VIVA MEDIKA
Jurnal Kesehatan, Kebidanan, dan Keperawatan

Homepage: http://ejournal.uhb.ac.id/index.php/vm
P-ISSN: 1979-2026 E-ISSN: 2656-1034
DOI: 10.35960/vm.v17i3.1490

Hepatoprotective Activity of Ethanol Extract of Mobe Leaves (Artocarpus lacucha Buch-Ham.) on Total Bilirubin, ALT, and AST Levels, and Macropathology of Liver in Rats Induced by Carbon Tetrachloride

Meiva Amelia Lubis[1], Aminah Dalimunthe [2, a*)], Panal Sitorus[2, b)], Denny Satria[2, c)]
[1]Pharmacy, Graduate School, Medan, Universitas Sumatera Utara
[2]Pharmacy, Lecturer, Medan, Universitas Sumatera Utara
Correspondence Author: aminah@usu.ac.id

ABSTRACT

This study evaluates the hepatoprotective effects of ethanol extract of mobe leaves (Artocarpus lacucha Buch-Ham.) in rats induced with carbon tetrachloride (CCl4). The objective was to determine the extract's efficacy in reducing liver damage markers, specifically total bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels, and to assess liver macropathology. The ethanol extract was prepared by maceration using 96% ethanol. Results showed that administering 400 mg/kg body weight (BW) of the extract significantly reduced total bilirubin, ALT, and AST levels in CCl4-induced rats. Additionally, liver macropathology observations indicated improved liver conditions in the treatment group. Phytochemical screening revealed bioactive compounds like flavonoids, alkaloids, tannins, saponins, triterpenoids, and glycosides, which are potential antioxidants and hepatoprotective agents. The significant contribution of this research is providing a scientific basis for using mobe leaf extract as a natural hepatoprotective agent, highlighting its potential for safe and effective herbal medicine. Further research and clinical trials are recommended to validate these findings and explore the extract's therapeutic applications.

Keywords: Hepatoprotective, Ethanol Extract of Mobe Leaves, Carbon Tetrachloride

1. INTRODUCTION

The liver plays a crucial role in various metabolic processes, including the detoxification of biological and xenobiotic compounds. As a result, the liver is often exposed to toxic substances that can cause damage. Additionally, oxidative stress and inflammation are major causes of various liver diseases (Dröge, 2002). Oxidative stress tends to activate macrophages and promote the release of pro-inflammatory cytokines such as Nuclear Factor Kappa Beta (NF-κB), which regulates the expression of various inflammatory mediators, including interleukins (IL-1β, IL-6) and Tumor Necrosis Factor (TNF-α). This process also induces cell apoptosis through the increased expression of Caspase 3 and 7, exacerbating liver disease (Chen et al., 2020).

Xenobiotic compounds, which are foreign substances not required by the body, undergo metabolic processes in the liver, producing hydroxyl radicals as by-products (Dalimartha, 1999). Carbon tetrachloride (CCl4) is a xenobiotic often used to induce lipid peroxidation and liver cell damage. In the body, CCl4 is converted into free radicals that damage cell membranes (Chodidjah et al., 2007).

The injury and toxicity caused by CCl4 occur rapidly due to its lipophilic nature,

***Aminah Dalimunthe.**
Tel.: -
Email: aminah@usu.ac.id

allowing CCl4 to penetrate cell membranes and distribute throughout organs. In the liver, CCl4 undergoes biotransformation through the endoplasmic reticulum (cytochrome P450), producing trichloromethyl (CCl3-) and Cl-radicals. The CCl3- radicals react with oxygen to form CCl3O2-, which leads to lipid peroxidation of cell membranes, membrane damage, and increased leakage of liver enzymes such as Alanine Transaminase (ALT) and Aspartate Transaminase (AST). The increase in enzyme leakage indicates the loss of membrane integrity and increased permeability, marking liver damage due to CCl4. This can also be confirmed through histopathological examination, showing degenerative changes and necrosis (Hariyanto, 2013).

Despite advances in modern medicine, there are still no successful therapeutic approaches to protect liver function or enhance liver cell regeneration effectively (Madrigal-Santillán ert al., 2014). Alternative and complementary preventive therapies using medicinal plants significantly contribute to human health through their promotive, curative, and rehabilitative properties. Hepatoprotectors are compounds with therapeutic effects for restoring, maintaining, and treating liver function damage (Fan ert al., 2018). Given the prevalence of liver diseases caused by oxidative stress and inflammation, there is an urgent need for effective and safe hepatoprotective agents.

The hepatoprotective activity of flavonoid compounds is associated with enhanced antioxidant defenses, such as increased activity of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), and Glutathione (GSH) levels (Domitrović ert al., 2011). These compounds can combat free radicals and induce cellular stress defense systems, making plants with antioxidant and anti-inflammatory activities a potential therapeutic strategy for preventing and treating liver damage (Tacker ert al., 2009)

Mobe leaves contain flavonoids, tannins, saponins, phenolics, steroids, and triterpenoids with anti-inflammatory, antidiabetic, antioxidant, antibacterial, and antifungal effects. This plant is known as a source of phenolic derivatives, specifically di- or tri-oxygenated and isoprenylated flavones at the C3 position (Indarto, 2015). (Jagtap & Bapat, 2010), the Artocarpus genus contains flavonoids that can inhibit chemical mediators released from mast cells, neutrophils, and macrophages. The Artocarpus family also contains secondary metabolites beneficial as hepatoprotective agents (Pandery & Bhatnagar, 2009).

