

Activity of *Moringa Oleifera* Lam on Liver Function and Histology in White Male Rats

Yufri Aldi^{*1}, Aisyah Aisyah¹, Rahmad Abdillah¹, Aditya Alqamal Alianta²

Yufri Aldi^{*1}, Aisyah Aisyah¹,
Rahmad Abdillah¹, Aditya
Alqamal Alianta²

¹Department of Pharmacology,, Faculty of
Medicine, Universitas Andalas, Padang, West
Sumatra, INDONESIA.

²Department of Socio-economic, Faculty of
Animal Science, Universitas Andalas, Padang,
West Sumatra, INDONESIA.

Correspondence

Yufri Aldi

Department of Pharmacology, Faculty of
Pharmacy, Universitas Andalas, Padang,
West Sumatra, INDONESIA.

E-mail: yufrialdi@phar.unand.ac.id

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ABSTRACT

Introduction: *Moringa oleifera* Lam has many pharmacological activities, such as immunostimulants.

Aim: This study aims to determine the safety profile of repeated use and routine use of *Moringa oleifera* Lam on liver function and histology. **Material and Method:** This study used 36 male white rats as test animals. *Moringa* leaves were administered orally as standardised ethanol extract at doses of 7, 21, and 140 mg/kg BW for 21 days. The liver function observed was the activity of the ALT enzyme. On days 8, 15, and 22, three animals from each group had their blood collected through the orbital sinuses of the eyes and sacrificed to excise the livers. The parameters observed were ALT enzyme activity, liver ratio value, and histological damage of liver tissue. The two-way ANOVA was used to analyse the data of ALT enzyme activity and organ ratio values, followed by DMRT. In contrast, descriptive analysis was used to describe liver histological damage. **Result:** Test animals had no toxic symptoms or sudden death during the study. The results showed that ALT enzyme activity was significantly affected by the dose and duration of administration of the ethanol extract of *Moringa* leaves ($p < 0.05$). However, on the organ ratio values, there were no significant effects of dose ($p > 0.05$), but significantly affected by duration of administration ($p < 0.05$). The liver histological descriptive analysis showed histological differences between the control and treatment groups. The 7 mg/kg BW and 21 mg/kg BW showed liver tissue with average to minimal damage. However, the dose groups at 140 mg/kg BW for 14 and 21 days showed changes in liver histology, although only minimal to mild impairment. **Conclusion:** It concluded that the administration of extract of *Moringa oleifera* Lam did not cause severe damage to the liver of white male rats.

Key words: *Moringa oleifera* L., Subacute toxicity, Alanine aminotransferase, Histology.

INTRODUCTION

Moringa oleifera Lam is one of the herbs that can be used as food and medicine. *Moringa oleifera* Lam originated from the sub-Himalayan regions of India, Pakistan, Bangladesh, and Afghanistan and then spread to various areas, including Indonesia. *Moringa oleifera* Lam is often called the miracle tree because almost all plant parts, including the leaves, have extraordinary benefits. *Moringa oleifera* Lam leaves are commonly used to treat hyperlipidemia, cancer, arthritis, prostate problems, and rheumatism.¹

Moringa oleifera Lam leaves contain secondary metabolite compounds, such as alkaloids, flavonoids, phenolics, and triterpenoids/steroids. Alkaloids act as antimicrobials, and phenols as antioxidant compounds.² *Moringa oleifera* Lam leaves contain quercetin, a flavonoid compound with antioxidant activity five times stronger than vitamin C and vitamin E. Antioxidants neutralise free radicals so that oxidative damage to biomolecules can be prevented.³ Other metabolites contained in *Moringa oleifera* Lam leaves include phenolic acids, carotenoids, polyphenols, isothiocyanates, phytates, glucosinolates, and phenolic acids.⁴

Moringa oleifera Lam leaves have many pharmacological activities, such as antimicrobial, antioxidant, antihyperlipidemic, anti-inflammatory, anticancer, antihyperglycemic, anti-hyperuricemia, and analgesic.⁵ Dillasamola *et al.*⁶ reported that *Moringa oleifera* Lam leaves have

immunomodulatory activity as immunostimulants ethanol extract of *Moringa* leaves at 10, 30, and 100 mg/kg BW doses. Immunomodulators are substances that alter the function of the immune system by dynamically controlling immune cells, such as cytokines. Immunomodulators work by restoring impaired immune function (immune restoration), improving immune system function (immunostimulation), and suppressing immune responses (immunosuppression). There are two types of immune responses when exposed to foreign substances: specific and non-specific. The specific immune response is the immune response that happens after exposure to specific foreign substances. In contrast, the non-specific immune response is the innate immune response that has existed since birth.⁷

Osman *et al.*⁸ reported that the LD50 of *Moringa oleifera* Lam leaves ethanol extract in rats was 6616.67 mg/kg BW. The traditional use of *Moringa oleifera* Lam leaves in the health sector, significantly as an immunostimulant, is increasing among the public to overcome various diseases. However, scientific research on the safety of long-term and regular use of *Moringa oleifera* Lam leaves is still limited. Thus, researchers are interested in conducting a subacute toxicity test of *Moringa* leaves for 21 days and observing liver histology and ALT enzyme activity in male white rats. The tested *Moringa oleifera* Lam leaves were extracted using standardised ethanol solvent. The results of this study are expected to be an overview of the safety of *Moringa oleifera* Lam in repeated use and routine use and can be used as a basis for further research.

