

Acute Toxicity Studies of *Mirabilis jalapa* L. Flower Extract in Mice

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Traditional medicine, derived from medicinal plants, has been used for generations as a form of treatment. The safety, quality, and efficacy of plants used for medicinal purposes are important considerations. *Mirabilis jalapa* L., also known as the four o'clock flower, is a traditional medicine with known benefits such as diuretic, anti-inflammatory, and antibacterial properties. However, its safety level remains uncertain. To address this, an acute toxicity test was conducted on the ethanol extract of *Mirabilis jalapa* L. flowers to determine its safety. The extraction process utilized a reflux method with 96% alcohol as a solvent, resulting in a standardized extract. Subsequent toxicity tests were performed on 12 female white mice, using doses of 300 and 2,000 mg/kg body weight. The results revealed an LD₅₀ value exceeding 2,000 mg/kg body weight for the ethanol extract of *Mirabilis jalapa* L. flowers, indicating its classification as "Practically Not Toxic."

INTRODUCTION

Traditional medicine has been practiced since ancient times and encompasses both advantages and disadvantages in addressing various illnesses. Medicinal plants have been recognized as key components of traditional medicine since ancestral times. These plants are commonly used by communities to treat a wide range of ailments, sourced from the surrounding areas. However, it is essential for plants used for medicinal purposes to meet specific criteria in terms of safety, quality, and effectiveness (World Health Organization, 2004). Therefore, research data on drug safety testing is crucial to ascertain the safety of these medicines (Saad et al., 2006).

Drug safety is a necessary condition and effort, where drugs that are considered safe can actually cause concern, even though they cannot be interpreted as completely safe for consumption. This relates to the side effects of drugs that occur in the community, and there have been many cases of undesired side effects from traditional medicines. Reactions such as nausea, drowsiness, itching, and lower back pain may occur. Therefore, it is important to understand the safety of plants used as medicine in the community (Posadzki et al., n.d.).

One such traditional medicine widely used by the community, despite the lack of known safety information, is the four o'clock flower (*Mirabilis jalapa* L.), also known as the ashar flower. This plant is recognized for its various beneficial properties, including diuretic, anti-inflammatory, antispasmodic, antiviral activity and antibacterial effects (Hanani et al., 2017). Traditionally, this flower has been used to treat inflammation, urinary tract disorders, boils, diarrhea, muscle pain, stomach cramps, and as an aphrodisiac (Raza et al., 2024) (Islam et al., 2021) (Peiris et al., 2022). Additionally, this flower is also used as a purgative (laxative) and emetic (vomit inducer), as well as to treat gastrointestinal diseases such as dysentery and other digestive disorders (Malakar & Biswas, 2021) (Bharskar & Mankar, 2022). Other recorded uses include treating skin infections, wounds, and as a tonic or body stimulant (Saha et al., 2020). In some cultures, the *Mirabilis jalapa* flower is also used to treat urinary tract infections, as a diuretic, and to treat parasitic infections (Bharskar & Mankar, 2022) (Saha et al., 2020). These traditional benefits are supported by the presence of active

compounds such as flavonoids, alkaloids, and phytosterols, which have been scientifically proven to have anti-inflammatory, antimicrobial, antioxidant, and antidiabetic activities (Raza et al., 2024)(Peiris et al., 2022)(Islam et al., 2021)(Bharskar & Mankar, 2022). Modern research shows that *Mirabilis jalapa* flower extract exhibits anti-inflammatory activity in vitro comparable to standard drugs, particularly in models of protein denaturation and red blood cell membrane stabilization (Kodical & Fernandes, 2020). Additionally, enzyme inhibition tests for *alpha-glucosidase* and *alpha-amylase* indicate significant antidiabetic potential, as well as antioxidant and antibacterial activity against skin pathogens such as *Staphylococcus aureus* and *Escherichia coli* (Fayehun et al., 2024). Ensuring drug safety is crucial to prevent any potential harm. However, there is currently a lack of supporting data regarding the safety of *Mirabilis jalapa* L. flower. Conducting an acute toxicity test of the ethanol extract can help determine the safety level of using *Mirabilis jalapa* L. flower as a medicinal treatment.

Based on the aforementioned background, it is imperative to perform an acute toxicity test on female white mice (*Mus musculus*) using the ethanol extract of *Mirabilis jalapa* L. flower in order to ascertain its safety as a medicinal treatment. An acute toxicity test is designed to evaluate the toxic effects of a substance following a single or repeated dose administered within a 24-hour period. The objective of this test is to determine the lethal dose (LD₅₀) that causes mortality in 50% of the experimental animals. In addition to determining the LD₅₀, observations are also made regarding the clinical symptoms exhibited by the experimental animals.(OECD, 2001).

METHOD

Simplisia and Extract Preparation

Fresh *Mirabilis jalapa* flowers were collected from Way Dadi District, Bandar Lampung. The plants were identified and authenticated at the Biology department of Lampung University. Five kg of fresh *Mirabilis jalapa* flowers were carefully selected, removing any impurities and damaged parts. The samples were then dried through aeration, resulting in 90 g of dry simplisia. The dried simplisia was subjected to extraction using the reflux method with 96% ethanol as a solvent. The liquid extract was further evaporated to obtain a thick extract. The extract was characterized through organoleptic tests and determination of loss on drying.

Animal Test

The study utilized female white mice aged 2-3 months, with a body weight ranging from 20-35 g. A total of 12 mice were obtained from the pharmacology laboratory of Cendikia Farma Husada Academy. The use of animals in this study was approved by the Health Research Ethics Commission of Politeknik Kesehatan Kementerian Kesehatan Medan, with the approval number: 010903/KEPK/POLTEKKES KEMENKES MEDAN 2022.