Previous research has tested the antioxidant activity of ethanol, ethyl acetate, and n-hexane extracts of mobe leaves using the ABTS (2,2'-Azinobis (3-Ethyl Benothiazoline)-6-Sulfonic Acid) method. The antioxidant activity was tested with a spectrophotometer at a wavelength of 734 nm, showing IC50 values of the ethanol extract at 87.547 µg/ml (strong effect), the ethyl acetate extract at 138.767 µg/ml (moderate effect), and the n-hexane extract at 558.094 µg/ml (very weak effect) (Pulungan, 2018).

Based on this background, this study provides a scientific basis for using ethanol extract of mobe leaves as a natural hepatoprotective agent. Mobe (A. lacucha) is a plant native to North Sumatra, Indonesia. This plant is efficacious as an antioxidant, antibacterial, antidiarrheal, anti-inflammatory, analgesic, antinociceptive, schistosomicidal, hepatoprotective, neuroprotective, cytotoxic, antiglycation, and anticholesterol, and can also be used for anti-aging and wound healing (Sitorus et al., 2022) The findings demonstrate the potential of mobe leaf extract in protecting the liver from toxic damage, which has not been extensively researched. Thus, this study opens opportunities for developing safer, effective, and affordable herbal preparations for liver disease therapy. Furthermore, these findings offer new insights into the therapeutic benefits of bioactive compounds in mobe leaves, encouraging further research and clinical trials to ensure their safety and efficacy as herbal medicine.

This study aims to determine the hepatoprotective activity of ethanol extract of mobe leaves in rats induced with CCl4. Oxidative stress is considered the main impact affecting hepatocytes exposed to CCl4. SOD-1 is an antioxidant that can inhibit free radicals causing lipid peroxidation in cell membranes, followed by the release of liver enzymes (ALT, AST) and a decrease in total bilirubin (TB)

levels, which are considered biomarkers of liver damage. Liver damage due to CCl4 can be further confirmed through histological examination of the liver, evaluated macroscopically to detect pathological changes. This research provides significant benefits to society and researchers. For society, the findings enrich information on the use of mobe leaves as medicinal plants with hepatoprotective potential, offering a safer, easily accessible, and affordable alternative treatment. For researchers, these findings open opportunities for further studies to develop standardized herbal preparations based on mobe leaf extract, effective in protecting and improving liver function, contributing to pharmacology and herbal medicine development.

2. METHODOLOGY

This study employed an experimental design that included the collection of plant materials, preparation of simplicia, and testing the hepatoprotective effects on liver biomarkers (ALT, AST), along with macropathological examination of the liver in rats treated as per the recommendations of the Health Research Ethics Committee No. 0226/KEPH-FMIPA/2023, dated March 30, 2023.

2.1 Research Location

The research was conducted in several laboratories at the University of North Sumatra, including the Laboratory of Pharmaceutical Biology, the University Hospital Laboratory, and the Histology Laboratory at the Faculty of Medicine, Universitas Suamtera Utara.

2.2 Equipment and Materials

The equipment used in this study included laboratory glassware, mortar and pestle, blender, analytical balance, drying oven, rotary evaporator, funnel, hot plate, desiccator, petri dishes, microtubes, inverted microscope, oral sonde, animal scales, surgical knives, centrifuge, syringes, EDTA tubes, gloves, and plastic pots.

Materials used in this study included mobe leaves (Artocarpus lacucha Buch-Ham.), silymarin, CCl4, corn oil, CMC-Na, distilled water, NaCl 0.9%, EDTA, 10% Natural Buffered Formalin (NBF), chloral hydrate, ferric chloride, lead acetate, concentrated sulfuric acid, concentrated hydrochloric acid, methanol, chloroform-isopropanol, acetic anhydride, toluene, 96% ethanol, Meyer's reagent, Bouchardat's reagent, Dragendorff's reagent, Lieberman-Bourchard's reagent, Molisch's reagent, xylol, adeps lanae, vaseline album, and 70% alcohol.

2.3 Procedure

2.3.1 Collection and Identification of Plant Materials

Mobe leaves were collected purposively from Laguboti District, Toba Samosir Regency, North Sumatra Province. The leaves used in this study were identified at the Herbarium Medanese (MEDA), University of North Sumatra.

2.3.2 Preparation of Mobe Leaf Simplicia

Fresh mobe leaves were collected, cleaned, washed, drained, and air-dried. After weighing the fresh weight, the leaves were dried in a drying oven at ±40°C until completely dry. The dried simplicia was then chopped, blended into a powder, and stored in tightly closed containers at room temperature to prevent moisture and contamination.

2.3.3 Ethanol Extraction of Mobe Leaves (EEDM)

The extract was prepared using maceration with ethanol as the solvent. Specifically, 500 grams of dried simplicia was soaked in 5 liters of 96% ethanol at a ratio of 1:10 for 6 hours with occasional stirring, then left to stand for 18 hours. The macerate was filtered, and the residue was re-macerated with an additional 5 liters of ethanol for 6 hours. The combined filtrates were concentrated using a rotary evaporator at a temperature of 40°C to obtain a thick extract. This process ensured the thorough extraction of bioactive compounds.

2.3.4 Characterization of Simplicia and Extract

The characteristics of the simplicia and extract were examined, including macroscopic

and microscopic analyses, determination of water content, water-soluble extract content, ethanol-soluble extract content, total ash content, and acid-insoluble ash content.

2.3.5 Macroscopic and Microscopic Examination

Macroscopic examination involved observing the shape, smell, and taste of mobe leaves and simplicia powder. Microscopic examination of the simplicia powder was performed using a microscope after adding chloral hydrate solution.

2.3.6 Determination of Water Content, Soluble Extract Content, and Ash Content

Water content was determined using the azeotropic (toluene distillation) method. Water-soluble and ethanol-soluble extract contents were determined by maceration and evaporation of the filtrate. Total ash content and acid-insoluble ash content were calculated after incineration and filtration.

