






## Research Paper

# Hepatoprotective Effect of the Ethanolic Extract of *Moringa oleifera* Leaves in Carbon Tetrachloride-induced Wistar Rats

Annisa Dwi Lestari<sup>1</sup>, R. Susanti<sup>1\*</sup>, Ari Yuniastuti<sup>1</sup>

<sup>1</sup> Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Semarang, Indonesia.



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### \* Corresponding author:

R. Susanti,

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Semarang, Indonesia.

E-mail: basanatha@mail.unnes.ac.id

## ABSTRACT

**Background:** Hepatotoxicity is liver damage caused by exposure to xenobiotics, resulting when the liver is unable to detoxify free radicals, such as reactive oxygen species (ROS), or other harmful metabolites. *Moringa oleifera* has been reported to possess preventive and therapeutic potential against various diseases due to its extract and bioactive components, particularly quercetin, which plays a significant role in its hepatoprotective effects. The present study aimed to evaluate the hepatoprotective potential of ethanolic *Moringa oleifera* leaf extract in carbon tetrachloride (CCl<sub>4</sub>)-induced rats.

**Methods:** A total of 25 male Wistar rats were divided into five groups: a negative control group (0.9% NaCl), a positive control group (CCl<sub>4</sub>), and three treatment groups receiving *Moringa oleifera* extract at doses of 250 mg/kg body weight (D1), 500 mg/kg body weight (D2), and 1000 mg/kg body weight (D3), all previously induced with 10 % CCl<sub>4</sub> solution administered at 1 mL/kg body weight. Observations were made on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, as well as relative liver weight.

**Results:** Administration of ethanolic *Moringa oleifera* leaf extract at all three doses significantly reduced AST and ALT levels compared to the CCl<sub>4</sub> group, indicating hepatoprotective effects. However, no significant differences in relative liver weight were observed among the treatment groups.

**Conclusion:** Ethanolic *Moringa oleifera* leaf extract exhibits hepatoprotective effects against CCl<sub>4</sub>-induced liver injury through the reduction of AST and ALT levels by 46.5% and 37%, respectively, although it does not influence relative liver weight.

**Keywords:** Alanine aminotransferase (ALT), Aminotransferase (AST), hepatoprotective, *Moringa oleifera*, Relative liver weight.

## Introduction

The liver plays a crucial role in maintaining the body's homeostasis by participating in bilirubin and cholesterol metabolism, glucose synthesis and storage, hormone metabolism, plasma protein secretion, and detoxification of both endobiotics and xenobiotics [1, 2]. Xenobiotics are exogenous compounds introduced into the body through environmental exposure, including pollutants, toxic substances, and chemical agents [3]. These foreign compounds, which cannot be synthesized endogenously, are commonly found in consumer products, such as artificial colorants, preservatives, industrial chemicals, and pharmaceuticals [4].

Hepatotoxicity refers to liver injury or dysfunction caused by xenobiotic or drug exposure during hepatic metabolism [5]. This condition may arise from excessive drug consumption or exposure beyond therapeutic limits, potentially leading to harmful effects on the liver. Industrial chemicals like carbon tetrachloride (CCl<sub>4</sub>),

widely used in laboratories and industries, are well-known hepatotoxins [6]. Impaired detoxification of free radicals, such as reactive oxygen species (ROS) and harmful metabolites, can result in liver damage [7]. Despite advances in medicine, no fully effective drugs are available to comprehensively enhance liver function, protect the organ, and facilitate hepatocyte regeneration. Therefore, exploring plant-based alternatives is essential to identify safer, more effective therapeutic agents for managing liver diseases [8].

