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Phytochemical Profile, Antioxidant Activity, and Hematological and Liver–Kidney Serum Markers of Lampung Green Robusta Coffee Extract in Lead-Induced Mice

Profil Fitokimia, Aktivitas Antioksidan, Parameter Hematologi dan Penanda Serum Hati–Ginjal Ekstrak Kopi Robusta Hijau Lampung pada Mencit Terinduksi Timbal

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ABSTRACT

Lampung Province is one of Indonesia's major robusta coffee–producing regions, and green coffee beans contain bioactive compounds with potential biological benefits. This study analyzed the phytochemical composition and antioxidant activity of ethanolic extract of Lampung green robusta coffee beans and evaluated its effects on hematological parameters and liver–kidney serum markers in lead acetate–induced mice. Phytochemical screening revealed alkaloids, flavonoids, phenolics, saponins, tannins, and terpenoids. Quantitative analysis showed total phenolics of 122.76 mg GAE/g extract, flavonoids of 12.23 mg QE/g extract, tannins of 208.86 mg TAE/g extract, and saponins of 1.32%. The extract exhibited very strong antioxidant activity (IC_{50} = 21.99 ppm). Lead acetate exposure (20 mg/kg BW for 14 days) decreased erythrocyte counts and increased leukocyte and urea levels. Administration of the extract (400 mg/kg BW) improved erythrocyte counts, modulated leukocyte levels, and reduced urea concentrations.

Kata kunci:
Antioksidan
Fitokimia
Kopi robusta
Parameter hematologi

ABSTRAK

Provinsi Lampung merupakan salah satu sentra utama produksi kopi robusta di Indonesia, dan biji kopi hijau mengandung senyawa bioaktif dengan potensi manfaat biologis. Penelitian ini menganalisis kandungan fitokimia dan aktivitas antioksidan ekstrak etanol biji kopi robusta hijau Lampung serta mengevaluasi pengaruhnya terhadap parameter hematologi dan penanda serum fungsi hati–ginjal pada mencit yang diinduksi timbal asetat. Skrining fitokimia menunjukkan adanya alkaloid, flavonoid, fenolik, saponin, tanin, dan terpenoid. Analisis kuantitatif menunjukkan kandungan fenolik total 122,76 mg GAE/g ekstrak, flavonoid 12,23 mg QE/g ekstrak, tanin 208,86 mg TAE/g ekstrak, dan saponin 1,32%. Ekstrak menunjukkan aktivitas antioksidan sangat kuat (IC_{50} = 21,99 ppm). Paparan timbal asetat (20 mg/kgBB selama 14 hari) menurunkan eritrosit serta meningkatkan leukosit dan urea. Pemberian ekstrak (400 mg/kgBB) meningkatkan eritrosit, memodulasi leukosit, dan menurunkan kadar urea.

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1. INTRODUCTION

Robusta coffee (*Coffea canephora*) represents the dominant coffee commodity in Indonesia, contributing a substantially larger proportion of national production than arabica coffee. Statistical reports indicate that between 2013 and 2022 robusta accounted for approximately 73.00% of Indonesia's coffee production (508.33 thousand tons), whereas arabica contributed about 27.00% (187.98 thousand tons) (Pusat Data dan Sistem Informasi Pertanian, 2022). Robusta coffee is cultivated widely across several Indonesian regions, with Lampung Province recognized as one of the country's most important production centers. In 2022, Lampung ranked as the second largest coffee-producing province after South Sumatra, with total production reaching 124.5 thousand tons (BPS-Statistics Indonesia, 2023). Coffee beans are known to contain abundant polyphenolic compounds, particularly chlorogenic acid, which contributes significantly to antioxidant activity by inhibiting oxidative reactions and limiting the formation of free radicals (Farhaty & Muchtaridi, 2016). Previous studies have shown that robusta coffee generally contains higher levels of chlorogenic acid than arabica coffee, which corresponds to its stronger antioxidant capacity and highlights its potential as a natural antioxidant source (Afriyanti et al., 2023).

The bioactive compounds present in robusta coffee beans from several regions in Indonesia—including South Sumatra, West Java, Central Java, and East Java—have been reported to include alkaloids, flavonoids, saponins, tannins, caffeine, and phenolic compounds (Nurhayati et al., 2023; Rubinadzari et al., 2022; Utami et al., 2018; Wahyudi & Wulandari, 2022; Wigati et al., 2019). These compounds are associated with diverse pharmacological properties such as immunomodulatory, antiviral, antifungal, antioxidant, anti-inflammatory, antibacterial, anticancer, and hepatoprotective activities (Assa et al., 2021; Hasan et al., 2022; Heath et al., 2017). However, the composition and concentration of these bioactive compounds are strongly influenced by post-harvest processing methods, particularly roasting. Roasting is known to alter the physicochemical characteristics of coffee beans and may substantially modify the stability and availability of several phytochemicals (Gloess et al., 2014). Previous findings indicate that dried green robusta beans contain higher concentrations of chlorogenic acids, total phenols, and caffeine compared with beans roasted at temperatures above 195°C. Moreover, green robusta beans generally exhibit stronger antioxidant activity, as indicated by lower IC_{50} values relative to roasted coffee processed under similar conditions (Herawati et al., 2019).