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MATERIALS AND METHOD

Tools

The tools used were *rotary evaporator* (Buchi*), analytical balance (Ohaus*), animal scales (Ohaus*), animal cages, measuring cups (Pyrex*), mortar and pestle, racks and test tubes, microtube, centrifuge (Rotovix*), 5010 v5+ photometer (Riele*), dropper pipette, filter paper, spatula, sonde (Terumo*), glass beaker (Pyrex*), ointment pot, tweezers (Lai Gooi TS*), surgical scissors, funnel, vial, watch glass, object glass (Onelab*), cover glass (Onelab*), incubator (Mettler In110*), *freezer* (Sharp SJ-246GC-SD*), *laminar airflow* (Sugold Sw-Cj-1c*), *water bath* (Mettler Wnb 22*), staining jar, dehydrator (Tissue-Tek VIP 5 Jr*), *embedding centre* (Microm*), *embedding cassette* (Macrosette*), timer, rotary microtome (Leica*), computer, and microscope (olympus*).

Material

The materials were *Moringa* leaves (*Moringa oleifera* L.), distilled water (Andeska laboratory), 70% distilled ethanol (Andalas laboratory), Hematoxylin and Eosin dye (Pupick Med), physiological NaCl (PT Widatra Bhakti), Na CMC 1%, ALT analyst reagent (DiaSys), formalin buffer (Leica), 80% alcohol (Brata Med), 95% alcohol (Brata Med), absolute ethanol (Brata Med), 80% xylol (Merck), 95% xylol (Merck), 100% xylol (Merck), paraffin (Merck), and Canada balsam (DPX mountant).

Preparation of extraction

5 kg of fresh *Moringa oleifera* Lam leaves samples were sorted and cleaned from impurities, then air-dried to obtain dry samples. Afterwards, powder making was carried out with a grinder until a fine powder of *Moringa oleifera* Lam leaves was obtained.⁹ The powder was macerated using 70% ethanol solvent (1:10) for 24 hours with three times repetitions. The powder was soaked for the first 6 hours while occasionally stirred and left undisturbed for 18 hours. All the macerates were collected and evaporated using a rotary evaporator to obtain a concentrated extract.

Characterization of *Moringa oleifera* Lam extract

Organoleptic test

The extracts obtained were tested organoleptically using sensory that assessed the extract's form, colour, taste, and odour.⁹

Yield

Calculation of yield was performed by weighing the *Moringa* leaf crude drug (simplified) to obtain mass (A), then weighing the obtained extract to obtain mass (B).⁹ The yield was calculated using the formula:

$$\text{Yield} = \frac{B}{A} \times 100\%$$

Determination of total ash content

Put 2-3 g of the extract in a crucible that has been incinerated and tared, gently incandescent until the charcoal is exhausted, cool and weighed. If, in this way, the charcoal cannot be removed, add hot water, stir, and filter through filter paper along with the filtering residue in the same crucible. Put the filtrate into the krus, evaporate and incandesce until the weight remains at 800 ± 25 °C. Calculate the ash content of the material dried in the air with the following equation.⁹

$$\text{Total ash content} = \frac{W_2 - W_0}{W_1 - W_0} \times 100\%$$

Determination of acid-insoluble ash content

Boil the ash obtained to determine total ash content with 25 mL of dilute hydrochloric acid LP for 5 minutes. Collect the acid-insoluble part, filter through ash-free filter paper, wash with hot water, and

incandesce in a crucible until the weight remains at 800 ± 25 °C. Calculate the acid-insoluble ash content of the air-dried material with the following equation.⁹

$$\text{Acid insoluble ash content} = \frac{W_2 - W_0}{W_1 - W_0} \times 100\%$$

Determination of moisture content (gravimetric method)

Weigh approximately 10 g of extract in a pre-weighed container. Dry at 105 °C for 5 hours and weigh. Continue drying and weighing at one-hour intervals until the difference between two consecutive weighings is less than 0.25%.⁹

$$\text{Moisture content} = \frac{(W_1 - W_0) - (W_2 - W_0)}{W_1 - W_0} \times 100\%$$

Phytochemical screening of *Moringa oleifera* Lam extract

Flavonoid examination

Put 1 mL of extract in a test tube, then add a few drops of concentrated HCl and a little Magnesium powder. The orange-red to purple-red indicates that the extract contains flavonoids.¹⁰

Phenolic examination

Put 1 mL of extract into a test tube, then add FeCl_3 1%. The formation of a blue-black colour characterises positive results.²

Saponin examination

Put 1 mL of test extract into the tube, and shake vertically for 10 seconds. If a stable foam forms, it is positive for saponins.¹¹

Steroid and triterpenoid screening

Dissolve the extract in 3 mL of chloroform, then add 2 mL of anhydrous acetic acid and 2 mL of concentrated sulfuric acid through the tube wall. If a brownish or violet ring is formed at the restriction of two solvents, it indicates the presence of triterpenoids, while if a bluish-green colour is formed, it indicates the presence of steroids.¹¹