Herbal medicines have long been used as primary healthcare in many cultures worldwide, offering bioactive compounds with therapeutic potential [9]. One such plant is *Moringa oleifera*, a species belonging to the genus *Moringa* (Family: Moringaceae), which thrives in tropical climates, including Indonesia. Almost all parts of *Moringa oleifera* (seeds, leaves, flowers, bark, roots, and fruits) contain diverse

bioactive components, including carotenoids, phenolics, alkaloids, glucosinolates, isothiocyanates, folates, tannins, saponins, and fatty acids. The primary phenolic constituents in *Moringa oleifera* leaves include flavonoids (e.g., quercetin, luteolin, myricetin, apigenin, and kaempferol), lignans, and phenolic acids (e.g., cumaroylquinic, caffeoylquinic, and feruloylquinic acids) [10]. These compounds contribute to various pharmacological properties, including antibacterial, anti-ulcer, antipyretic, antiepileptic, anticancer, and anti-inflammatory activities [11].

Of particular interest is the hepatoprotective effect of *Moringa oleifera*. Its phenolic and flavonoid compounds possess anti-inflammatory and antioxidant properties, preventing lipid peroxidation, reducing oxidative stress markers, and lowering liver enzyme levels [6]. Consequently, *Moringa oleifera* may protect the liver from oxidative stress caused by excessive free radical exposure.

Transaminases, intracellular enzymes released into circulation following cell injury, are commonly used as biomarkers of liver damage. Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are extensively accepted indicators of liver injury, with elevated levels reflecting hepatocellular cytolysis [12, 13]. Additionally, changes in liver weight may indicate treatment-related alterations, such as hepatocellular hypertrophy caused by xenobiotic exposure [14].

The present study investigates the effects of *Moringa oleifera* leaf ethanolic extract on liver function parameters, including serum AST and ALT levels and relative liver weight, to provide evidence supporting its hepatoprotective potential.

## Materials and Methods

### Preparation of *Moringa oleifera* leaf extract

Fresh *Moringa oleifera* leaves were obtained from Tanjung Mas, Semarang, Central Java, Indonesia. Leaves were dried at 50°C and ground into powder. Extraction was performed by maceration using 70% ethanol as the solvent. The filtrate was concentrated with a rotary evaporator at 40°C until a thick extract was obtained, then oven-dried and reground before being dissolved according to the required doses.

### Assessment of total flavonoid content (TFC) and total phenolic content (TPC)

The TFC was assessed using an aluminium chloride-based colorimetric assay. The sample extract was mixed with AlCl<sub>3</sub> reagent and incubated at room temperature, then the absorbance was measured at 430 nm against a reagent blank. Quercetin was used as the calibration standard, and TFC was expressed as milligrams of quercetin equivalent per gram extract (mg QE/g).

In addition, TPC was assessed using the Folin Ciocalteu colorimetric assay. The extract was mixed with Folin–

Ciocalteu reagent and allowed to react briefly, after which a sodium carbonate solution was added. The mixture was incubated at room temperature, protected from light, and its absorbance was later recorded using a UV–Vis spectrophotometer at 765 nm. Gallic acid was employed to generate the calibration curve, and TPC was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). The TFC and TPC were conducted at the Integrated Laboratory FMIPA, Universitas Negeri Semarang, Indonesia.

### Preparation of CCl<sub>4</sub> solution

A 10% CCl<sub>4</sub> solution was prepared by mixing 0.4 mL of pure CCl<sub>4</sub> with 3.6 mL of mineral oil. The solution was administered at a dose of 1 mL/kg body weight, equivalent to 0.2 mL for a rat weighing approximately 200 g.

### Experimental animals and treatments

A total of 25 male Wistar rats were acclimatized for seven days before being randomly assigned into five groups: normal control (K), positive control CCl<sub>4</sub> without extract (K+), and three treatment groups receiving *Moringa oleifera* extract at doses of 250 (D1), 500 (D2), and 1000 (D3) mg/kg body weight.

Dose selection was guided by previously published studies demonstrating the hepatoprotective activity and safety of *Moringa oleifera* leaf extract within similar dose ranges in rat models. Previous toxicity evaluations indicated that *Moringa oleifera* leaf supplementation is safe at doses up to 1000 mg/kg body weight, whereas adverse effects appear at much higher levels, 3000 mg/kg body weight [15]. Within this established safe range, three doses were selected to evaluate potential dose-dependent hepatoprotective responses in the present study.