Despite the growing body of research examining the pharmacological potential of robusta coffee, scientific investigations focusing specifically on green robusta coffee beans remain relatively limited. This limitation is particularly evident for robusta coffee originating from Lampung Province, which represents a major coffee-producing region in Indonesia but remains underexplored in terms of its phytochemical composition and biological activity. In addition, studies evaluating the potential effects of green robusta coffee bean extracts on hematological parameters and serum markers related to liver and kidney function in experimental models are still scarce.

Oxidative stress is known to contribute to tissue injury and dysfunction in several organs, including the liver and kidneys. The presence of antioxidant compounds in plant-derived extracts may therefore influence physiological responses associated with oxidative damage. Based on this rationale, further investigation of Lampung robusta coffee is needed to better characterize its phytochemical properties and biological potential. Therefore, this study aimed to analyze the phytochemical composition and antioxidant activity of the ethanolic extract of Lampung green robusta coffee beans and to evaluate its effects on hematological parameters as well as liver and kidney serum markers in mice exposed to lead acetate.

2. METHODS

2.1. Preparation of Green Robusta Coffee Beans

Green robusta coffee beans were obtained from Sukamandi Village, Pesawaran Regency, Lampung Province (5°32'46.6"S; 105°03'42.5"E). Botanical identification was performed at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, confirming the species as robusta coffee (*Coffea canephora* Pierre ex A. Froehner). The green coffee beans were ground using an ORION Herb & Spice Grinder to obtain a fine powder prior to extraction.

2.2. Extraction and Ethanol-Free Test

Extraction was performed using the maceration method. Approximately 300 g of powdered green robusta coffee beans (simplicia) were macerated in 1.5 L of 96% ethanol (1:5 w/v) in a glass container for 72 hours with daily agitation. The residue was subsequently re-macerated using the same procedure. The combined filtrates were concentrated using a rotary evaporator (BUCHI Rotary Evaporator B-100) at 40°C and 30 rpm to obtain the crude ethanolic extract (Khairani et al., 2024a).

An ethanol-free test was conducted to confirm the absence of residual ethanol in the extract. The esterification method was applied by placing 1 mL of extract in a test tube, followed by the addition of two drops of H_2SO_4 and two drops of CH_3COOH, then heating the mixture. The absence of the characteristic ester odor indicated that the extract was ethanol-free (Tivani et al., 2021). Validation was performed by adding two drops of H_2SO_4 and $K_2Cr_2O_7$ to 1 mL of extract followed by heating. The absence of a

color change to blue indicated that ethanol was not present in the extract (Sukadiasa et al., 2023).

2.3. Phytochemical Screening

Phytochemical screening was conducted to detect the presence of phenolics, flavonoids, tannins, saponins, steroids, terpenoids, and alkaloids in the extract. A total of 0.05 g of extract was mixed with 5 mL chloroform and 5 mL distilled water (1:1), shaken thoroughly, and allowed to stand until two distinct layers formed. The aqueous upper layer was used for phenolic, flavonoid, and saponin analysis, while the chloroform layer was used for steroid and terpenoid analysis. Tannin and alkaloid tests were conducted using separate procedures (Arnelio et al., 2025; Octaviani et al., 2019).

Phenolic compounds were identified by adding 1% $FeCl_3$ to the aqueous layer; a blue coloration indicated a positive reaction. Flavonoids were detected by adding magnesium powder and HCl to the aqueous layer, with yellow-orange to red coloration indicating their presence. Saponins were identified by vigorous shaking of the aqueous layer to produce stable foam lasting 3–5 minutes.

Steroid and terpenoid detection was performed using the chloroform layer. The filtrate was evaporated and treated with CH_3COOH, H_2SO_4, and Liebermann–Burchard reagent. Red coloration indicated terpenoids, whereas green or blue coloration indicated steroids. Tannins were detected by adding 1% $FeCl_3$ to an aqueous extract solution, producing a green-black color (Saskia et al., 2025). Alkaloids were analyzed by mixing the extract with HCl and distilled water followed by heating and filtration; the addition of Bouchardat reagent producing a brown-black precipitate confirmed the presence of alkaloids (Komala et al., 2025).