2.3.7 Phytochemical Screening of Simplicia and Extract

Phytochemical screening was conducted to detect the presence of secondary metabolites such as flavonoids, alkaloids, saponins, tannins, glycosides, and steroids/triterpenoids in the simplicia powder and ethanol extract of mobe leaves. The screening procedure involved color reactions and precipitation using specific reagents.

2.4 Biochemical Serum Test

2.4.1 Preparation of Suspensions and CCl4 Induction

Suspensions of 1% CMC-Na, EEDM, and silymarin were prepared with varying doses. CCl4 induction was carried out by mixing CCl4 with corn oil at a ratio of 1:1.

2.4.2 Preparation of Experimental Animals

Thirty male rats, averaging 200 grams in weight, were used in this study. The rats were divided into six treatment groups, acclimatized for one week, and weighed.

2.4.3 Animal Treatment Procedures

The rats were treated as follows:

- Group I: EEDM 400 mg/kg BW + CCl4
- Group II: EEDM 200 mg/kg BW + CCl4
- Group III: EEDM 100 mg/kg BW + CCl4
- Group IV: EEDM 50 mg/kg BW + CCl4
- Group V: Silymarin 100 mg/kg BW + CCl4 (positive control)
- Group VI: Na CMC 1% + CCl4 (negative control)

After 14 days, the rats were sacrificed using ketamine anesthesia, and blood was drawn from the heart for analysis of liver biomarkers (TB, ALT, AST) at the Universitas Sumatera Utara Hospital Laboratory.

2.5 Measurement of Biomarkers and Macropathological Examination of the Liver

2.5.1 Measurement of Liver Damage Biomarkers

Total bilirubin levels were measured using a photometric method with Vanadiate Oxidating (VOX) reagent at a wavelength of 450 nm and a temperature of 37 ºC. ALT and AST levels were measured using a photometric method with a Portable Microlab 300 LX photometer.

2.5.2 Macropathological Examination of the Liver

Macropathological examination of the liver was conducted to observe pathological changes in the liver tissue, including color, shape, and consistency.

2.6 Data Analysis

The data obtained were analyzed using SPSS IBM 26 software with the one-way ANOVA method. A p-value <0.05 was considered significant ($\alpha = 0.05$)

3. Results and Discussion

3.1 Plant Identification

The identification of the plant was carried out at the Herbarium Medanese (MEDA) at the University of North Sumatra, confirming that

the plant used was Mobe (Artocarpus lacucha Buch-Ham.). This identification supports the validity of the specimen used in the study.

3.2 Preparation of Ethanol Extract of Mobe Leaves

This study employed the maceration extraction technique using 96% ethanol as the solvent. The extraction process yielded a thick extract, which was then weighed to calculate the yield percentage of the ethanol extract of mobe leaves. The yield of the ethanol extract was 14.28%, indicating the efficiency of the extraction procedure.

3.3 Characterization of Simplicia

The characterization of simplicia was conducted to determine the physical and chemical properties of the mobe leaf powder. The results of the characterization are presented in Table 1. The determination of water content, water-soluble extract content, ethanol-soluble extract content, total ash content, and acid-insoluble ash content was performed according to the standards outlined in the Indonesian Herbal Pharmacopoeia (Ministry of Health, 2000). The characterization of the mobe leaf simplicia included both macroscopic and microscopic examinations, as well as determinations of water content, water-soluble extract content, ethanol-soluble extract content, total ash content, and acid-insoluble ash content. Macroscopically, the mobe leaves were found to be round and elongated with smooth edges and a hairy underside. Microscopically, the simplicia powder exhibited covering hairs and vascular tissue with anisocytic stomata. The water content of the simplicia was determined to be 5.32%, meeting the requirement of less than 12% as set by the Indonesian Herbal Pharmacopoeia. The water-soluble and ethanol-soluble extract contents were 12.82% and 12.27%, respectively, indicating the presence of bioactive compounds that dissolve in these solvents. The total ash content was 7.19%, while the acid-insoluble ash content was 0.68%, both of which comply with the standards and indicate that the simplicia does not contain hazardous heavy metals in amounts exceeding safety limits.

3.4 Phytochemical Screening of Simplicia and Extract

Phytochemical screening was conducted to identify the presence of secondary metabolites in the simplicia and ethanol extract of mobe leaves. The results of the phytochemical screening are shown in Table 1. (Chahyadi ert al., 2014);(Salama ert al., 2012)).

Table 1. Phytochemical Screening Results of Ethanol Extract of Mobe Leaves

No	Compound Group	Reagent	Theory	Screening Result	Simplicia	Extract
1	Alkaloids	Meyer, Bouchardat, Dragendorff	White/yellow, brown, black, brick-red precipitate	White,brown, brick-red precipitate	+	+
2	Flavonoids	Mg powder, concentrated HCl, amyl alcohol	Yellow, orange, red in amyl alcohol	Orange in amyl alcohol	+	+
3	Tannins	10% FeCl3	Blue/greenish black	Greenish black	+	+
4	Saponins	Hot water	Permanent foam formation	Permanent foam	+	+
5	Triterpenoids	Liberman-Burchard	Blue, purple	Blue, purple	+	+
6	Glycosides	Molisch	Purple ring formation	Purple ring	+	+

Table 1 details the results of phytochemical screening, showing the presence of alkaloids, flavonoids, tannins, saponins, triterpenoids, and glycosides in both simplicia and extract of mobe leaves, indicating their potential bioactive properties.