Alkaloid screening

Take a few mg of extract and add a few drops of Wagner's reagent. Positive results are characterised by forming a brown precipitate.²

Preparation of test preparations

Dose planning

The ethanol extract of *Moringa oleifera* Lam will be administered to male white rats at 7, 21, and 140 mg/kg BW. The dose was followed by the immunostimulant activity test of *Moringa oleifera* Lam leaf ethanol extract, which uses 10, 30, and 100 mg/ kg BW in male white mice. (Dillasamola *et al.*, 2018).. There is a difference in the highest dose used because the researchers want to observe the activity when the dose was increased to 140 mg/kg BW and whether it caused any toxic effects on the liver. The test formulations will be administered orally daily for seven days, 14 days, and 21 days.

Preparation of 1% Na CMC suspension

Weigh 500 mg of Na CMC and sprinkle it on 10 mL of hot water in a heated mortar. Leave it for 15 minutes and then grind until homogeneous. Next, add aquadest until the volume is 50 mL.

Preparation of suspension of *Moringa* leaf ethanol extract (*Moringa oleifera* L.)

Suspend 1 g of extract into 50 mL of 1% Na CMC. Then, dilute the test suspension according to the concentrations of each designed dose. The

volume of the test formulation injection should be adjusted based on the body weight of the test animals.

Administration of test preparations

The animals will be divided into four groups as follows:

Group I: Only given 1% Na CMC

Group II: Given the extract at a dose of 7 mg/kg BW

Group III: Given the extract at a dose of 21 mg/kg BW

Group IV: Given the extract at a dose of 140 mg/kg BW

Each group consists of 9 rats, further divided into three subgroups: subgroup A (treated for seven days), subgroup B (treated for 14 days), and subgroup C (treated for 21 days). Each subgroup consists of 3 rats.

On days 8, 15, and 22, the animals are anesthetised and partially conscious, and blood samples are taken through the orbital sinus of the eye to determine the ALT activity. Then the animals were sacrificed by inhalation anaesthesia using ether. They were then dissected, and their liver was taken to prepare histological slides using the paraffin method. These slides were observed under a microscope.

Analysis of ALT activity

Blood serum collection

Thirty-six test animals were sacrificed and collected the blood on days 8, 15, and 22 after the experiment. The blood was collected through the orbital sinus of the eye. Then, the blood was collected in a gel activator tube and centrifuged for 10 minutes at 3000 rpm to obtain serum. The clear solution of serum was then pipetted using a micropipette and transferred into microtubes. A 5010 v5+ photometer immediately read the serum to check the ALT levels of the test animals.

Monoreagent preparation

Mix four parts of reagent 1 with 1 part of reagent 2 (20 mL of reagent 1 + 5 mL of reagent 2) = mono reagent.¹²

ALT level examination

100 µL serum and 1000 µL mono reagent were put into a test tube to be homogenised for 1 minute. Measure the blank's absorbance value before checking the serum's ALT levels. Then, the serum and mono reagent mixture was immediately measured for absorbance using a 5010 v5+ photometer right after the 1st minute at a wavelength of 340 nm. Record the results shown on the photometer.¹²

Liver histology analysis

Histology preparation

To begin with, sacrifice the rats, remove the liver, and rinse with physiological NaCl solution. Transfer the organ into a formalin buffer solution. Then, cut the liver organs into several sections representing the entire liver. Organ tissues were dehydrated with 80%, 95%, and absolute alcohol solutions for 1 hour each. In addition, transfer the object into a solution of absolute alcohol: xylol (1:1), xylol 1 and xylol 2, each for 1 hour for clarification.

Furthermore, place the object into the infiltration solution in an incubator at 56-60°C. Xylol: paraffin, paraffin I, paraffin II, and paraffin III (each for half an hour). Next, embedding, the object is inserted into a metal mould or paper box filled with liquid paraffin that was heated in an incubator and then allowed to cool and freeze. Section by placing a paraffin block in a holder, then thinly slice with a microtome knife (5-6 µm).

After that, the glass slide is rubbed with Mayer's albumin. Place several incisions on it, drip with water, and stretch on a hot plate until the incisions expand. Staining with hematoxylin Erlich and eosin was performed as follows: Xylol I, xylol II (5 minutes each), absolute alcohol I, absolute II, 80%, 95%, and 100% (3 minutes each), Ehrlich's hematoxylin for 10 minutes, then washed with running water. Next, the preparations were dipped in eosin for 5 minutes, absolute alcohol III and absolute IV (each for 2 minutes), xylol III and xylol IV for 1 minute, and washed with running water. Mounting (closure) so that the tissue does not dry, eating is dripped with adhesive in the form of balsam Canada, covered with cover glass, and then dried. And then, label the preparation to the right of the object, examine it under a microscope and take a microscopic photograph.¹³

Examination of liver histology preparations

Examination of liver histology preparations with 400x magnification. The assessment of histological damage is semi-quantitative and is based on scoring (values) according to the observed changes in the liver organ under a microscope, then scoring based on the matrix in Table 1.¹⁴

Data analysis

The data were analysed using a two-way ANOVA statistical test between time (duration of administration) and dosage. Furthermore, the data were analysed with Duncan's Multiple Range Test). This process was conducted with IBM SPSS.