2.4. Determination of Total Phytochemical Content

Total phenolic content (TPC) and total tannin content (TTC) were determined using the Folin–Ciocalteu method. Ten milligrams of extract were dissolved in 10 mL methanol. One milliliter of this solution was mixed with 0.4 mL Folin–Ciocalteu reagent, allowed to stand for 4–8 minutes, and then combined with 4 mL of 7% Na_2CO_3 solution. Distilled water was added to a final volume of 10 mL, and the mixture was incubated at room temperature for 2 hours. Absorbance was measured at 744.8 nm using a spectrophotometer (A&E Lab). Gallic acid (10–50 mg/L) and tannic acid (2–10 mg/L) calibration curves were used to calculate total phenolic and tannin contents using the regression equation $y = ax + b$ (Ahmad et al., 2015; Sanjani et al., 2022; Kesuma et al., 2022).

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method. One hundred milligrams of extract were dissolved in methanol and mixed with $AlCl_3$, CH_3COOK, and distilled water to a final volume of 10 mL. The mixture was incubated for 30 minutes in the dark, and absorbance was measured at 431 nm. Quercetin standard solutions (10–50 mg/L) were used for calibration (Chandra & Handayani, 2024).

Total saponin content was measured by treating 100 mg of extract with 25% H_2SO_4 followed by autoclaving at 110°C for 120 minutes. The sample was extracted with ether, dried, and reacted with anisaldehyde and sulfuric acid before spectrophotometric analysis at 435 nm. Saponin standards (12.5–200 mg/L) were used to calculate the total saponin content using linear regression (Handayani et al., 2020).

2.5. Antioxidant Activity Assay

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. The extract was prepared in eight concentration levels (1000–7.8 ppm), while ascorbic acid was used as a positive control (150–1.171 ppm). Each sample (100 µL) was placed in a 96-well microplate and mixed with 100 µL of DPPH solution (125 µM in methanol). After incubation at 30°C for 30 minutes in the dark, absorbance was measured at 514 nm using an ELISA reader spectrophotometer (Thermo Scientific Varioskan Flash). The percentage of inhibition was calculated and used to determine IC_{50} values using linear regression (Prastya et al., 2019).

2.6. Ethical Approval and Experimental Animals

The study protocol was approved by the Ahmad Dahlan University Research Ethics Committee (REC-UAD/02/02/01-2025/007 and REC-UAD/02/02/01-2025/009). Male BALB/c mice aged 2–3 months and weighing 25–30 g were used as experimental animals. The mice were obtained from CV. Kencana Animal Breeding dan Riset Toksikologi (Bandung Regency, West Java).

Animals were acclimatized for seven days with free access to food and water and randomly assigned to five treatment groups (**Table 1**). The experimental treatments were administered daily for 14 days. Test substances (extract or vitamin C) were given orally in 0.3 mL distilled water, followed one hour later by oral administration of lead acetate (20 mg/kg BW).

On day 15, blood samples were collected from the tail vein to determine erythrocyte and leukocyte counts. The animals were then euthanized, and blood samples were collected via cardiac puncture into EDTA tubes. Serum was obtained by centrifugation at 6000 rpm for 10 minutes and used for liver and kidney marker analyses.

2.7. Hematological Analysis

Erythrocyte and leukocyte counts were determined using a hemocytometer. Blood samples were diluted with Hayem's solution (200×) for erythrocytes and Turk's solution (20×) for leukocytes. After homogenization, 10 µL of the diluted suspension was placed on the hemocytometer and observed under a microscope at 400× magnification. Erythrocytes were counted in five central squares, whereas leukocytes were counted in four corner squares (Khairani et al., 2024b).

Table 1. Experimental groups and treatments

Group	Treatment
K1	0.3 mL distilled water per day (normal control)
K2	0.3 mL distilled water per day + lead acetate (20 mg/kg BW) (negative control)
K3	Vitamin C (0.036 mg/g BW) + lead acetate (20 mg/kg BW) (positive control)
D1	Extract (200 mg/kg BW) + lead acetate (20 mg/kg BW)
D2	Extract (400 mg/kg BW) + lead acetate (20 mg/kg BW)

2.8. Liver Serum Markers

Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) levels were measured using diagnostic reagents (Glory Diagnostic). Working reagents were prepared by mixing reagent 1 and reagent 2 in a 4:1 ratio. Serum samples (100 µL) were mixed with the reagent and incubated for one minute. Absorbance was measured at 340 nm using a spectrophotometer, and readings were repeated after 1, 2, and 3 minutes to determine enzyme activity (Nofita et al., 2020).

2.9. Kidney Serum Markers

Kidney function was evaluated by measuring serum creatinine and urea (BUN) levels. Creatinine analysis used picric acid and alkaline buffer reagents, while urea was measured using buffered urease and coenzyme reagents (Glory Diagnostic). Absorbance readings were obtained using a spectrophotometer following the procedures described by Tandi et al. (2020).

