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Original Research Article

Acute Oral Toxicity Assessment of *Morinda citrifolia* L. (Noni) Leaves in Experimental Mice

Jon Farizal [1], Zamharira Muslim [2*], Rahmad Abdillah [3]

[1] *Department of Technology Laboratorium Medic, Poltekkes Kemenkes Bengkulu, Bengkulu, Indonesia.*
[2] *Department of Pharmacy, Poltekkes Kemenkes Bengkulu, Bengkulu, Indonesia.*
[3] *Department of Pharmacology, Faculty of Pharmacy, Universitas Andalas, Padang, Indonesia.*

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ABSTRACT

Noni leaves (*Morinda citrifolia L.*) have been widely utilized in Indonesia as a traditional remedy. This *in vivo* study aimed to assess the acute oral toxicity of the ethanol extract of *M. citrifolia* in male BALB/c mice. The subjects were divided into five dosage groups: 5 mg/kg body weight (Group A), 50 mg/kg body weight (Group B), 300 mg/kg body weight (Group C), 2000 mg/kg body weight (Group D), and 5000 mg/kg body weight (Group E), alongside a control group (Group F). The extract was administered orally, and toxicological effects were monitored over 14 days, including behavioral changes, toxic symptoms, mortality, food and water intake, and changes in body and organ weights. No mortality was observed, and the estimated LD_{50} of *M. citrifolia* extract was above 5000 mg/kg body weight. However, excessive urination was noted in Groups B, C, D, and E, and hypoactivity (sedative effect) was observed in Groups D and E. Body and organ weights tended to increase post-intervention. Histopathological analysis of the liver showed ballooning and inflammation. These findings suggest that the ethanol extract of *M. citrifolia L.*, when administered orally, is relatively non-toxic.

Keywords: Acute Oral Toxicity, *Morinda citrifolia* L, *In Vivo*, Noni Leaves, Liver toxicity

Introduction

The use of conventional medicine is often associated with adverse effects, leading researchers to explore alternative natural therapeutic options. These circumstances underscore the challenges faced by modern medicine, including the need to substantially revise medical training curricula to emphasize and leverage the benefits of ethnomedicine, prioritize practical knowledge application, and advance therapeutic approaches.[1]

Morinda citrifolia L., commonly known as noni, is a traditional medicinal plant with a rich history in various cultures and is widely found in Southeast Asia and Polynesia.[2,3] Research has demonstrated that noni possesses diverse pharmacological and biological effects, including antioxidant, antimicrobial, anticancer, anti-inflammatory, and hypotensive properties.[4–7] In previous studies, the ethanol extract of *M. citrifolia* L. leaves has been utilized to assess parasitemia levels.[8] Furthermore, noni has shown potential benefits in treating degenerative, autoimmune, psychological, gastric, and bone-related diseases, as well as promoting wound healing.[9] Studies indicate that nearly all parts of the noni plant, including the roots, stems, leaves, and fruits, can serve as herbal remedies due to their rich content of proxeronins and phytonutrients.[10] The purpose of toxicity testing of substances from natural materials is to evaluate their safety and potential side effects, ensuring rational utilization and development while minimizing the risks associated with pharmacological applications in clinical settings.[11] Consequently, this study seeks to evaluate the acute toxicity of *M. citrifolia* L. leaf extract in accordance with the OECD Guidelines for Testing of Chemicals in toxicology

***Corresponding author. E mail: muslim@poltekkesbengkulu.ac.id
Tel: +6285263634060

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Materıals and Methods

Plant collection and Identification

M. citrifolia L. leaves were collected from Bengkulu Province, Indonesia is located between 2°16' N to 3°31' N and between 101°01' to 103°41' E. Samples were identified by The Taxonomies at the Biology Herbarium, Faculty of Mathematics and Natural Sciences, Bengkulu University (Certificate No. 78/UN30.28.LAB.BIOLOGI/PM/ 2017). Sample selection ensured that the plants were over one year old, and that only mature but not aged leaves were collected. Approximately 5 kg of leaf samples were harvested, dried to a moisture content of 5.31%, and mashed.