RESULTS AND DISCUSSION

Extraction process

The extraction process was performed using the maceration method. The maceration method was chosen because it allows for a large sample size with a simple procedure, requiring no special treatment, and is suitable for heat-sensitive compounds.¹⁵ This maceration process uses a brown bottle placed in a place protected from light. This process aimed to avoid the decomposition of the active substance structure. *Moringa oleifera* Lam leaf simplisia powder was macerated using 70% ethanol as the solvent. Ethanol was chosen because it is a relatively safe universal solvent that can dissolve almost all polar, semi-polar, and non-polar compounds. 70% ethanol was selected because the study utilised dried samples with relatively low water content. The 30% water content of this solvent serves to help break the cell wall so that ethanol penetration into the cell is faster and more optimal.¹⁶

The maceration process was carried out for 24 hours with three repetitions with a ratio of sample and solvent of 1:10. The powder

Table 1: Liver histology damage score.

Score	Description
0	Normal - no hepatocyte necrosis
1	Minimal-mild Focal, limited to the centrilobular region Less than ¼ of affected lobules are necrotic
2	Mild-moderate Focal and multifocal Central to midzonal lobular region ½ affected lobules are necrotic
3	Moderate to severe Multifocal (centrilobular-portal region) ¾ > X > ½ affected lobules are necrotic
4	Severe Multifocal X > ¾ affected lobules are necrotic
5	Severe (whole lobules) Hepatocyte loss from the central vein to the portal area extends to adjacent lobules.

was soaked for the first 6 hours while occasionally stirred and left undisturbed for 18 hours. All the macerates were collected and evaporated using a rotary evaporator to obtain a concentrated extract weighing 116.1 grams.

Organoleptic test of purified extract

The organoleptic examination of the ethanol extract of *Moringa oleifera* Lam leaves revealed a concentrated extract with a dark greenish-brown colour, distinct aroma, and a bitter taste. These results comply with the organoleptic standards for *Moringa oleifera* leaf extract in the Indonesian Herbal Pharmacopoeia.⁹

Yield of purified extract

Furthermore, calculating the percentage yield of *Moringa oleifera* Lam leaf ethanol extract yielded 17.86%. The yield was calculated to determine the percentage of extract obtained from the initial sample weight and determine the solvent's ability to attract active substances in the sample. The percentage value of the yield obtained has met the standard yield percentage in the Indonesian Herbal Pharmacopoeia, which is not less than 9.2%.⁹

Preliminary examination of chemical content (phytochemical screening)

The ethanol extract of *Moringa oleifera* leaves is a method used to determine the metabolic content in a plant. Qualitative phytochemical screening of the ethanol extract of *Moringa* leaves indicated the presence of phenolic compounds, flavonoids, alkaloids, and steroids/triterpenoids. Demographic conditions, nutrition and the environment where a plant grows are factors that cause differences in the content of chemical compounds.

Examination of moisture content, total ash content and acid-insoluble ash content

The examination of moisture content in the ethanol extract of *Moringa oleifera* leaves resulted in 9.15%. This moisture content value meets the requirements specified in the Indonesian Herbal Pharmacopoeia, which should not exceed 10%.⁹ The examination of total ash content yielded 5.01%. The ash content value meets the requirements in the Indonesian Herbal Pharmacopoeia, which is no more than 9%.⁹ Meanwhile, the examination of acid-insoluble ash content yielded 0.469%. This acid-insoluble ash content value complies with the requirements in the Indonesian Herbal Pharmacopoeia, which should not exceed 0.9%.⁹

Administration of test preparations

Each group consisted of 9 rats divided into three subgroups: Group A, which was treated for seven days; Group B, which was treated for 14 days; and Group C, which was treated for 21 days. Each subgroup consisted of 3 rats. Rats in Group 1 were designated as the control group and were given a 1% Na CMC suspension. The 1% Na CMC suspension was used as a suspending agent because it is inert, non-toxic, non-irritating, and produces a stable solution. Groups II, III, and IV were administered test preparations at 7 mg/kg BW, 21 mg/kg BW, and 140 mg/kg BW, respectively.

On days 8, 15, and 22, the animals were half-conscious, and their blood samples were taken through the orbital sinus of the eye to determine ALT levels. Subsequently, the animals were euthanised by inhalation anaesthesia using ether, then dissected, and the liver was taken to make histological preparations using the paraffin method and observed under a microscope.

Analysis of ALT activity

ALT enzyme is a specific indicator of liver damage. ALT enzyme is produced in the liver and released into the blood, where its level is

directly proportional to the condition of the liver. Higher levels in the blood indicate more severe liver damage.¹⁷ The examination of ALT levels was carried out using a 5010 v5+ photometer with an enzymatic method. The principle of determining ALT kinetics was based on IFCC (International Federation of Clinical Chemistry) recommendations, where a chemical reaction occurs between L-alanine contained in reagent 1 with 2-oxoglutarate contained in reagent 2. The ALT enzyme catalysed the chemical reaction in the experimental animals' serum. This chemical reaction produces L-glutamate and pyruvate. Then the pyruvate formed will be reduced by NADH contained in reagent 2 with the help of the LDH catalyst in reagent 1 to produce D-lactate (Greiner Diagnostic GmbH).¹⁸ According to BPOM, the standard value of ALT levels in rats is 10-50 IU/L.¹⁹

Based on the statistical analysis of ALT levels using two-way ANOVA, it was found that the average ALT level was significantly influenced by the dose and duration of *Moringa oleifera* Lam leaf extract administration ($p < 0.05$). However, the interaction between the dose and duration of *Moringa oleifera* Lam leaf extract administration did not significantly affect the average ALT level ($p > 0.05$).