3.5 Health Research Ethics Approval

This study involved male white rats (200-240 grams) obtained from the Pharmacology Laboratory at the University of North Sumatra. The rats were housed in ventilated cages with controlled temperature and humidity and were given pellet feed and water. The rats were acclimatized for seven days before the experiment. All experimental procedures were approved by the animal research ethics committee of FMIPA – University of North Sumatra (No. 0226/KEPH-F-MIPA/2023) according to the "Guide for the Care and Use of Laboratory Animals."

3.6 Hepatoprotective Activity Testing of Ethanol Extract of Mobe Leaves

3.6.1 Measurement of Liver Damage Biomarkers

Liver damage biomarkers were evaluated by measuring parameters such as total bilirubin (TB), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in blood serum. Increased levels of TB, ALT, and AST in the blood indicate liver damage (Huang et al., 2017). To assess the hepatoprotective activity of the ethanol extract of mobe leaves, TB, ALT, and AST were measured on day 15 after the rats were given CCl4 1 ml/kg BW.

Before measurement, the rats were sacrificed using ketamine anesthesia, and blood was drawn from the heart to obtain serum and plasma. Blood serum was centrifuged for 15 minutes at 3000-4000 rpm to separate the supernatant and sediment. The supernatant was used to measure TB, ALT, and AST levels using commercial kits. The data were analyzed using one-way ANOVA and Turkey Post-Hoc tests with SPSS software.

3.6.1.1 Total Bilirubin Measurement in Rat Serum

Serum bilirubin is a sensitive biomarker for diagnosing liver disorders. Bilirubin is a breakdown product of hemoglobin that conjugates with glucuronic acid in hepatocytes to increase its water solubility. Elevated total bilirubin levels in the serum of rats given CCl4 result from the release of bilirubin from liver cytosol into the bloodstream due to increased cell membrane permeability caused by hepatocyte damage (Shanmugam et al., 2013).

The one-way ANOVA analysis of total bilirubin levels in rat blood serum showed a significance value of = 0.000 ($p < 0.05$) among treatment groups, indicating significant differences in mean total bilirubin levels. The mean total bilirubin levels are shown in Table 2.

Table 2. The Mean total of bilirubin levels

Group	Mean TB (µmol/L) ± SD
EEDM 400 mg/kg BB + CCl4 1 ml/kg BB	0,15 ± 0,00ab
EEDM 200 mg/kg BB + CCl4 1 ml/kg BB	0,18 ± 0,00ab
EEDM 100 mg/kg BB + CCl4 1 ml/kg BB	0,19 ± 0,00ab
EEDM 50 mg/kg BB + CCl4 1 ml/kg BB	0,22 ± 0,01ab
Silymarin 100 mg/kg BB + CCl4 1 ml/kg BB	0,14 ± 0,00a
Na CMC 1 % + CCl4 1 ml/kg BB	0,27 ± 0,00b

Table 2 shows significant differences ($p<0.05$) in total bilirubin reduction after EEDM administration compared to the negative control. The statistical results indicate that total bilirubin levels in the silymarin control group were lower than those in the other treatment groups induced with CCl4 1 ml/kg BW. The negative control group had the highest mean total bilirubin level (0.27 ± 0.00 µmol/L), indicating that CCl4 1 ml/kg BW induced liver damage marked by increased total bilirubin levels.

The administration of the ethanol extract of mobe leaves for 14 days in rats induced with CCl4 1 ml/kg BW significantly reduced total bilirubin levels. At a dose of 400 mg/kg BW, total bilirubin levels decreased to 0.15 ± 0.00 µmol/L, while a dose of 50 mg/kg BW reduced total bilirubin levels to 0.22 ± 0.01 µmol/L. The effect of treatment on total bilirubin levels is illustrated in the following figure.

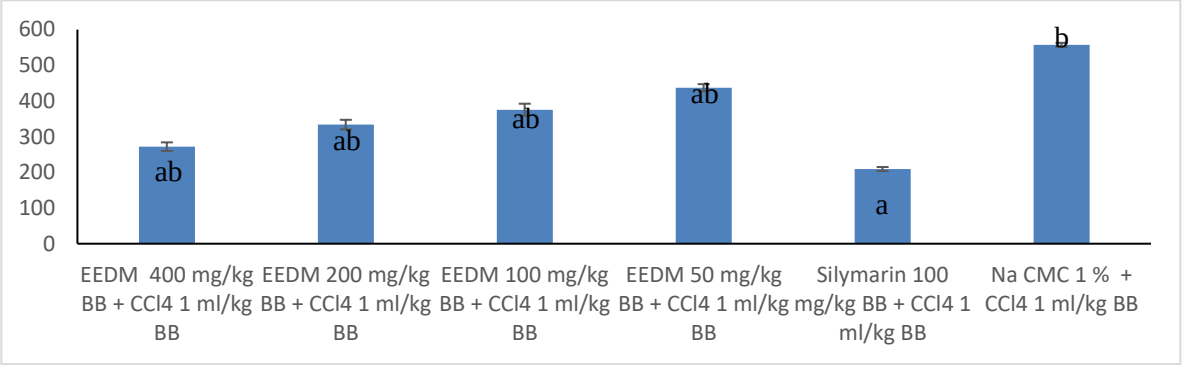

Figure 1. illustrates the effect of EEDM on total bilirubin levels. The Effect of EEDM on TB
a. Sig (P) < 0.05 indicates a significant difference compared to the negative control group.
b. Sig (P) < 0.05 indicates a significant difference compared to the Silymarin control group.

3.6.1.2 Alanine Aminotransferase Measurement in Rat Serum

Alanine aminotransferase (ALT) is an enzyme that catalyzes the conversion of alanine and α-ketoglutarate into glutamate and pyruvate, contributing to cellular nitrogen metabolism and hepatic gluconeogenesis. Increased ALT enzyme levels in blood serum indicate liver damage (Yadav et al., 2022).