On day 1, CCl<sub>4</sub> was administered intraperitoneally. From the following day, the extract was given orally once daily for 14 consecutive days. All procedures were conducted in accordance with the guidelines of the Health Research Ethics Committee, Faculty of Medicine, Universitas Negeri Semarang, Indonesia, and approved under ethical clearance No. 263/KEPK/FK/KLE/2024.

### Assessment of AST and ALT levels

On the last day, blood samples were collected via the orbital sinus and centrifuged to obtain serum. The AST and ALT levels were measured using Diasys® enzymatic kits ASAT (GOT) FS (Cat. No. 1 2601 99 10 021) and ALAT (GPT) FS (Cat. No. 1 2701 99 10 021), read with a UV–Vis spectrophotometer at a wavelength of 340 nm in the Laboratory of the Centre for Food and Nutrition Studies, Universitas Gadjah Mada, Indonesia.

### Relative liver weight analysis

On day 16, rats were euthanized, and the liver was excised, cleaned, and weighed using a digital analytical balance. Relative liver weight (RLW) was calculated as:

$$RLW = \frac{\text{absolute organ weight (g)}}{\text{body weight at sacrifice (g)}} \times 100$$

### Data analysis

Relative liver weights were calculated using Microsoft Excel. The AST and ALT measurements were performed at the Laboratory of the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Indonesia. Moreover, the Shapiro–Wilk test was employed to assess normality, and Levene’s test was used for homogeneity of variance in IBM SPSS Statistics (version 27) software. Parametric data were analyzed by one-way ANOVA followed by Tukey’s post-hoc test, while non-parametric data were analyzed using the Kruskal–Wallis test followed by the Mann–Whitney U test to determine significant differences

between groups. The TFC and TPC were analyzed once as descriptive parameters at the Integrated Laboratory FMIPA, Universitas Negeri Semarang. Statistical analysis was not performed on TFC and TPC data, as these represent single-value determinations of extract characteristics.

## Results

### Total phenolic and flavonoid content of *Moringa oleifera* leaf ethanolic extract

The phytochemical characterization of the *Moringa oleifera* leaf ethanolic extract confirmed measurable levels of TFC and high levels of TPC. The extract contained 8.84 mg QE/g sample of TFC and 110 mg GAE/g sample of TPC (Table 1). Following the extract characterization, statistical analysis for the biological parameters was then carried out, beginning with the assessment of data normality.

**Table 1.** Phytochemical characterization of *Moringa oleifera* leaf extract

Parameter	Value	Unit
Total Flavonoid Content (TFC)	8.84	mg QE/g extract
Total Phenolic Content (TPC)	110	mg GAE/g extract

### Effect of *moringa oleifera* leaf ethanolic extract on AST levels in CCl<sub>4</sub>-induced rats

The Shapiro–Wilk normality test indicated that AST levels were normally distributed ( $p > 0.05$ ), and Levene’s homogeneity test showed homogeneous variance ( $p = 0.161$ ). According to these results, the analysis was continued using one-way ANOVA, which demonstrated

significant differences between groups ( $p < 0.001$ ). The Tukey post-hoc test indicated that the control group (K) differed significantly from all treatment groups, with the most pronounced difference compared to the positive control group (K+). Among the treatment groups, the K+ group differed most significantly from the D3 group. The comparison of AST levels between groups is shown in Table 2.

**Table 2.** Analysis results of AST levels

Group	Mean±Standard Deviation (SD)	Normality Test	Homogeneity Test	ANOVA Test
K	37.29±0.63 <sup>a</sup>	0.425		
K+	79.53±1.26 <sup>e</sup>	0.503		
D1	50.30±0.73 <sup>d</sup>	0.498	0.161	<0.001
D2	45.25±1.21 <sup>c</sup>	0.386		
D3	42.53±0.55 <sup>b</sup>	0.819		

Note: Values followed by different superscript letters (a, b, c, d, e) indicate significant differences.