Furthermore, the DMRT result showed a non-significant difference in mean ALT levels in the 7 mg/kg BW dose group against the control group. However, in the dose group of 21 and 140 mg/kg BW, there is a significant difference in average ALT levels compared to the control group, with an increase in ALT levels beyond the normal range.

Duncan's test on the duration of *Moringa oleifera* leaf extract administration showed a significant difference in the average ALT level between the group treated with the extract for seven days and the groups treated with the extract for 14 days and 21 days. The groups treated with the extract for 14 days and 21 days did not show a significant difference in the average ALT level, but there was an increase in the ALT level above the normal range.

Based on the obtained range of results, the average \pm SD ALT levels for the control group and the groups administered *Moringa oleifera* Lam leaf extract at doses of 7, 21, and 140 mg/kg BW were 49.78 ± 2.11 ; 51.44 ± 1.59 ; 61.34 ± 3.50 ; 63.11 ± 5.40 U/L. Meanwhile, the average \pm SD ALT levels for the duration of administration of 7 days, 14 days, and 21 days were 53.83 ± 5.41 ; 56.92 ± 6.19 ; 58.50 ± 8.25 U/L (Table 2). Based on Table 2, the ALT levels slightly increased above the normal range. It happened due to lipophilic compounds in *Moringa oleifera* Lam leaf extract, such as alkaloids and flavonoids, which can damage the liver by disrupting the liver cell membrane and increasing membrane permeability, increasing ALT levels.²⁰

Liver ratio value calculation

Calculating the liver organ ratio values is used as a supporting parameter. It was because changes in organ weight are sensitive indicators of organ changes caused by chemicals. A compound can affect both an animal's organs and overall body weight. Therefore, a ratio value called relative organ weight was calculated by dividing the weight of each animal by its body weight.²¹

Rats sacrificed by inhalation anaesthesia using ether were dissected to take the liver and then weighed to calculate the liver organ ratio. The liver organ ratio observations showed no significant effect of dose and dose interaction with duration of administration ($p > 0.05$). However, the duration of *Moringa oleifera* Lam leaf ethanol extract had a significant effect on the ratio of liver organs ($p < 0.05$).

Furthermore, DMRT results on the duration of ethanol extract administration from *Moringa oleifera* Lam leaves showed a significant difference in the average value of organ ratios in the test group given moringa leaf ethanol extract for seven days, 14 days and 21 days. The 21-day administration of the ethanol extract from *Moringa oleifera* Lam

Table 2: Effect of dose and duration of moringa leaf extract administration on average ALT enzyme activity (U/L) of male white rats.

Dosage	Mean ALT levels (U/L) on day \pm SD			Mean \pm SD
	7 Days	14 Days	22 Days	
Control	48,67 \pm 3,06	50,67 \pm 1,15	50,00 \pm 2,00	49,78 \pm 2,11
7 mg/kg BW	50,00 \pm 1,00	52,00 \pm 2,00	52,33 \pm 0,58	51,44 \pm 1,59
21 mg/kg BW	59,67 \pm 3,06	60,67 \pm 2,08	63,67 \pm 4,73	61,34 \pm 3,50
140 mg/kg BW	57,00 \pm 2,94	64,33 \pm 1,53	68,00 \pm 2,65	63,11 \pm 5,40
Mean \pm SD	53,83 \pm 5,41	56,92 \pm 6,19	58,50 \pm 8,25	

Table 3: The effect of dosage and duration of *Moringa oleifera* Lam leaf extract administration on the liver organ ratio values in male white rats.

Dosage	Mean \pm SD			Mean \pm SD
	7 Days	14 Days	21 Days	
Control	0,046 \pm 0,008	0,040 \pm 0,005	0,029 \pm 0,004	0,038 \pm 0,009
7 mg/kg BW	0,046 \pm 0,006	0,038 \pm 0,005	0,035 \pm 0,008	0,040 \pm 0,008
21 mg/kg BW	0,047 \pm 0,007	0,039 \pm 0,008	0,031 \pm 0,009	0,039 \pm 0,010
140 mg/kg BW	0,046 \pm 0,004	0,037 \pm 0,004	0,030 \pm 0,006	0,038 \pm 0,008
Mean \pm SD	0,046 \pm 0,006	0,039 \pm 0,005	0,031 \pm 0,006	

leaves significantly decreased the average liver-organ ratio, followed by the 14-day and 7-day administrations.