Statistical analysis showed a decrease in liver function in rats due to the induction of CCl4 1 ml/kg BW. The one-way ANOVA results showed a significance value of = 0.000 ($p < 0.05$) among treatment groups, indicating significant differences in ALT levels in rat blood serum. The mean ALT levels are shown in Table 3.

Table 3. The Mean ALT levels

Group	Mean ALT (U/L) ± SD
EEDM 400 mg/kg BB + CCl4 1 ml/kg BB	255 ± 7,97 ab
EEDM 200 mg/kg BB + CCl4 1 ml/kg BB	286 ± 13,50 ab
EEDM 100 mg/kg BB + CCl4 1 ml/kg BB	335,60 ± 13,94 ab
EEDM 50 mg/kg BB + CCl4 1 ml/kg BB	427,80 ± 7,46 ab
Silymarin 100 mg/kg BB + CCl4 1 ml/kg BB	224,60 ± 7,80 a
Na CMC 1 % + CCl4 1 ml/kg BB	505,20 ± 5,26 b

Table 3 presents the mean ALT levels in various treatment groups, indicating significant differences ($p<0.05$) in ALT reduction after EEDM administration compared to the negative control group. The ALT levels in the silymarin control group were lower than those in the other treatment groups. The negative control group showed the highest ALT level (505.20 ± 5.26 U/L), indicating severe liver damage due to CCl4 induction.

The administration of the ethanol extract of mobe leaves for 14 days in rats induced with CCl4 significantly reduced ALT levels. At a dose of 400 mg/kg BW, ALT levels decreased to 255.0 ± 7.97 U/L, while a dose of 50 mg/kg BW reduced ALT levels to 427.80 ± 7.46 U/L. The effect of treatment on ALT levels is illustrated in the following figure.

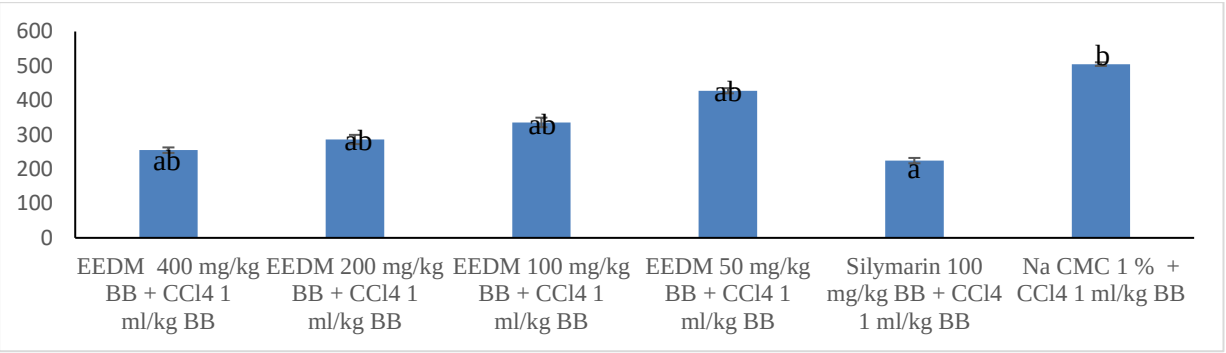

Figure 2. Shows the effect of EEDM on ALT levels. The Effect of EEDM on ALT
a. Sig (P) < 0.05 indicates a significant difference compared to the negative control group.
b. Sig (P) < 0.05 indicates a significant difference compared to the Silymarin control group.

3.6.1.3 Aspartate Aminotransferase Measurement in Rat Serum

Aspartate aminotransferase (AST) or serum glutamic oxaloacetic transaminase (SGOT) is found in almost all body tissues except bone. Increased AST levels in serum indicate liver cell or tissue damage (Yadav ert al., 2022).

Statistical analysis showed a decrease in liver function in rats due to the induction of CCl4 1 ml/kg BW. The one-way ANOVA results showed a significance value of = 0.000 ($p < 0.05$) among treatment groups, indicating significant differences in AST levels in rat blood serum. The mean AST levels are shown in Table 4.

Table 4. Results of Average AST Levels Measurement in Rat Blood Serum

Group	AST Mean (U/L) ± SD
EEDM 400 mg/kg BB + CCl4 1 ml/kg BB	272,00 ± 12,04 ab
EEDM 200 mg/kg BB + CCl4 1 ml/kg BB	333,80 ± 13,48 ab
EEDM 100 mg/kg BB + CCl4 1 ml/kg BB	375,40 ± 17,01 ab
EEDM 50 mg/kg BB + CCl4 1 ml/kg BB	437,40 ± 9,81 ab
Silymarin 100 mg/kg BB + CCl4 1 ml/kg BB	209,20 ± 5,67 a
Na CMC 1 % + CCl4 1 ml/kg BB	557,40 ± 5,67 b

Table 4 shows significant differences ($p<0.05$) in AST reduction after EEDM administration compared to the negative control. The AST levels in the silymarin control group were lower than those in the other treatment groups. The negative control group showed the highest AST level (557.40 ± 5.67 U/L), indicating severe liver damage due to CCl4 induction.

The administration of the ethanol extract of mobe leaves for 14 days in rats induced with CCl4 significantly reduced AST levels. At a dose of 400 mg/kg BW, AST levels decreased to 272.00 ± 12.04 U/L, while a dose of 50 mg/kg BW reduced AST levels to 437.40 ± 9.81 U/L. The effect of treatment on AST levels is illustrated in the following figure.