### Effect of *moringa oleifera* leaf ethanolic extract on ALT levels in CCl<sub>4</sub>-induced rats

The ALT levels were normally distributed according to the Shapiro–Wilk test; however, the Levene test indicated that the data were not homogeneous ( $p < 0.05$ ). Therefore, the analysis was continued using the non-parametric Kruskal–Wallis test. The results revealed significant

differences between groups ( $p < 0.05$ ). The Mann–Whitney U test showed significant differences between several group pairs ( $p < 0.05$ ). Descriptive analysis indicated that among the treatment groups, the D3 group had the lowest ALT level (24.76 U/L), which was close to that of the control group (K) (19.32 U/L), as shown in Table 3.

**Table 3.** Analysis results of ALT levels

Group	Mean±Standard Deviation (SD)	Normality Test	Homogeneity Test	Kruskal-Wallis Nonparametric Test
K	19.32±0.41 <sup>a</sup>	0.314		
K+	39.32±1.88 <sup>e</sup>	0.533		
D1	30.30±0.74 <sup>d</sup>	0.487	0.001	<0.001
D2	27.96±0.55 <sup>c</sup>	0.809		
D3	24.76±1.14 <sup>b</sup>	0.153		

Note: Values followed by different superscript letters (a, b, c, d, e) indicate significant differences.

### Effect of *Moringa oleifera* leaf ethanolic extract on relative liver weight in CCl<sub>4</sub>-induced rats

Relative liver weight data were homogeneous based on Levene's test, but not all data were normally distributed

according to the Shapiro–Wilk test ( $p < 0.05$ ). Therefore, the analysis continued with the Kruskal–Wallis test, which showed no significant differences between the treatment groups ( $p > 0.05$ , Table 4).

**Table 4.** Analysis Results of Relative Liver Weight

Group	Mean±Standard Deviation (SD)	Normality Test	Homogeneity Test	Kruskal-Wallis Nonparametric Test
K	3.86±0.54 <sup>a</sup>	0.040		
K+	3.92±0.23 <sup>a</sup>	0.265		
D1	3.92±0.27 <sup>a</sup>	0.815	0.332	0.078
D2	4.11±0.42 <sup>a</sup>	0.120		
D3	4.50±0.33 <sup>a</sup>	0.606		

Note: Values followed by the same superscript letter (a) indicate no significant difference.

## Discussion

Hepatotoxicity can result from the entry of xenobiotic compounds, such as drugs at toxic doses or industrial chemicals like CCl<sub>4</sub>, into the body. In this study, CCl<sub>4</sub> was used to induce liver injury by intraperitoneal injection in male Wistar rats.

The AST and ALT serve as key biomarkers to assess liver function and detect hepatocellular injury. While AST is found in multiple tissues, ALT is predominantly cytosolic and liver-specific. Hepatic injury leads to the release of these enzymes into circulation, with their serum levels correlating with the extent of tissue damage [16, 17]. In addition to enzyme elevation, liver injury can cause morphological changes, such as increased relative liver weight.

The AST and ALT levels demonstrated a significant rise in the CCl<sub>4</sub> only group (K<sup>+</sup>) compared with the control (K) (Tables 2 and 3). Although values remained within the normal range for rats, the two- to three-fold increase in ALT suggests hepatocellular damage [18]. These findings are consistent with previous studies indicating that CCl<sub>4</sub> administration elevates transaminase levels [19, 20]. In contrast, relative liver weight did not differ significantly between groups (Table 4).

*Moringa oleifera* leaves contain potent antioxidants, including quercetin, kaempferol, ascorbic acid, β-carotene, isothiocyanates, polyphenols, and rutin, which can be efficiently extracted with 70% ethanol [21, 22]. Phenolic compounds identified in the extract include p-hydroxybenzoic acid, sinapic acid, caffeic acid, gallic acid, and others, while flavonoids like catechin, rutin, and quercetin are among the most bioactive constituents [23].

Administration of *Moringa oleifera* leaf ethanolic extract significantly reduced serum AST and ALT levels ( $p < 0.001$ ), with enzyme activities approaching control values, suggesting hepatoprotective effects (Table 3). This protective action is probably due to the diverse bioactive compounds present in the extract. Similar hepatoprotective effects have been reported in acetaminophen-induced toxicity models, in which

*Moringa oleifera* leaf extract improved histopathology and reduced liver enzymes [24]. Additionally, *Moringa oleifera* leaf extract reduced ALT, AST, and ALP levels in cadmium chloride-induced hepatotoxicity [25].