2.10. Statistical Analysis

Data were analyzed using SPSS version 25.0 for Windows. One-way analysis of variance (ANOVA) was applied to determine differences among treatment groups. When significant differences were observed, post hoc analysis was conducted using the Least Significant Difference (LSD) test with a significance level of $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Profile of Green Robusta Coffee Bean Extract

Phytochemical screening indicated that the ethanolic extract of Lampung green robusta coffee beans contained several classes of secondary metabolites, including phenolics, flavonoids, tannins, saponins, alkaloids, and terpenoids, whereas steroid compounds were not detected (**Table 2**). Quantitative analysis presented in **Table 2** showed that the extract contained total phenolics of 122.76 ± 0.55 mg GAE/g extract, total flavonoids of 12.23 ± 0.13 mg QE/g extract, total tannins of 208.86 ± 1.47 mg TAE/g extract, and total saponins of 1.32 ± 0.005%. Among the quantified compounds, tannins were present at the highest level, followed by phenolic compounds, while flavonoids and saponins were detected in comparatively lower concentrations. These findings are consistent with previous reports showing that robusta coffee beans contain various phytochemical compounds, particularly phenolic and flavonoid groups, which have been identified in coffee extracts from several regions in Indonesia (Nurhayati et al., 2023; Rubinadzari et al., 2022; Utami et al., 2018; Wahyudi & Wulandari, 2022; Wigati et al., 2019).

3.2. Antioxidant Activity of Green Robusta Coffee Bean Extract

The antioxidant activity of the ethanolic extract of Lampung green robusta coffee beans was evaluated using the DPPH radical scavenging assay. The relationship between extract concentration and the percentage of radical inhibition is presented in **Figure 1A**. Increasing concentrations of the extract generally produced higher levels of DPPH inhibition, indicating a concentration-dependent scavenging effect. At the highest tested concentration (1000 ppm), the extract showed an inhibition value of approximately 90%, whereas lower concentrations resulted in progressively reduced inhibition levels. A similar pattern was observed for the positive control, ascorbic acid, although inhibition occurred at considerably lower concentrations, reflecting its stronger antioxidant potency.

Table 2. Phytochemical composition of ethanolic extract of Lampung green robusta coffee beans

Phytochemical compound	Qualitative screening	Quantitative content (Mean ± SD)
Phenolics	+	122.76 ± 0.55 mg GAE/g extract
Flavonoids	+	12.23 ± 0.13 mg QE/g extract
Tannins	+	208.86 ± 1.47 mg TAE/g extract
Saponins	+	1.32 ± 0.005 %
Alkaloids	+	–
Terpenoids	+	–
Steroids	–	–

Note: (+) detected; (–) not detected or not quantified. GAE = gallic acid equivalent; QE = quercetin equivalent; TAE = tannic acid equivalent. Quantitative values are presented as mean ± SD (n = 3).

The IC_{50} values obtained from the DPPH assay are shown in **Figure 1B**. The ethanolic extract exhibited an IC_{50} value of 21.99 ± 4.90 ppm, whereas ascorbic acid showed a lower IC_{50} value of 4.47 ± 0.17 ppm. Statistical analysis using an independent t-test indicated that the difference between the two samples was statistically significant ($t = 5.05$, $p = 0.0072$). These results indicate that ascorbic acid displayed stronger antioxidant activity than the extract under the conditions of the assay. Nevertheless, the IC_{50} value obtained for the extract remains within the category of very strong antioxidant activity, as compounds with IC_{50} values below 50 ppm are commonly classified as having very strong antioxidant capacity (Molyneux, 2004).

The strong antioxidant activity observed in the Lampung green robusta coffee extract is consistent with its phytochemical profile presented in **Table 2**, which shows the presence of phenolics, flavonoids, tannins, and other secondary metabolites. Polyphenolic compounds are widely recognized for their ability to donate electrons or hydrogen atoms to neutralize free radicals and interrupt oxidative chain reactions. Similar phytochemical groups have previously been reported in robusta coffee beans from several regions in Indonesia, where phenolic compounds and related metabolites contribute substantially to the antioxidant properties of coffee extracts (Nurhayati et al., 2023; Rubinadzari et al., 2022; Utami et al., 2018; Wahyudi & Wulandari, 2022; Wigati et al., 2019).

3.3. Effects on Hematological Parameters

The effects of Lampung green robusta coffee bean extract on hematological parameters in mice exposed to lead acetate are presented in **Figure 2**. Normal erythrocyte counts in mice range from 5.0–9.5 $\times 10^6/mm^3$, while leukocyte counts range from 12.1–15.9 $\times 10^3/mm^3$ (Kusumawati, 2004; Suckow et al., 2001). In the present study, the group receiving lead acetate alone (K2) showed the lowest erythrocyte count and the highest leukocyte count among all groups. The erythrocyte value in K2 fell below the normal reference range, whereas the leukocyte count exceeded the typical range observed in healthy mice. These results are consistent with previous studies indicating that lead exposure can decrease red blood cell counts while increasing leukocyte levels (Sugiharto et al., 2020). Similar findings were reported by Thuong et al. (2023), who observed reductions in both the number and size of erythrocytes together with a time-dependent increase in leukocyte counts in lead-exposed mice.