Administration of *Moringa oleifera* Lam leaf extract dosage in the treatment group at 140 mg/kg BW decreased the liver-organ ratio compared to the control group. The average \pm SD values of the liver organ ratio in the control group, dosage of 7 mg/kg BW, 21 mg/kg BW, and 140 mg/kg BW were 0.038 \pm 0.009, 0.040 \pm 0.008, 0.039 \pm 0.010, and 0.038 \pm 0.008, respectively. Meanwhile, the average \pm SD values of the liver organ ratio for 7 days, 14 days, and 21 days were 0.046 \pm 0.006, 0.039 \pm 0.005, and 0.031 \pm 0.006, respectively (Table 3). An anomaly in the control group parameter was the difference in liver organ ratio values. It was due to the experimental animals having varying body weights. Differences in organ weight are often accompanied by differences in body weight between treatment groups, making interpreting organ weight more difficult. Therefore, the liver-organ ratio is only used as a supporting parameter that helps indicate cell damage.

Liver histology analysis

Liver tissue preparations were observed using a light microscope at a magnification 400x. The 400x magnification was used to observe changes in cell morphology. Liver histology damage scores were assessed globally on tissue preparations by evaluating the degree of hepatocyte necrosis. If no hepatocyte necrosis was observed in normal cells, a score of 0 was assigned. If the cells had minimal-mild damage, focal, limited to the centrilobular region, with $<1/4$ of affected lobules being necrotic, the score was 1. If the cells had mild-moderate damage, focal-multifocal, central-mid zonal lobular region, with $1/2$ affected lobules necrotic, the score was 2. If the cells have moderate to severe damage, multifocal, centrilobular-portal region with more than $1/2$ of the lobule and less than $3/4$ of the lobule affected by necrosis, a score of 3 was given. If the cells have severe damage, multifocal, with more than $3/4$ of the lobule affected by necrosis, a score of 4 was assigned. If cells showed severe damage (across the entire lobule), hepatocyte loss in the central vein area extending to the portal area and spreading to surrounding lobules, a score of 5 was given.¹⁴ The examination results were described descriptively by comparing the histological damage in the liver between the treatment group and the control group.

Histology of the liver of the test animals for 7 days showed that the control group and the dose of 7 mg/kg BW showed liver tissue with parenchyma containing hepatocytes in lobules arranged regularly within normal limits so that it was given a score of 0 (Figure 3. (a) and

(b)). However, at doses of 21 mg/kg BW and 140 mg/kg BW, minimal focal damage was observed in the centrilobular area, with less than $1/4$ of the lobule showing necrosis, resulting in a score of 1 (Figure 3. (c) and (d)).

Histology of the liver of the test animals for 14 days showed that the control group and the dose of 7 mg/kg BW showed liver tissue with parenchyma containing hepatocytes in lobules arranged regularly within normal limits so that it was given a score of 0 (Figure 4. (a) and (b)). While at a dose of 21 mg/kg, BW found minimal damage, focal, in the centrilobular area with less than $1/4$ of the lobule affected by necrosis, resulting in a score of 1 (Figure 4. (c)). At the highest dose of 140 mg/kg BW, there was mild damage, focal, in the mid-zonal lobular area, with the lobule showing necrosis, characterised by cloudy cytoplasm