Relative liver weight increased in the CCl<sub>4</sub> and treatment groups, but without statistical significance (Table 4). This increase may reflect the accumulation of fat or glycogen, or hepatocellular hypertrophy or hyperplasia as an adaptive response to injury [26]. Hepatocellular hypertrophy following xenobiotic exposure is a reversible, non-adverse change, especially in the absence of necrosis or elevated ALT [27]. Therefore, liver weight changes in treated groups likely represent a metabolic adaptation rather than damage.

Although *in vivo* oxidative stress markers (e.g., MDA, SOD, or GSH) were not directly quantified in this study, the mechanism of hepatoprotection is strongly suggested to be antioxidant-mediated. The CCl<sub>4</sub> induces toxicity primarily through the generation of free radicals [28, 29]. The phytochemical analysis in this study revealed a high TPC of 110 mg GAE/g and a measurable TFC of 8.84 mg QE/g (Table 1). The TPC value obtained in this study was higher than that reported in a previous study by [30], which observed 55.97±3.10 mg GAE/g in a leaf ethanolic extract of *Moringa oleifera*. However, the TFC value was slightly lower than their reported 11.30±0.06 mg QE/g. Another study by [31] reported a TFC value of 7.79 mg QE/g for a *Moringa oleifera* leaf water extract, which is slightly lower than the TFC value obtained in this study. This variation in phytochemical content emphasises the importance of considering extract characteristics when assessing the physiological impact.

Phenolic compounds are well-documented to act as potent free radical scavengers [32]. Therefore, the significant reduction in AST and ALT levels observed is likely attributed to the antioxidant capacity of the

high phenolic concentration in the extract, which counteracts the oxidative cascade initiated by CCl<sub>4</sub>. In addition, flavonoids have been broadly reported to exert hepatoprotective effects through their anti-inflammatory and antioxidant properties, including the ability to reduce hepatic inflammation, suppress oxidative stress, and stabilize hepatocyte membranes, thereby preventing the leakage of liver enzymes, such as AST and ALT, into circulation [33, 34]. Consistent with previous findings, higher levels of phenolics and flavonoids in plant extracts are frequently associated with stronger antioxidant and anti-inflammatory activities [34, 35].

The highest dose of *Moringa oleifera* leaf ethanolic extract of 1000 mg/kg body weight (D3) produced the most significant hepatoprotective effect, as evidenced by substantial reductions in serum AST and ALT activities (Tables 2 and 3). Compared with the CCl<sub>4</sub>-only treated group (K+), AST and ALT of the D3 group decreased by 46.5% and 37%, respectively, indicating liver function toward normal control values (K); this dose is considered safe and non-genotoxic [36].

## Conclusions

Administration of *Moringa oleifera* ethanolic extract significantly reduced serum AST and ALT levels in Wistar rats induced with 10 % CCl<sub>4</sub> solution administered at 1 mL/kg body weight. The optimal results were observed in the D3 group (1000 mg/kg body weight), with AST and ALT levels decreased by 46.5% and 37%, respectively, compared to the CCl<sub>4</sub>-only-treated group (K+). However, the administration of *Moringa oleifera* ethanolic extract did not significantly affect the relative liver weight in rats exposed to CCl<sub>4</sub>.

## Data Access and Responsibility

The authors confirm that this article contains original work and accept full responsibility for its content.

## Ethical Considerations

All experimental procedures involving animals were approved by the Ethics Committee of Universitas Negeri Semarang, No. 263/KEPK/FK/KLE/2024.

## Authors' Contributions

ADL: Conducted experiments, data collection, data analysis, and manuscript drafting. RS: Supervised the research, provided guidance on methodology, data analysis, and manuscript review.

AY: Supervised the research, assisted in the general idea, data analysis, and manuscript review.

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## Conflict of Interests

The authors declare that there is no conflict of interest.

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