Following oral exposure, lead (Pb) can be absorbed through the gastrointestinal tract and transported via the bloodstream. In circulation, lead is primarily associated with erythrocytes and plasma proteins (Andjelkovic et al., 2019). Lead exposure is known to induce oxidative stress through increased production of reactive oxygen species (ROS) and disruption of antioxidant defense mechanisms. This imbalance between ROS generation and elimination can lead to oxidative damage to cellular components, including membrane lipids, proteins, and DNA, ultimately affecting tissue and cellular function (El-Sayed et al., 2006; Patra et al., 2011; Vural Aydin, 2024). A decrease in erythrocyte counts may therefore be associated with intravascular hemolysis caused by oxidative damage and lipid peroxidation in circulating red blood cells. In contrast, elevated leukocyte counts may reflect an inflammatory response triggered by lead exposure or increased leukocyte production in lymphoid organs (Alwaleedi, 2016; Maryam et al., 2025).

The administration of green robusta coffee bean extract produced different patterns in hematological parameters depending on the dose. As shown in **Figure 2A**, mice receiving 400 mg/kg BW extract (D2) exhibited erythrocyte counts that were closer to those observed in the control groups compared with the lead-treated group (K2). In contrast, the group receiving 200 mg/kg BW extract (D1) showed lower erythrocyte values that remained closer to the lead-treated group. For leukocyte counts (Figure 2B), the extract-treated groups showed values that were lower than those observed in K2 but remained variable among animals, and the differences were not statistically significant. These findings indicate that under the experimental conditions used in this study, administration of green robusta coffee bean extract was associated with variations in hematological parameters, particularly in erythrocyte counts.

The observed pattern may be related to the bioactive compounds present in green robusta coffee beans. Robusta coffee is known to contain several active constituents, including polyphenols such as chlorogenic acids, alkaloids such as caffeine, and saponins, which have been reported to exhibit anti-inflammatory properties (Frost-Meyer & Logomarsino, 2012; Warman et al., 2024). Chlorogenic acid, the major phenolic compound in green coffee, has been reported to reduce reactive oxygen species production. Previous studies have also shown that green coffee extracts may reduce inflammatory responses, as indicated by decreased TNF-α expression (Hwang et al., 2014; Nugraha et al., 2023).

3.4. Liver and Kidney Serum Markers

The liver serum markers measured in this study were serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) levels in mice. These enzymes are commonly used to evaluate liver injury because alterations in their serum activity may indicate hepatocellular damage (Girish et al., 2025; Yuneldi et al., 2018). The results of SGPT and SGOT measurements are presented in **Figure 3A** and **Figure 3B**. In the present study, SGPT values ranged from 36.86 to 91.76 U/L, whereas SGOT values ranged from 134.06 to 169.75 U/L across the experimental groups. Statistical analysis showed that there were no significant differences in SGPT or SGOT levels among the groups ($p > .05$). All measured values remained within the reported physiological ranges for BALB/c mice (SGPT: 22–146 U/L; SGOT: 68–180 U/L) (Kurtz & Travlos, 2017).

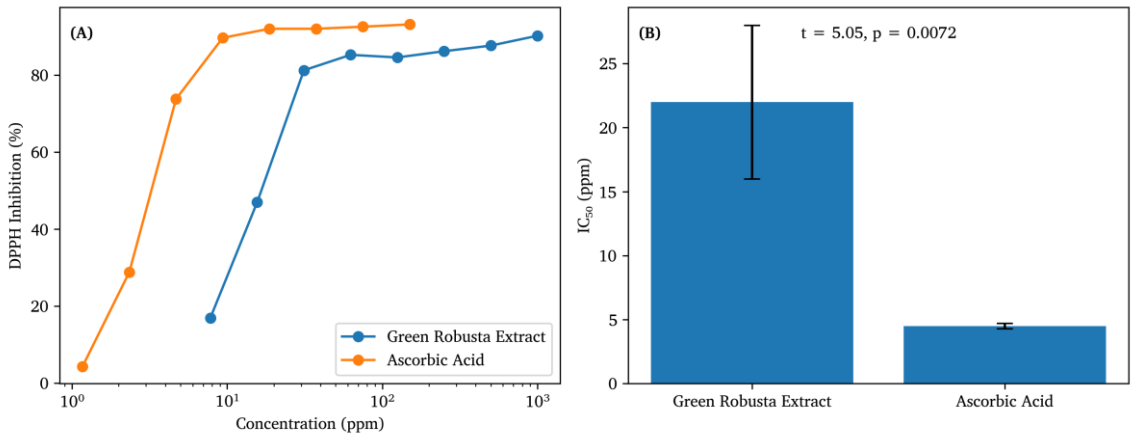

Figure 1. Antioxidant activity of ethanolic extract of Lampung green robusta coffee beans. (A) Dose–response relationship between concentration and DPPH radical scavenging activity of the extract compared with ascorbic acid. (B) Comparison of IC_{50} values of the extract and ascorbic acid. Data are presented as mean ± SD (n = 3). A significant difference between samples was observed using an independent t-test ($p < 0.05$).