Extraction was performed on 800 g of the dried material using the maceration method with 2400 ml of 70% ethanol. Following maceration, a rotary evaporator (Buchi® R210) was used at 60º C to concentrate the extract. The extraction process adhered to the standards of the Indonesian Herbal Pharmacopoeia, 2nd Edition.[12]

Animal Handling

Healthy male BALB/c mice aged 6–8 weeks and weighing 20–30 g were used for the experiment. Animal care took place at the Animal House, Faculty of Pharmacy, Andalas University. The mice were housed individually and acclimatized for two weeks under controlled conditions (50% ± 10% humidity, 22°C ± 1°C temperature, and a 12-hour light-dark cycle). Animals were given standard pellet food and water *ad libitum*, following the Guidelines for the Care and Use of Laboratory Animals. All procedures minimized animal suffering and optimized the number of animals used. This study received ethical approval from the Health Research Ethics Committee of the Health Polytechnic of the Ministry of Health, Bengkulu (Ethical Statement No. KEPK/143/04/2023).

Acute Oral Toxicity

Experimental animals were divided into six groups, each consisting of five mice, and were treated over 14 days. Doses of *M. citrifolia* L. extract varied as follows: 5 mg/kg (Group A), 50 mg/kg (Group B), 300 mg/kg (Group C), 2000 mg/kg (Group D), 5000 mg/kg (Group E), and

a control group (Group F) that received a carboxymethylcellulose (CMC) Na suspension. The extract was administered orally following a 3–4 hour fasting period. Acute oral toxicity testing followed OECD Guideline 420 for the testing of chemicals. After the intervention period, animals were euthanized, and liver tissues were preserved in 10% buffered formalin or neutral buffered formalin (NBF). NBF known for its ability to maintain the structural integrity of tissues, which is essential for accurate histological examination. [13]

Histopathology Processing

An automated tissue processor was employed to prepare formalin-fixed liver tissue samples from mice treated with *Morinda citrifolia* L. extract, along with control samples. The process began with two fixation steps: initially immersing the tissue in 10% buffered formalin for two hours, followed by rinsing it in distilled water for one hour to remove the fixative. Next, the tissue was dehydrated through a graded series of alcohol concentrations at 70%, 80%, 96%, and 100%, each step lasting one hour and repeated three times. Following dehydration, the tissue samples underwent a clearing process in two stages using Xylol I and Xylol II, each for 1.5 hours. The cleared tissue was then infiltrated with liquid paraffin for two hours at 56–58 °C. Embedding followed, in which the tissue was placed into a cassette and allowed to cool for 0.5–1 hour before the paraffin fully solidified. Once impregnated, the tissue paraffin blocks were sectioned via microtomy, producing thin sections as specified by the protocol. These sections were then mounted on glass slides and stained with Hematoxylin and Eosin for histopathological examination.[14] The liver tissue was assessed for signs of necrosis, apoptosis, inflammation, and hepatocyte ballooning.

Histopathological assessment

A liver biopsy is an essential procedure for evaluating structural changes and the degree of liver damage, offering vital histological information that informs diagnosis and treatment.[15] Indicators of hepatic injury, including cellular necrosis and hepatocyte ballooning, were observed. The Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) scoring system was applied to assess the severity of liver damage (Table 1).[16] Macroscopic assessments, including liver size and the morphological integrity of hepatic cells, were conducted by anatomical pathologists in the Anatomical Pathology Laboratory.

Table 1: Histopathological Assessment of Liver Tissue According to the NASH CRN Scoring System[25]

Parameters	Grade	Observed Findings
Lobular Inflammation	0	None
	1	<2 foci/200x HPF
	2	2-4 foci/200x HPF
	3	>4 foci/200x HPF
Ballooning	0	None
	1	Few ballon cells (≤ 50%)
	2	Many cells/prominent (> 50 %)

Statistical Analysis

The data obtained included behavioral responses and toxic effects observed following administration of the test preparation in comparison with the control. Results are presented as mean ± standard deviation (SD), and differences between groups were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's post hoc test using SPSS Version 25. Statistical significance was considered at $p < 0.05$.