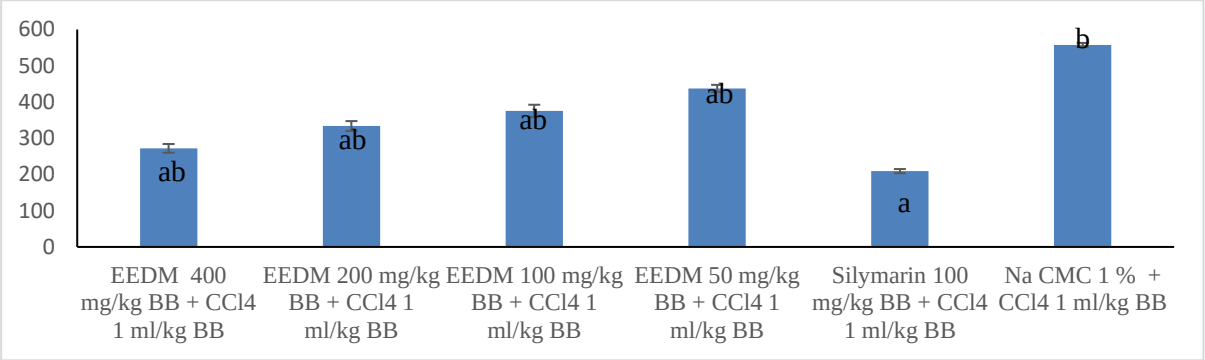

Figure 3. The Effect of EEDM on AST
a. Sig (P) < 0.05 indicates a significant difference compared to the negative control group.
b. Sig (P) < 0.05 indicates a significant difference compared to the Silymarin control group.

3.6.2 Macropathological Observation of the Liver

Macropathological observation of the liver of male white rats was conducted for each treatment group after being given EEDM for 14 days and induced with CCl4. Observations included the color, consistency, and surface of the liver, as presented in Table 6 and Figure 4.

Table 5. Results of Macropathological Observations of Rat Liver Organs

Group	Color	Consistency	Surface
EEDM 400 mg/kg BW + CCl4 1 ml/kg BW	Dark red	Elastic	Smooth
EEDM 200 mg/kg BW + CCl4 1 ml/kg BW	Pale red	Elastic	Smooth with white spots
EEDM 100 mg/kg BW + CCl4 1 ml/kg BW	Pale red	Elastic	Smooth with white spots
EEDM 50 mg/kg BW + CCl4 1 ml/kg BW	Pale red	Elastic	Smooth with white spots
Silymarin 100 mg/kg BW + CCl4 1 ml/kg BW	Dark red	Elastic	Smooth
Na CMC 1% + CCl4 1 ml/kg BW	Pale red	Elastic	Smooth with white spots

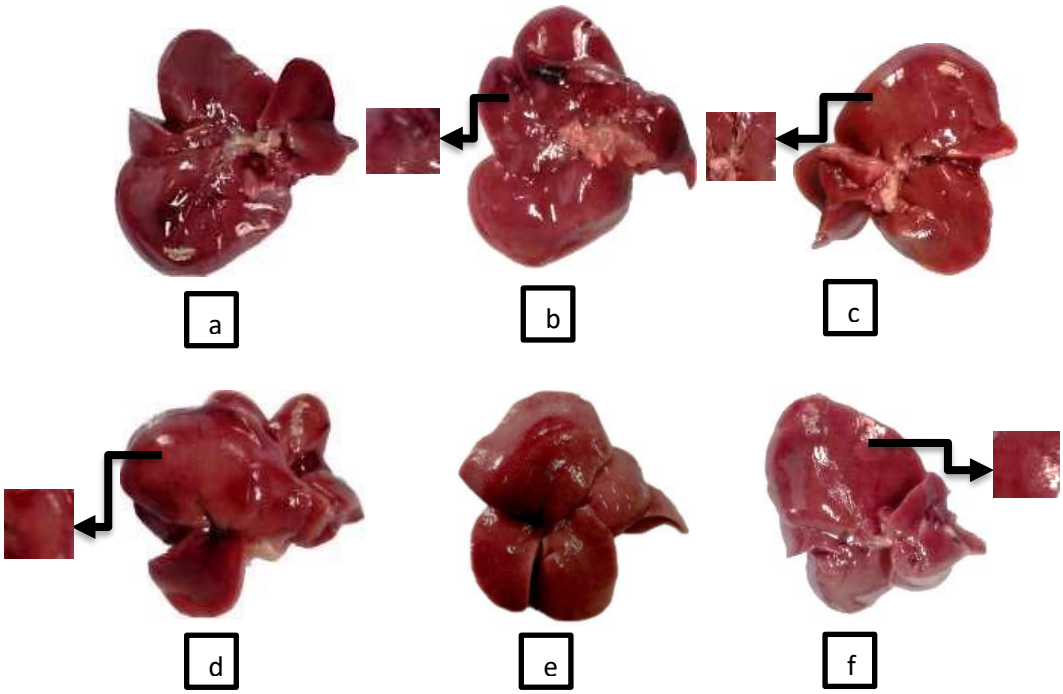

Figure 4. Macropathology of rat liver organ.
(a) negative control (Na CMC 1 % + CCl4 1 ml/kg BB), (b) positive control (Silymarin 100 mg/kg BB + CCl4 1 ml/kg BB), (c) EEDM 50 mg/kg BB + CCl4 1 ml/kg BB, (d) EEDM 100 mg/kg BB + CCl4 1 ml/kg BB, (e) EEDM 200 mg/kg BB + CCl4 1 ml/kg BB, (f) EEDM 400 mg/kg BB + CCl4 1 ml/kg BB.