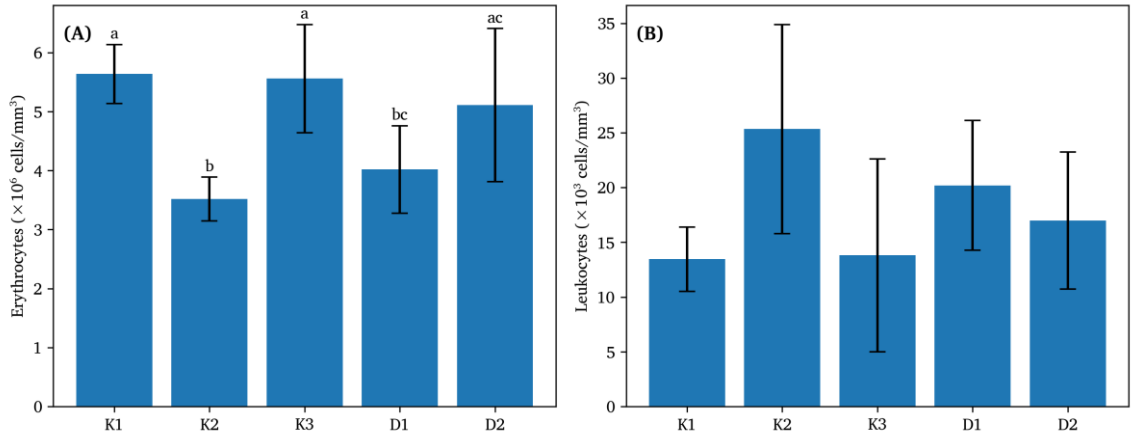

Figure 2. Hematological parameters in mice exposed to lead acetate and treated with Lampung green robusta coffee bean extract. (A) Erythrocyte counts and (B) leukocyte counts in experimental groups: K1 (normal control), K2 (lead acetate), K3 (vitamin C + lead acetate), D1 (extract 200 mg/kg BW + lead acetate), and D2 (extract 400 mg/kg BW + lead acetate). Data are presented as mean ± SD (n = 3). Different superscript letters indicate significant differences among groups based on one-way ANOVA followed by LSD post hoc test ($p < .05$).

Lead exposure has been reported to cause toxicity in hematopoietic tissues and other organs, including the liver. Exposure to lead can alter specific enzymatic activities and affect several hematological and biochemical parameters in mice (Das et al., 2024). SGPT is a cytoplasmic enzyme found in hepatocytes and is released into the circulation when hepatocellular membrane damage occurs. SGOT is located in both the cytosol and mitochondria; therefore, increased SGOT levels may indicate mitochondrial injury in liver cells (Alvarez & Whittemore, 2009; Gowda et al., 2009). In the present study, administration of lead acetate at 20 mg/kg BW for 14 days did not significantly influence SGPT or SGOT levels in either the control or treatment groups. This exposure period represents a subacute toxicity model designed to observe early biological responses. Under these conditions, the duration of lead exposure may have been insufficient to cause detectable hepatocellular enzyme leakage into the bloodstream. Previous studies have reported different outcomes when the exposure period is extended. For instance, administration of lead acetate at a similar dose for 21 days has been reported to significantly increase SGPT and SGOT levels (Yuniarti et al., 2021). Lead-induced liver injury is generally associated with oxidative stress, which can promote lipid, protein, and DNA oxidation as well as inflammatory responses in hepatic tissue (Chen et al., 2019). Damage to hepatocytes may subsequently lead to the release of these enzymes into the bloodstream, thereby increasing serum enzyme levels (Nursasmi et al., 2024).

In the present study, administration of green robusta coffee bean extract did not produce significant changes in SGPT or SGOT levels. Nevertheless, coffee and its major bioactive compounds have been reported to influence liver-related biochemical parameters in several experimental studies. Compounds such as caffeine and chlorogenic acid possess antioxidant properties and have been associated with hepatoprotective activity in experimental models. For example, ethanol extract of green robusta coffee beans has been reported to reduce elevated ALT (SGPT) levels in mice subjected to MSG-induced hepatotoxicity,

although the reduction did not reach the level observed in the normal control group (Arisca, 2024). Other studies have also reported that coffee administration may inhibit transforming growth factor beta (TGF-β), thereby reducing liver fibrosis in experimental animals. Coffee administration has also been shown to significantly reduce SGPT levels in Wistar rats treated with thioacetamide (Arauz et al., 2013). In addition, medium-roasted robusta coffee has been reported to reduce hepatocyte damage in histopathological examinations of the liver in aspirin-induced male Sprague Dawley rats (Ananti et al., 2024). Water extract of robusta coffee beans, as a source of antioxidants, has also been reported to have potential in preventing liver dysfunction in carbon tetrachloride–induced hepatitis models (Sukohar & Sastramihardja, 2012).