Results and Discussion

Oral administration of *Morinda citrifolia L.* ethanol extract to test animals did not result in any mortality over the 14-day observation period (Table 2). Therefore, the results of this study determined that the LD_{50} of Morinda citrifolia L extract exceeded 5000 mg/kg body weight (bw). Previous research on the acute oral toxicity of a combination of *Curcuma xanthorrhiza Roxb*, *Phyllanthus niruri* L., and *Morinda citrifolia* L. similarly estimated the LD_{50} to be above 5000 mg/kg b.w.[17] Another study reported no toxic effects at doses exceeding 2000 mg/kg b.w. from freeze-dried infusions in Sprague-Dawley rats.[18] However, in this study, behavioral and physiological changes were observed within the first 6 hours (Table 1). Symptoms included tremors and excessive grooming in all intervention groups, while hypoactivity was noted in groups receiving more than 300 mg/kg b.w., 2000 mg/kg b.w., and 5000 mg/kg b.w. Excessive urination was recorded in groups B, C, D, and E. Some studies noted a reduction in locomotor activity among rats treated with high doses of M. citrifolia extracts. This decrease in movement can be a sign of sedation or lethargy, which may result from neurotoxic effects of the extract.[19] Excessive grooming, or barbering, is a stress indicator in rats, representing both a coping and stress response.[20]

On day 14, a tendency for increased body weight was observed in the animals (Table 3). The highest increase occurred in group C (dose 300 mg/kg b.w.), where weight rose from 26.80 ± 1.51 g to 29.60 ± 1.17 g. A significant increase was also seen in group E with the high dose (5000 mg/kg b.w.), with weight rising from 28 ± 1.00 g to 29.56 ± 1.3 g. The negative control group also showed an increase in body weight (from 24.40 ± 1.14 g to 25.92 ± 1.13 g), although not as pronounced as in the treatment groups. Generally, these data indicate that the *M. citrifolia* extract did not lead to significant weight loss, suggesting the absence of acute toxicity that could cause severe weight reduction. Statistical analysis showed no significant differences in body and organ weight changes between test groups ($p > 0.05$) and the control group, although an increase in average body weight was observed in each group compared to day 0. Assessing body and organ weights in experimental animals is essential for evaluating toxicity, as changes in these weights are indicators of normal organ function and can also signal variations in nutritional status.[21]

Overall, the mice's food intake was good, which corresponded with the observed increase in body weight. This weight gain may be attributed to the phytochemical components in the plant.[22] Food intake increased in the treatment groups compared to the control group (F), accompanied by a simultaneous rise in water intake (Figure 1). The extract did not affect the mice's body weight or frequency of food and water consumption.

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Table 2: Toxicity Symptoms in Animals Treated with *M. citrifolia* L. Leaf Extract During the First 6 Hours

Group *		A (5 mg/kg b.w)	B (50 mg/kg b.w)	C (300 mg/kg b.w)	D (2000 mg/kg b.w)	E (5000 mg/kg b.w)	F (Negative Control)
Toxic symptoms	↑Respiratory rate	0	0	0	0	0	0
	Piloerection	0	0	0	0	0	0
	Hypersalivation	0	0	0	0	0	0
	Diarea	0	0	0	0	0	0
	Hyperlakrimation	0	0	0	0	0	0
	Hyperurination	0	2	2	3	2	0
Behavior symptoms	Hypoactivity	0	0	3	4	5	0
	Tremor	3	3	4	4	5	0
	Excessive grooming	5	5	5	5	5	0
	Seizure	0	0	0	0	0	0
	Coma	0	0	0	0	0	0

*Each group has five mice.

Table 3: Average Body and Organ Weights of Mice Treated with *M. citrifolia* L Leaf Extract Over 14 Days