Figure 4 shows the macropathology of rat liver organs across different treatment groups. (a) Negative control (Na CMC 1% + CCl4 1 ml/kg BW), (b) Positive control (Silymarin 100 mg/kg BW + CCl4 1 ml/kg BW), (c) EEDM 50 mg/kg BW + CCl4 1 ml/kg BW, (d) EEDM 100 mg/kg BW + CCl4 1 ml/kg BW, (e) EEDM 200 mg/kg BW + CCl4 1 ml/kg BW, (f) EEDM 400 mg/kg BW + CCl4 1 ml/kg BW. The positive control group had dark red livers with a smooth and even surface, while the negative control group showed white spots on the liver surface and a paler color. The macropathology of the livers in the treatment groups given EEDM 400 mg/kg BW showed dark red livers approaching the positive control group with a smooth and even surface.

3.7 Discussion

Macropathological observations showed that the administration of EEDM at a dose of 400 mg/kg BW and silymarin at 100 mg/kg BW could improve the macropathological condition of male white rat livers induced with CCl4. Normal liver organs are dark red with a smooth and even surface due to the high blood content. Exposure to toxins or drug overdose can cause liver damage, marked by a paler color and a spotted liver surface.

CCl4 is a toxic chemical metabolized in the liver by cytochrome P450 2E1 into trichloromethyl radicals, which react with oxygen to form highly reactive trichloromethylperoxy radicals, leading to increased reactive oxygen species (ROS) and decreased antioxidant defenses. This causes lipid peroxidation, mitochondrial damage, DNA damage, and hepatocyte necrosis, indicated by changes in liver color and surface (Cahaya Widya Putri & Rahman, 2021).

3.7.1 Comparison with Previous Studies

The findings of this study are consistent with previous research on the hepatoprotective effects of plant extracts. For example, Domitrović et al. (2011) reported that berberine exhibited hepatoprotective activity by inhibiting TNF-α, COX-2, and iNOS expression in CCl4-intoxicated mice, reducing liver damage markers and improving liver histopathology. Similarly, the hepatoprotective activity of flavonoids is well-documented, with studies showing their ability to enhance antioxidant defenses such as increased activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH) levels, combating free radicals and inducing cellular stress defense systems (Fan et al., 2018; Tacke et al., 2009).

The results of this study align with the findings of Purlungan (2018), who demonstrated the antioxidant activity of ethanol extract of mobe leaves using the ABTS method. The IC50 values indicated a strong effect, suggesting the potential of mobe leaves as a natural source of antioxidants and hepatoprotective agents. This study further confirms the therapeutic potential of mobe leaves by demonstrating significant reductions in total bilirubin, ALT, and AST levels in CCl4-induced rats, corroborating the hepatoprotective effects observed in previous studies

3.7.2 Limitations of the Study

Despite the promising results, this study has several limitations. First, the study was conducted on a limited number of rats, which may affect the generalizability of the findings. Future studies should include a larger sample size to validate these results. Second, the study focused on biochemical and macropathological assessments without detailed histopathological analysis, which could provide more comprehensive insights into the liver's structural changes. Third, the study did not investigate the molecular mechanisms underlying the hepatoprotective effects of EEDM, such as the specific pathways involved in antioxidant and anti-inflammatory activities. Finally, the study was conducted in an animal

model, and the findings may not directly translate to human applications. Clinical trials are necessary to evaluate the safety and efficacy of mobe leaf extract in human subjects.

3.7.3 Future Directions

Based on the results of this study, further research is recommended to explore the molecular mechanisms of the hepatoprotective effects of the ethanol extract of mobe leaves. Investigating the specific pathways involved in reducing oxidative stress and inflammation could provide deeper insights into its therapeutic potential. Additionally, conducting histopathological analyses would help to better understand the structural changes in the liver tissue. Future studies should also consider larger sample sizes and include clinical trials to evaluate the safety and efficacy of mobe leaf extract in humans, potentially leading to the development of natural, affordable, and environmentally friendly alternatives for liver disease therapy.

CONCLUSION

This study demonstrates that the ethanol extract of mobe leaves (Artocarpus lacucha Buch-Ham.) has significant hepatoprotective effects in rats induced with carbon tetrachloride (CCl4). Administration of the ethanol extract at a dose of 400 mg/kg body weight (BW) effectively reduced total bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels in blood serum. Additionally, the macropathological condition of the liver improved, approaching normal conditions similar to those in the positive control group treated with silymarin.

The findings of this study not only reaffirm the traditional use of mobe leaves as a medicinal plant but also provide a scientific basis for its potential development as a natural hepatoprotective agent. The significant reduction in liver damage markers and improvement in liver condition suggest that mobe leaf extract could be an effective alternative to synthetic hepatoprotective drugs, which often come with adverse side effects. The presence of bioactive compounds such as flavonoids, alkaloids, tannins, saponins, triterpenoids, and glycosides indicates that mobe leaves possess strong antioxidant and anti-inflammatory properties, making them a valuable resource in herbal medicine.

RECOMMENDATIONS

Based on the results of this study, further research is recommended on the molecular mechanisms of the hepatoprotective effects of the ethanol extract of mobe leaves. Additionally, clinical trials on humans are needed to evaluate the safety and efficacy of this extract as herbal medicine. Developing herbal products based on the ethanol extract of mobe leaves could offer a natural, affordable, and environmentally friendly alternative for liver disease therapy.