Kidney function in this study was evaluated by measuring serum creatinine and urea levels, which are widely used indicators of renal function. Creatinine is a metabolic byproduct of creatine that is filtered by the glomeruli without tubular reabsorption, and increased serum levels may indicate impaired glomerular function (Laksmi et al., 2014). Urea is a product of protein metabolism synthesized in the liver and excreted by the kidneys; abnormal serum concentrations may therefore indicate impaired renal function (Rahmawati, 2018). The results of creatinine and urea measurements are shown in **Figure 3C** and **Figure 3D**. In this study, creatinine levels ranged from 0.30 to 0.33 mg/dL, while urea levels ranged from 19.53 to 31.43 mg/dL. Statistical analysis showed no significant differences among the groups for either creatinine or urea levels ($p > .05$). All creatinine values remained within the reported normal range for mice (0.2–0.9 mg/dL) (Mus et al., 2023), indicating that lead acetate exposure at 20 mg/kg BW for 14 days did not impair glomerular function under the experimental conditions used in this study.

These findings are consistent with Mohammadi et al. (2015), who also reported no increase in creatinine levels following lead exposure at 60 mg/kg BW for 14 days. However, longer exposure periods (21–28 days) or higher doses of lead (20–50 mg/kg BW) have been reported to increase creatinine levels (Cutami et al., 2024; Ovie et al., 2023; Purlinda et al., 2024). With respect to urea levels, the mean values in all groups except K2 remained within the reported normal range for mice (13.9–28.3 mg/dL) (Kusumawati, 2004). The higher urea value observed in group K2 may reflect early alterations in kidney function associated with lead exposure. Elevated blood urea levels can occur as a result of renal tubular damage caused by epithelial cell necrosis or impaired tubular reabsorption (Mus et al., 2023; Rosai, 2004). Lead exposure has also been reported to trigger the formation of reactive oxygen species, contributing to oxidative stress and potential renal tissue damage. A portion of absorbed lead may be excreted from the body, while the remainder may accumulate in renal tissue and interfere with kidney function (Zulaikhah & Wibowo, 2020).

