica* herbal teas. J Sci Food Agric 91(15): 2731-2739. https://doi.org/10.1002/jsfa.4454

Asrani SK, Devarbhavi H, Eaton J, Kamath PS (2019) Burden of liver diseases in the world. J Hepatol 70(1):151-171. https://doi.org/10.1016/j.jhep.2018.09.014

Ben Ari Z, Avlas O, Pappo O, Zilbermints V, Cheporko Y, Bachmetov L, Zemel R, Shainberg A, Sharon E, Grief F, Hochhauser E (2012) Reduced hepatic injury in toll-like receptor 4-deficient mice following D-galactosamine/lipopolysaccharide-induced fulminant hepatic failure. Cell Physiol Biochem 29(1-2): 41-50. https://doi.org/10.1159/000337585

Brinkhaus B, Lindner M, Schuppan D, Hahn EG (2000) Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*. Phytomedicine 7(5): 427-448. https://doi.org/10.1016/s0944-7113(00)80065-3

Choi MJ, Zheng HM, Kim JM, Lee KW, Park YH, Lee DH (2016) Protective effects of *Centella asiatica* leaf extract on dimethylnitrosamine-induced liver injury in rats. Mol Med Rep 14(5): 4521-4528. https://doi.org/10.3892/mmr.2016.5809

Cioffi E, Comune L, Piccolella S, Buono M, Pacifico S (2023) Quercetin 3-O-glucuronide from Aglianico vine leaves: A selective sustainable recovery and accumulation monitoring. Foods 12(14): 2646. https://doi.org/10.3390/foods12142646

Gupta S, Bhatt P, Chaturvedi P (2018) Determination and quantification of asiaticoside in endophytic fungus from *Centella asiatica* (L.) Urban. World J Microbiol Biotechnol 34(8): 111. https://doi.org/10.1007/s11274-018-2493-9

Higuchi N, Kato M, Kotoh K, Kohjima M, Aishima S, Nakamuta M, Fukui Y, Takayanagi R, Enjoji M (2007) Methylprednisolone injection via the portal vein suppresses inflammation in acute liver failure induced in rats by lipopolysaccharide and d-galactosamine. Liver Int 27(10): 1342-1348. https://doi.org/10.1111/j.1478-3231.2007.01590.x

Hong W, Hwang-Bo J, Jeon H, Ko M, Choi J, Jeong YJ, Park JH, Kim I, Kim TW, Kim H, Kang SC (2021) A comparative study of the hepatoprotective effect of *Centella asiatica* extract (CA-HE50) on lipopolysaccharide/d-galactosamine-induced acute liver injury in C57BL/6 mice. Nutrients 13(11): 4090. https://doi.org/10.3390/nu13114090

Humason GL (1979) Animal Tissue Techniques, 4th edn. San Francisco: WH Freeman and Company.

Jaeschke H (2000) Reactive oxygen and mechanisms of inflammatory liver injury. J Gastroenterol Hepatol 15(7): 718-724. https://doi.org/10.1046/j.1440-1746.2000.02207.x

Jafari S, Jafari SM, Ebrahimi M, Kijpatanasilp I, Assatarakul K (2023) A decade overview and prospect of spray drying encapsulation of bioactives from fruit products: Characterization, food application and *in vitro* gastrointestinal digestion. Food Hydrocolloids 134: 108068. https://doi.org/10.1016/j.foodhyd.2022.108068

Jirillo E, Caccavo D, Magrone T, Piccigallo E, Amati L, Lembo A, Kalis C, Gumenscheimer M (2002) The role of the liver in the response to LPS: Experimental and clinical findings. J Endotoxin Res 8(5): 319-327. https://doi.org/10.1179/096805102125000641

Jorge OA, Jorge AD (2005) Hepatotoxicity associated with the ingestion of *Centella asiatica*. Rev Esp Enferm Dig 97(2): 115-124. https://doi.org/10.4321/s1130-01082005000200006

Kilinç H (2021) Isolation and characterization of secondary metabolites from apolar fraction of *Scabiosa sicula* and evaluation of their antioxidant activities. Gümüşhane Üniversitesi Fen Bilimleri Dergisi 11(3): 934-942. https://doi.org/10.17714/gumusfenbil.888739

Kim SJ, Kim JK, Lee DU, Kwak JH, Lee SM (2010) Genipin protects lipopolysaccharide-induced apoptotic liver damage in D-galactosamine-sensitized mice. Eur J Pharmacol 635(1-3): 188-193. https://doi.org/10.1016/j.ejphar.2010.03.007

Krishnaiah D, Bono A, Sarbatly R, Nithyanandam R, Anisuzzaman SM (2015) Optimisation of spray drying operating condition of *Morinda citrifolia* L. fruit extract using response surface methodology. J King Saud Univ Eng Sci 27 (1): 26-36. https://doi.org/10.1016/j.jksues.2012.10.004

Lala V, Zubair M, Minter DA (2023) Liver function tests. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. https://www.ncbi.nlm.nih.gov/books/NBK482489/

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25(4): 402-408. https://doi.org/10.1006/meth.2001.1262

Lu J, Chen C, Gai R, Qiu H, Wu Y, He Q, Yang X (2021) Protective effects and possible mechanisms of *Centella asiatica* (L.) urban extract against acute and chronic liver injury: Evidence from *in vivo* and *in vitro* studies. Phytother Res 35(5): 2785-2796. https://doi.org/10.1002/ptr.7024

Ministry of Health, Republic of Indonesia (2008) Farmakope herbal Indonesia 1st ed, pp. 311-314.