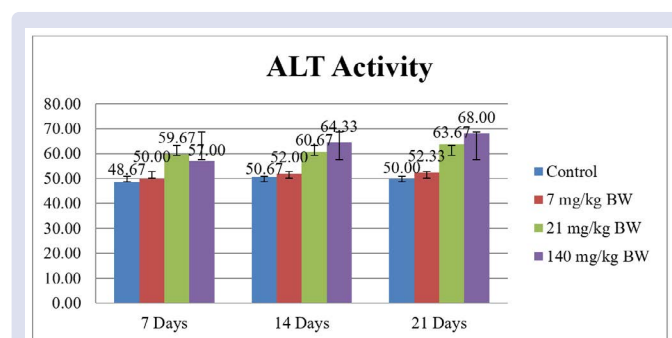
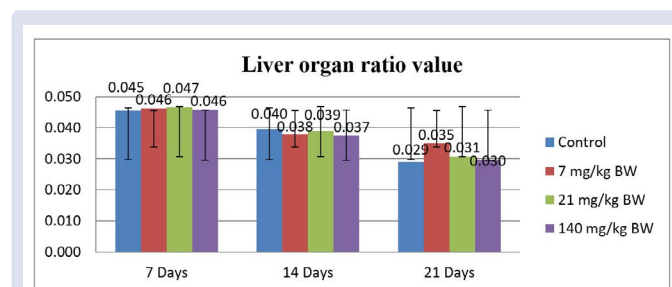
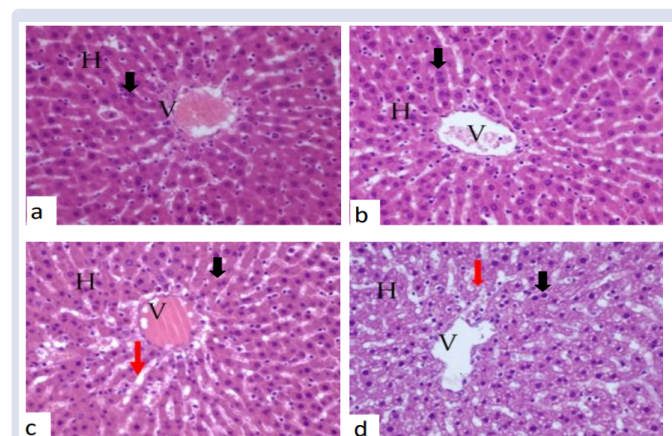
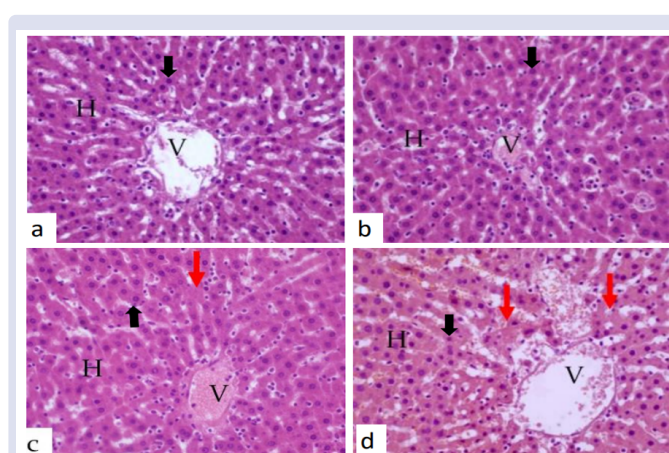
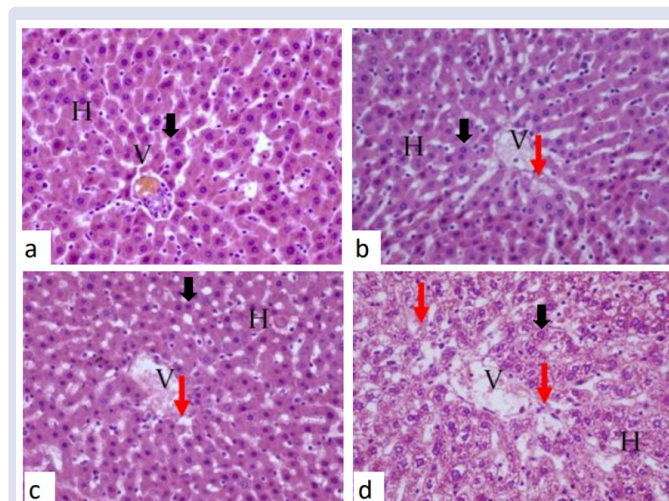
**Figure 1: Graph of the effect of dose and duration of *Moringa oleifera* Lam leaf extract administration on average ALT activity in male white rats.****Figure 2: Graph of the effect of dosage and duration of *Moringa oleifera* Lam leaf extract administration on the liver organ ratio values in male white rats.****Figure 3: Histological overview of the liver after 7 days of administration. (a) Control group; (b) 7 mg/kg BW dosage; (c) 21 mg/kg BW; (d) 140 mg/kg BW; (V) Central vein; (H) Hepatocytes; black arrow = normal cells; red arrow = necrosis.**

Table 4: Histological liver score data from 5 fields of view (FOV).

Dosage (mg/kg BW)	Duration administration	of Histological damage scores					Mean	Description
		FOV 1	FOV 2	FOV 3	FOV 4	FOV 5		
Control	7 days	0	0	0	1	0	0,2	Normal
7	7 days	1	0	0	0	0	0,2	Normal-minimal damage
21	7 days	1	0	1	0	0	0,4	Normal-minimal damage
140	7 days	1	0	0	1	0	0,2	Normal-minimal damage
Control	14 days	0	0	1	0	0	0,2	Normal
7	14 days	1	0	0	0	0	0,2	Normal-minimal damage
21	14 days	1	1	1	0	0	0,6	Normal-minimal damage
140	14 days	1	1	1	0	2	1	Minimal damage
Control	21 days	0	0	0	1	0	0,2	Normal
7	21 days	1	0	0	0	1	0,4	Normal-minimal damage
21	21 days	1	1	1	0	1	0,8	Minimal damage
140	21 days	2	0	1	2	1	1,2	Minimal-mild damage

**Figure 4:** Histological overview of the liver after 14 days of administration. (a) Control group; (b) 7 mg/kg BW dosage; (c) 21 mg/kg BW; (d) 140 mg/kg BW; (V) Central vein; (H) Hepatocytes; black arrow = normal cells; red arrow = necrosis.**Figure 5:** Histological overview of the liver after 21 days of administration. (a) Control group; (b) 7 mg/kg BW dosage; (c) 21 mg/kg BW; (d) 140 mg/kg BW; (V) Central vein; (H) Hepatocytes; black arrow = normal cells; red arrow = necrosis.

and nuclear lysis, with oedema present around the sinusoids, resulting in a score of 2 (Figure 4. (d)).

Liver histology of test animals for 21 days showed that in the control group, the liver tissue appeared to have parenchyma containing hepatocytes in lobules arranged regularly within normal limits, giving it a score of 0 (Figure 5. (a)). While in the dose group of 7 mg/kg BW and dose of 21 mg/kg BW found minimal damage, focal, in the centrilobular area with less than $\frac{1}{4}$ lobules showing necrosis, resulting in a score of 1 (Figure 5. (b) and (c)). At the highest dose of 140 mg/kg BW, there was mild focal damage in the mid-zonal lobular area, with less than $\frac{1}{2}$ of the lobule subjected to necrosis, so it was given a score of 2 (Figure 5. (d)).