REFERENCES

Cahaya Widya Putri, W., & Rahman, H. (2021). Uji Aktivitas Hepatoprotektor Ekstrak Etanol Daun Rambutan (Nephelium lappaceum L.) pada Mencit Putih Jantan yang Diinduksi Parasetamol Hepatoprotective Activity of Rambutan Leaf (Nephelium lappaceum L.) Ethanol Extract in Male Mice Induced by Paracetamol. In *Jurnal Farmasi Indonesia* (Vol. 18, Issue 2). http://journals.ums.ac.id/index.php/pharmacon

Chahyadi, A., Hartati, R., Wirasutisna, K. R., & Elfahmi. (2014). Boesenbergia Pandurata Roxb., An Indonesian Medicinal Plant: Phytochemistry, Biological Activity, Plant Biotechnology. *Procedia Chemistry*, *13*, 13–37. https://doi.org/https://doi.org/10.1016/j.proche.2014.12.003

Chen, C., Zhu, Z., Mao, Y., Xu, Y., Du, J., Tang, X., & Cao, H. (2020). HbA1c may contribute to the development of non-alcoholic fatty liver disease even at normal-range levels. *Bioscience Reports*, *40*(1). https://doi.org/10.1042/BSR20193996

Chodidjah, Widayati, E., & Utari. (2007). Pengaruh pemberian air rebusan meniran (Phyllanthm niruriLinn) terhadap gambaran histopatologi hepar tikus wistar yang terinduksi CCL4. *JurnalAnatomi Indonesia*, *2*(1), 8–12.

Dalimartha, S. (1999). *Ramuan tradisional untuk pengobatan hepatitis*. Penebar Swadaya.

Domitrović, R., Jakovac, H., & Blagojević, G. (2011). Hepatoprotective activity of berberine is mediated by inhibition of TNF-α, COX-2, and iNOS expression in CCl4-intoxicated mice. *Toxicology*, *280*(1–2), 33–43. https://doi.org/https://doi.org/10.1016/j.tox.2010.11.005

Dröge, W. (2002). Free radicals in the physiological control of cell function. In *Physiological Reviews* (Vol. 82, Issue 1, pp. 47–95). American Physiological Society. https://doi.org/10.1152/physrev.00018.2001

Fan, Y., Yue, X., Xue, F., Cui, J., Brauth, S. E., Tang, Y., & Fang, G. (2018). Auditory perception exhibits sexual dimorphism and left telencephalic dominance in Xenopus laevis. *Biology Open*, *7*(12). https://doi.org/10.1242/bio.035956

Hariyanto, M. B. (2013). Pengaruh pemberian buah melon (Cucumis melo L.) personde terhadap gambaran histopatologi zona sentrilobular hepar tikus putih (Rattus norvegicus) galur Wistar yang diinduksi CCl4 (karbon tetraklorida). In *(Sarjana Thesis)*. Universitas Brawijaya.

Indarto, I. (2015). Isolasi dan Identifikasi Senyawa Fenolik dari Kulit Akar Tumbuhan Artocarpus Dadah Miq 63-74. *Jurnal Ilmiah Pendidikan Fisika Al-Biruni*, *4*(2), 205–217. https://doi.org/10.24042/jpifalbiruni.v4i2.93

Jagtap, U. B., & Bapat, V. A. (2010). Artocarpus: a review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, *129*(2), 142–166. https://doi.org/10.1016/j.jep.2010.03.031

Madrigal-Santillán, E., Madrigal-Bujaidar, E., Álvarez-González, I., Sumaya-Martínez, M. T., Gutiérrez-Salinas, J., Bautista, M., Morales-González, Á., García-Luna Y González-Rubio, M., Aguilar-Faisal, J. L., & Morales-González, J. A. (2014). Review of natural products with hepatoprotective effects. In *World Journal of Gastroenterology* (Vol. 20, Issue 40, pp. 14787–14804). WJG Press. https://doi.org/10.3748/wjg.v20.i40.14787

Pandey, A., & Bhatnagar, S. P. (2009). Preliminary phytochemical screening and antimicrobial studies on Artocarpus lookcha Roxb. *Journal Acient Science of Life*, *28*(4), 21–24.

Rusmaladewi, A., & Istanto, W. (2014). Pengaruh pemberian N-acetylcysteine terhadap kadar SGOT dan SGPT pada tikus Wistar yang diberi parasetamol. *Medica Hospitalia: Journal of Clinical Medicine*, *2*(2), 78–83. https://doi.org/10.36408/mhjcm.v2i2.96

Salama, S. M., Bilgen, M., Al Rashdi, A. S., & Abdulla, M. A. (2012). Efficacy of Boesenbergia rotunda treatment against thioacetamide-induced liver cirrhosis in a rat model. *Evidence-Based Complementary and Alternative Medicine*, *2012*. https://doi.org/10.1155/2012/137083

Sitorus, P., Keliat, J. M., Asfianti, V., Muhammad, M., & Satria, D. (2022). A Literature Review of Artocarpus lacucha Focusing on the Phytochemical Constituents and Pharmacological Properties of the Plant. In *Molecules* (Vol. 27, Issue 20). MDPI. https://doi.org/10.3390/molecules27206940

Tacke, F., Luedde, T., & Trautwein, C. (2009). Inflammatory pathways in liver homeostasis and liver injury. *Clinical Reviews in Allergy & Immunology*, *36*(1), 4–12. https://doi.org/10.1007/s12016-008-8091-0

Timbrel, J. A. (2008). *Principles of biochemical toxicology* (4th ed.). New York: Informa Healthcare. pp. 308-311.

Yadav, S., Jangra, R., Sharma, B. R., & Sharma, M. (2022). Current Advancement in Biosensing techniques for determination of Alanine aminotransferase and Aspartate aminotransferase-a Mini Review. In *Process Biochemistry* (Vol. 114, pp. 71–76). Elsevier Ltd. https://doi.org/10.1016/j.procbio.2022.01.010