Parameters	Group					
	A (5 mg/kg b.w)	B (50 mg/kg b.w)	C (300 mg/kg b.w)	D (2000 mg/kg b.w)	E (5000 mg/kg b.w)	F (Na CMC 1%)
Body Weight						
Initial Weight (g)	22.6 ± 0.54	25.40 ± 1.51	26.80 ± 1.09	25.40 ± 1.51	28 ± 1.00	24.40 ± 1.14
Final Weight (g)	24.8 ± 1.6	26.75 ± 2.4	29.60 ± 1.17	26.83 ± 3.89	29.56 ±1.3	25.92 ± 1.13
Organ Weight						
Heart (g)	0.13±0.00	0.13±0.01	0.14±0.002	0.12±0.02	0.13±0.02	0.13±0.01
Kidney (g)	0.35±0.02	0.36±0.02	0.52±0.43	0.39±0.11	0.46±0.02	0.34±0.03
Liver (g)	1.73±0.13	1.54±0.46	2.02±0.07	1.59±0.49	1.74±0.22	1.52±0.30
Lymph (g)	0.16±0.05	0.07±0.03	0.1±0.00	0.1±0.03	0.17±0.04	0.12±0.01

Data are expressed as means ± SD of five mice in each group.

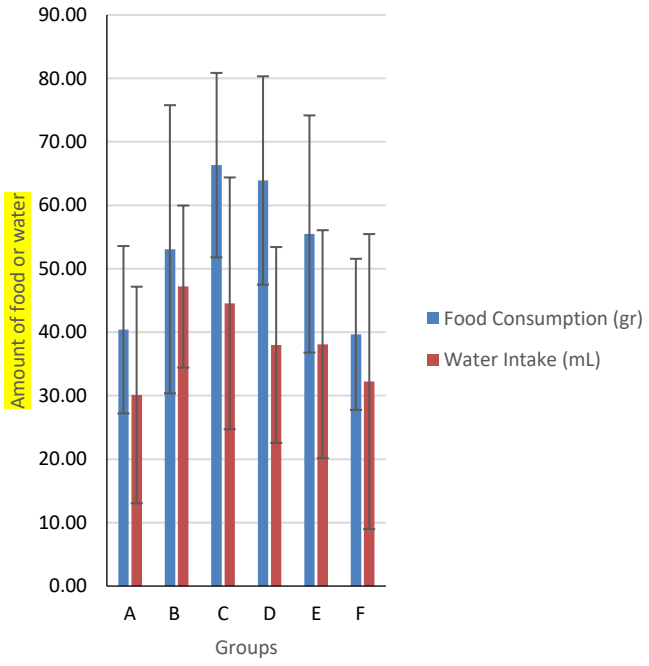

Figure 1: Food Consumption and Water Intake of Animals Treated with *M. citrifolia* L Leaf Extract During the Experimental Period

Examination of liver histopathology in all groups revealed hepatocyte damage (Figure 2). Enlarged degeneration, indicating inflammation and necrosis, was observed in the highest dose group, and lobular inflammation and cellular enlargement appeared across all intervention groups (Table 4). Enlargement, or ballooning degeneration, is a notable histopathological feature observed in liver tissue, typically indicative of hepatocellular injury (Figure 2). This morphological change is commonly associated with various liver diseases, especially those linked to metabolic disorders or inflammatory processes. Ballooning degeneration refers to an abnormal swelling of hepatocytes, where the affected cells appear enlarged and deviate from their normal shape, often exhibiting significant cytoplasmic vacuolization. This phenomenon generally arises as a response to cellular stress or hepatocyte damage. Marked glycogen accumulation and steatosis in ballooned hepatocytes suggest metabolic reprogramming rather than simple cellular death. In 43.8% of cirrhotic liver samples, ballooning degeneration has been associated with preneoplastic deformations.[23]

In the histopathological preparations of liver tissue viewed microscopically, ballooning degeneration is identified by characteristic hepatocyte swelling, vacuolization, and cytoplasmic spacing.[24] Hepatocyte swelling is distinguished by significantly enlarged liver cells, while vacuolization indicates the presence of large vacuoles within the hepatocyte cytoplasm, often reflecting fat or fluid accumulation. The decreased cytoplasmic density appears sparser, with a loss of typical structure. Although ballooning degeneration is widely regarded as a reliable marker of liver damage, it may also indicate the presence of viable cells undergoing metabolic alterations, thereby complicating the assessment of hepatic pathology.[23] A product is considered safe at a given dose if, within a 14-day observation period, there are no significant behavioral or toxic effects observed in animals compared to controls, and no animal deaths occur during the experiment.