Nolan T, Hands RE, Bustin SA (2006) Quantification of mRNA using real-time RT-PCR. Nat Protoc 1(3): 1559-1582. https://doi.org/10.1038/nprot.2006.236

Okselni T, Septama AW, Pamungkas RA, Rahmi EP, Efdi M, Koketsu M (2023) A systematic review and meta-analysis extraction techniques to reach the optimum asiaticoside content from the edible plant of *Centella asiatica*. S Afr J Bot 155: 261-273. https://doi.org/10.1016/j.sajb.2023.02.019

Pang X, Qiao Q, Vonglorkham S, Feng Z, Pang L, Chen S, Wang D, Lao L, Lin X, Wei J (2020) Asiatic acid ameliorates acute hepatic injury by reducing endoplasmic reticulum stress and triggering hepatocyte autophagy. Biomed Pharmacother 129: 110375. https://doi.org/10.1016/j.biopha.2020.110375

Park DW, Jeon H, Kwon JE, Lee YG, So R, Choe TH, Jeong YJ, Kang SC (2021) Hepatoprotective effect of *Centella asiatica* 50% ethanol extract against acetaminophen-induced acute liver injury in BALB/c mice. Toxicol Res 37(2): 261-275. https://doi.org/10.1007/s43188-020-00063-0

Park JH, Kim KH, Lee WR, Han SM, Park KK (2012) Protective effect of melittin on inflammation and apoptosis in acute liver

failure. Apoptosis 17(1): 61-69. https://doi.org/10.1007/s10495-011-0659-0

Sun B, Wu L, Wu Y, Zhang C, Qin L, Hayashi M, Kudo M, Gao M, Liu T (2020) Therapeutic potential of *Centella asiatica* and its triterpenes: A review. Front Pharmacol 11: 568032. https://doi.org/10.3389/fphar.2020.568032

Swandiny GF, Primahana G, Prastya ME, Ariani N, Susanti D, Hanafi M, Abdillah S (2023) An ethnopharmacology study of Indonesian medicinal plants in Gunung Sari village as dipeptidyl peptidase-IV inhibitor. Pharmacia 70(2): 365–373. https://doi.org/10.3897/pharmacia.70.e104437

Tian Z, Chen Y, Yao N, Hu C, Wu Y, Guo D, Liu J, Yang Y, Chen T, Zhao Y, He Y (2018) Role of mitophagy regulation by ROS in hepatic stellate cells during acute liver failure. Am J Physiol Gastrointest Liver Physiol 315(3): G374-G384. https://doi.org/10.1152/ajpgi.00032.2018

Tian Z, Yao N, Wu Y, Wang F, Zhao Y (2022) Association between plasma level of superoxide dismutase and survival of patients with acute-on-chronic liver failure. BMC Gastroenterol 22(1): 50. https://doi.org/10.1186/s12876-022-02126-y

Tilg H, Kaser A, Moschen AR (2006) How to modulate inflammatory cytokines in liver diseases. Liver Int 26(9): 1029-1039. https://doi.org/10.1111/j.1478-3231.2006.01339.x

Tsutsui S, Hirasawa K, Takeda M, Itagaki S, Kawamura S, Maeda K, Mikami T, Doi K (1997) Apoptosis of murine hepatocytes induced by high doses of galactosamine. J Vet Med Sci 59(9): 785-790. https://doi.org/10.1292/jvms.59.785

Vidyaniati P, Ariyoga A, Satramihardja HS (2009) Effect of Indian pennywort methanol extract compared with vitamin E on hepatitis rats model. Majalah Kedokteran Bandung 42(3): 101–107. http://dx.doi.org/10.15395/mkb.v42n3.19

Wang Y, Gao LN, Cui YL, Jiang HL (2014) Protective effect of danhong injection on acute hepatic failure induced by lipopolysaccharide and d-galactosamine in mice. Evid Based Complement Alternat Med 2014: 153902. https://doi.org/10.1155/2014/153902

**AUTHOR CONTRIBUTION:**

| Contribution | Yuliani T | Dewijanti ID | Angelina M | Fajriah S | Dewi RT | Primahana G | Randy A | Fitriani S | Sani Y |
|---|---|---|---|---|---|---|---|---|---|
| Concepts or ideas | x | | | x | | | | | |
| Design | x | | x | | | | | | |
| Definition of intellectual content | x | | x | x | | | | | |
| Literature search | x | | | x | x | x | x | | x |
| Experimental studies | | x | | | x | x | x | x | x |
| Data acquisition | | x | | | x | | x | x | x |
| Data analysis | x | | | | | | | | |
| Statistical analysis | x | | | | | | | | |
| Manuscript preparation | x | | | | | x | | | |
| Manuscript editing | x | | x | x | x | x | x | | |
| Manuscript review | x | x | x | x | x | x | x | x | x |

**Citation Format:** Yuliani T, Dewijanti ID, Angelina M, Fajriah S, Dewi RT, Primahana G, Randy A, Fitriani S, Sani Y (2025) Spray-dried *Centella asiatica* ethanol extract preserves liver functions in Gal/LPS-induced hepatitis rats through anti-inflammatory and antioxidant properties. J Pharm Pharmacogn Res 13(4): 1232–1244. https://doi.org/10.56499/jppres24.2242_13.4.1232

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

**Open Access:** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/ licenses/by/4.0/), which permits use, duplication, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.