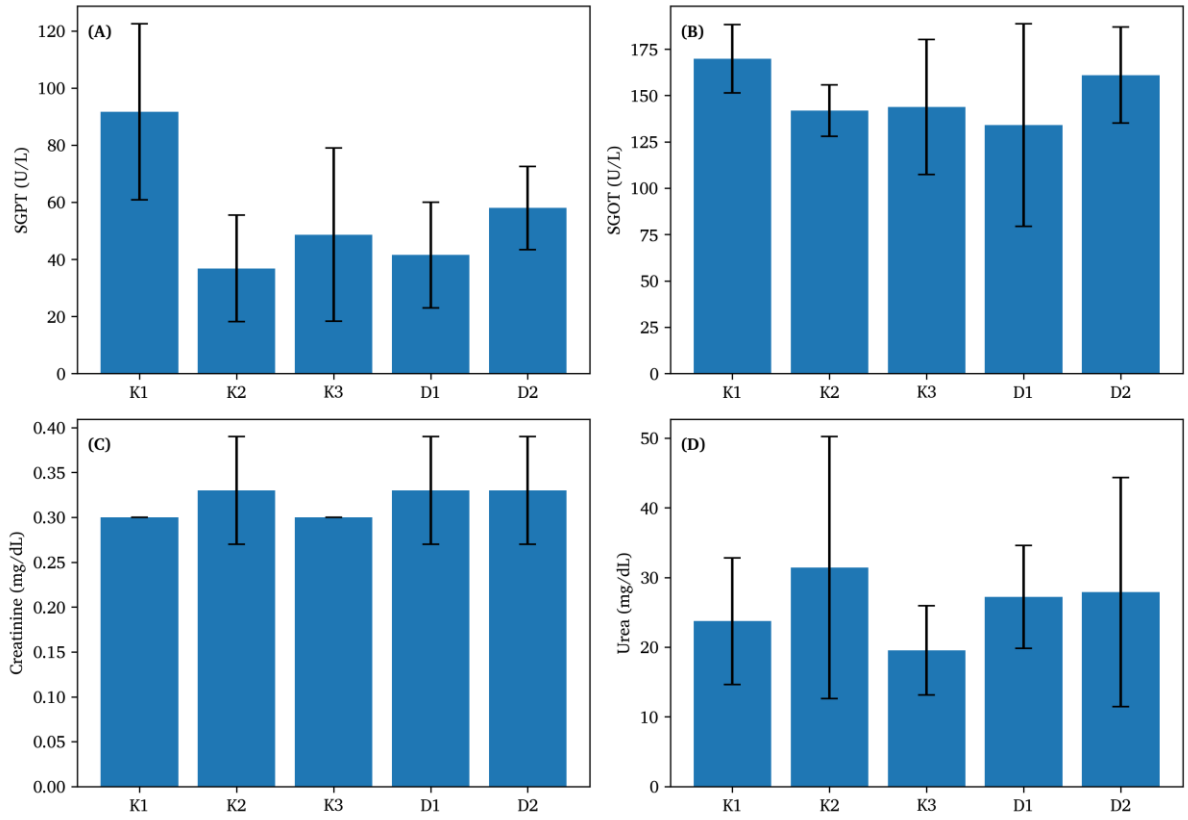

Figure 3. Liver and kidney serum markers in mice exposed to lead acetate and treated with Lampung green robusta coffee bean extract. (A) SGPT levels, (B) SGOT levels, (C) creatinine levels, and (D) urea levels in experimental groups: K1 (normal control), K2 (lead acetate), K3 (vitamin C + lead acetate), D1 (extract 200 mg/kg BW + lead acetate), and D2 (extract 400 mg/kg BW + lead acetate). Data are presented as mean ± SD (n = 3).

In the extract-treated groups (D1 and D2), urea levels remained within the physiological reference range and were lower than the value observed in the K2 group, although the differences were not statistically significant. The potential influence of robusta coffee extract on renal-related serum markers may be associated with the presence of polyphenolic compounds with antioxidant properties (Chairgulprasert & Kongsuwankeeree, 2017). Previous studies have reported that administration of green robusta coffee bean extract can reduce serum creatinine and blood urea nitrogen levels in models of cisplatin-induced nephrotoxicity (Leta et al., 2021). Green coffee has also been reported to improve the general condition of rats experiencing renal apoptosis induced by cisplatin, which has been attributed to its antioxidant and anti-apoptotic effects (El-Deen et al., 2019). In addition, Lampung robusta coffee bean extract has been reported to reduce histopathological damage in the proximal tubules of the kidneys of male Sprague-Dawley rats induced by monosodium glutamate (Salsabila et al., 2024). These findings suggest that the polyphenol content and antioxidant capacity of green robusta coffee bean extract may contribute to modulation of renal-related biochemical parameters. However, under the subacute lead exposure model used in the present study, these effects were limited and did not result in statistically significant differences in creatinine or urea levels.

4. CONCLUSION

The ethanolic extract of Lampung green robusta coffee beans exhibited a diverse phytochemical composition consisting of phenolics, flavonoids, tannins, saponins, alkaloids, and terpenoids, which corresponded with its very strong antioxidant activity in the DPPH assay. In the subacute lead exposure model, lead acetate induced alterations in hematological and biochemical parameters, particularly by reducing erythrocyte counts and increasing leukocyte and urea levels. Administration of the extract, especially at 400 mg/kg body weight, was associated with erythrocyte values closer to normal conditions, lower leukocyte levels compared with the lead-treated group, and urea concentrations within the physiological range. These findings highlight the potential of Lampung green robusta coffee as a natural source of antioxidant compounds capable of modulating hematological responses and selected renal-related serum markers under lead-induced oxidative stress conditions. Further investigations integrating longer exposure models, expanded sample sizes, and molecular or histopathological analyses will be essential to elucidate the underlying protective mechanisms and to better define the biomedical relevance of green robusta coffee.

AUTHOR CONTRIBUTIONS

Conceptualization, I.A.K.; methodology, I.A.K., J.P.A., and K.F.; validation, H.A.N.A. and R.F.; formal analysis, I.A.K.; investigation, I.A.K., J.P.A., and K.F.; resources, I.A.K.; data curation, J.S.M. and E.N.R.; writing—original draft preparation, I.A.K.; writing—review and editing, H.A.N.A., R.F., J.S.M., and E.N.R.; visualization, J.P.A. and K.F.; supervision, H.A.N.A. and R.F.; project administration, I.A.K. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

The animal study protocol was approved by the Research Ethics Committee of Ahmad Dahlan University (approval numbers: REC-UAD/02/02/01-2025/007 and REC-UAD/02/02/01-2025/009).

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

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

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used DeepL to assist with translation, Paperpal to improve sentence structure and grammar, and ChatGPT (OpenAI) to enhance the clarity and readability of the text. After using these tools, the authors carefully reviewed, edited, and verified the entire manuscript to ensure that it accurately reflects their own ideas, analyses, and interpretations. The authors take full responsibility for the integrity and originality of the published work.

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