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Table 4: Histopathological Assessment of Liver Following *M. citrifolia* L Extract Intervention (Each Group = 3 Samples)

Groups	Sample No.	Lobular Inflamation			Balloning%	
		Mild	Moderate	Serve	Less	More
A	1	1				<50
(5 mg/kg BW)	2	1				<50
	3	1				<50
B	1		2			<50
(50 mg/kg BW)	2		2			<50
	3		3			<50
C	1		3			<50
(300 mg/kg BW)	2		3			<50
	3		3			<50
D	1			4		50
(2000 mg/kg BW)	2			4		50
	3			4		50
E	1			4 necrosis		>50
(5000 mg/kg BW)	2			5 necrosis		>50
	3			5 necrosis		50
F	1	0				<50
(Na CMC 1%)	2	1				<50
	3	0				<50

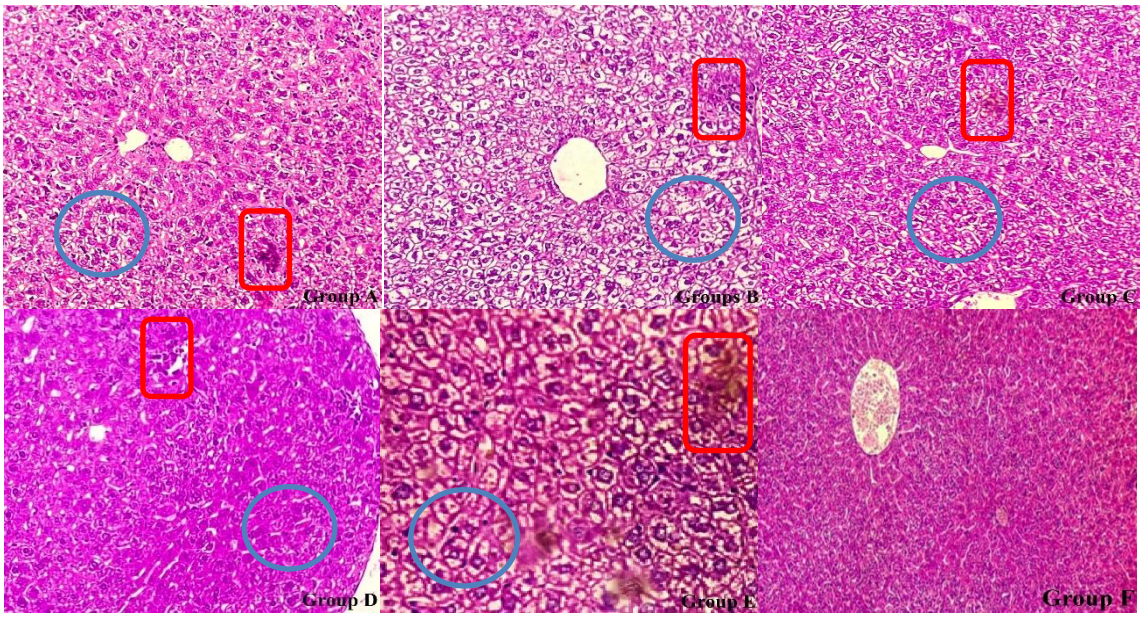

Figure 2: The Impact of Acute Oral Toxicity of *M. citrifolia* L. Extract on Liver Histomorphology in Male BALB/c Mice was Investigated. Histological Sections Were Visualized using Hematoxylin-eosin Staining and Examined Under an Optical Microscope at 100x Magnification. Red Squares Denote Areas of Inflammation, while Blue Circles Highlight Regions of Ballooning Degeneration.

Conclusıon

Oral acute toxicity tests ethanol extract of *Morinda citrifolia* L. in mice showed no mortality, indicating a relatively low toxicity profile with LD_{50} values categorized as non-toxic. However, certain toxic symptoms were observed, including hyperurination, behavioral changes, tremors, and excessive grooming. In addition, high doses of the extract caused significant liver damage, as evidenced by hepatocyte bulging and inflammation. Further research should focus on identifying the specific compounds responsible for these side effects and determining safe dosage limits.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors confirm that the work presented in this article is original, and they assume full responsibility for any claims related to its content.

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