The observed liver tissue preparations were taken from the area near the central vein. The central vein allows more evident observation of damage due to its larger size than other blood vessels. Additionally, the central vein is where blood enters the liver tissue.¹⁶ Cells close to the central vein contain a high concentration of metabolites due to the area around the central vein being more often damaged than the peripheral area. In this study, the shape of the central vein was observed to widen with increasing dosage and duration of administration. However, at the 21-day duration, the central vein appeared to shrink. It is due to stasis in the sinusoids, which begins to experience oedema. The table below presents the liver histology scores from 5 fields of view (FOV).

Histological assessment of the toxicity effect of *Moringa oleifera* Lam leaf ethanol extract on liver histology showed histological differences between the control group and the treated group. In the negative control group, the parenchyma consisted of hepatocyte cells arranged regularly in trabeculae and separated by sinusoids, with no signs of degeneration or inflammation. In the low and medium-dose treatment groups, 7 mg/kg BW and 21 mg/kg BW showed a picture of liver tissue within normal limits to minimal damage (Table 4). It indicates that *Moringa oleifera* Lam leaf extract is relatively safe at doses 1 and 2. However, administering a high dose for 14 and 21 days showed histological changes in the liver, although only minimal to mild damage was observed (Table 4). It suggests potential adverse effects of *Moringa oleifera* Lam leaf extract at high doses and long term on liver tissue. Throughout the 21-day study period, no toxic symptoms were observed in the test animals, and no sudden deaths were recorded.

CONCLUSIONS

Based on the description above, it concluded that administering ethanol extract of *Moringa oleifera* Lam to male white rats increased

average ALT levels at a dose of 140 mg/kg BW for 21 days but still within normal limits. Furthermore, administering ethanol extract of *Moringa oleifera* Lam at 7 and 21 mg/kg BW doses for 7, 14, and 21 days showed regular liver tissue observations within the normal range.

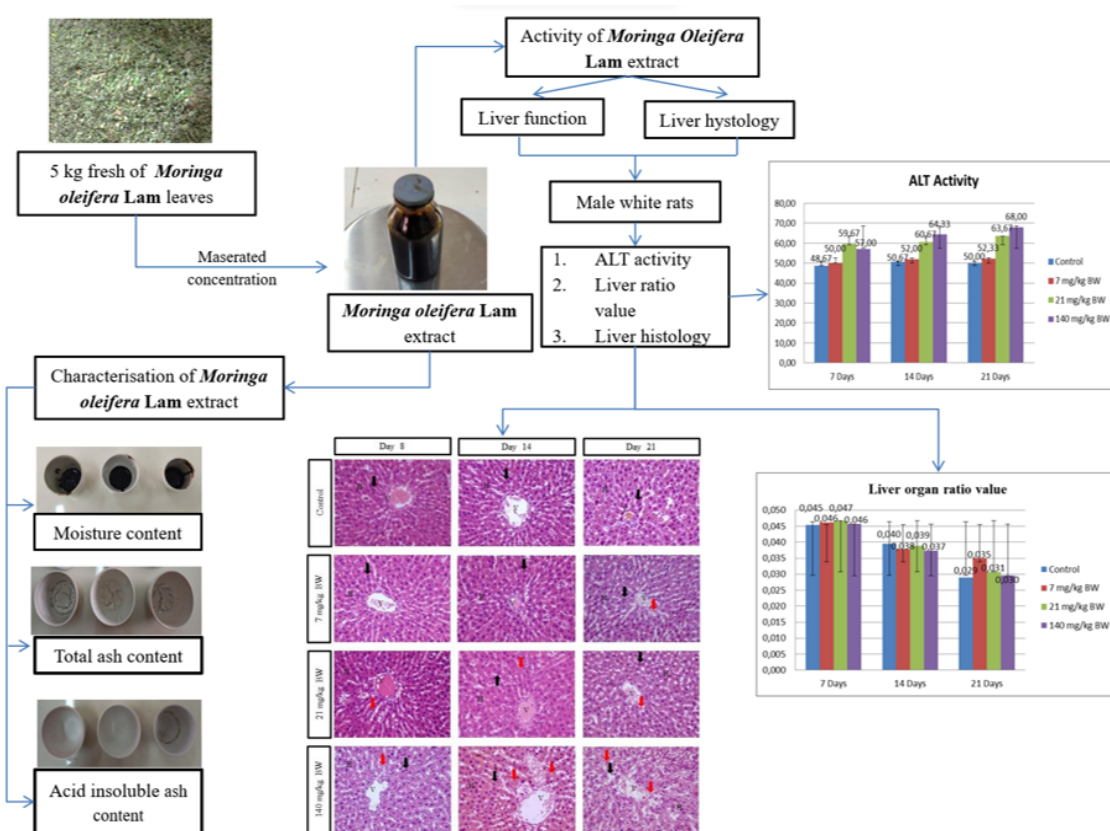
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GRAPHICAL ABSTRACT



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