Acute Toxicity Test Procedure

The oral acute toxicity test was conducted following the fixed dose method as outlined in the Food and Drug Administration regulation No. 10 of 2022 (Peraturan Badan Pengawas Obat Dan Makanan Nomor 10 Tahun 2022 Tentang Pedoman Uji Toksisitas Praklinik Secara In Vivo, 2022), which provides guidelines for in vivo preclinical toxicity testing. This method is suitable for substances with a moderate level of toxicity, and the selected dose should not result in death, severe pain, or irritative/corrosive effects. A group of test animals of the same sex was administered graded doses using the fixed doses method, including: 5; 50; 300; and 2,000 mg/kg body weight (with the option to increase the dose up to 5,000 mg/kg body weight). The initial dose was determined based on preliminary tests, aiming to induce mild toxicity symptoms without causing severe toxic effects or death. The procedure continued until the toxic dose level was reached, up to a maximum of 1 death, or until no toxic effects were observed at the highest dose or lower doses.

RESULT AND DISCUSSION

Extract Characterization

The evaporation process yielded a thick extract weighing approximately 12.01 g. The extract exhibits a dark brown color, possesses a distinctive aroma, and has an oily texture. Furthermore, the extract demonstrated a drying shrinkage of 20%.

Preliminary Test

In order to establish the appropriate dose range for the acute toxicity study, preliminary tests were conducted. These tests involved two dose groups, each consisting of one test animal. The test animals were acclimatized for a period of 6 days prior to the administration of the treatment, in order to adapt to the environmental conditions. The treatment involved the oral administration of the ethanol extract of *Mirabilis jalapa* L. flowers, with a single dose administered within a 24-hour period. The doses used were 300 mg/kg BW and 2,000 mg/kg BW, as shown in table 1. Observations of the test animals at a dose of 300 mg/kg BW indicated that they exhibited normal activity. However, the test animals receiving a dose of 2,000 mg/kg BW showed a decrease in activity and weakness. Based on the results of the preliminary tests, it was determined that the highest dose range inducing clinical toxic symptoms in mice was 2,000 mg/kg BW. Consequently, the main test was conducted using a dose of 2,000 mg/kg BW.

Table 1. Preliminary Acute Toxicity Test

Groups	Dosage	Sample	Clinical Symptoms	Number of deaths
I	300 mg/kg BW	1	Normal activity	0
II	2,000 mg/kg BW	1	Decrease activity, weakness and silence	0

Main Test

After the completion of the preliminary test, the main test was conducted using a dose of 2,000 mg/kg BW. The main test consisted of two groups: the negative control group and the treatment group, with each group consisting of 5 mice (*Mus musculus*). Prior to the treatment, the test animals were acclimatized for 6 days. Subsequently, the treatment group received the ethanol extract of *Mirabilis jalapa* L. flowers orally, once within a 24-hour period, at a dose of 2,000 mg/kg BW. On the other hand, the control group received an oral administration of CMC Na suspension, also once within 24 hours.

Table 2. The Results of Main Test for Acute Toxicity

Group	Mice	Clinical Symptoms	Number of Death
Negative control	1	Normal activity	0
	2	Normal activity	0
	3	Normal activity	0
	4	Normal activity	0
	5	Normal activity	0
Dose 2,000 mg/kg BW	1	Normal activity	0
	2	Decrease activity, weakness. After 12 hours the mice returned to normal activity	0
	3	Normal activity	0
	4	Decrease activity, tremors, weakness. After 12 hours the mice returned to normal activity	0
	5	Normal activity	0

Observations were conducted every 4 hours during the initial 24-hour period to monitor the clinical condition of the test animals. The observations continued for a total of 14 days to determine if any of the test animals had died. In the control group, no toxic symptoms or deaths were observed within the first 24 hours. However, in the treatment group, changes in behavior were observed in mice 2 and 4, including decreased activity, weakness, and tremors. However, after 12 hours of treatment, the mice returned to normal activity, and no deaths were recorded among the test animals. It is important to note that respiratory system toxicity, changes in activity, cardiac activity, and disorders in the central and autonomic nervous systems, such as paralysis and coma, are considered critical symptoms in toxicity tests (Asare et al., 2012).

Observation of Body Weight for 14 Days

During the 14-day observation period, body weight was monitored as an additional parameter to assess the effects of the treatment. Daily measurements of the test animal’s body weight were taken throughout the observation period. In the negative control group, the average body weight of the test animals before treatment was 26.8 g, and after 14 days, it decreased to 25.8 g. On the other hand, in the treatment group, the average body weight before treatment was 29.8 g, and by day 7, there was an increase in body weight, reaching an average of 30.35 g by the end of the observation period. The detailed results can be found in Table 3. The observation of the test animal’s body weight revealed that there was an increase in body weight in the treatment group, while the control group exhibited a tendency towards a decrease in body weight. Body weight is influenced by the balance between calorie intake and energy expenditure. If the calorie intake exceeds the energy expenditure, body weight tends to increase, and vice versa. The increase in body weight observed in the treatment group indicates that the administration of ethanol extract of *Mirabilis jalapa* L flower did not induce toxic symptoms during the extended 14-day observation period (Khairani et al., 2022).

Table 3. Weight Observation Results for 14 Days

Group	Mice	Days			Average body weight	
		1	7	14		
Body weight (g)						
Negative control	1	26	26	26	26.8 g	
	2	27	27	27		
	3	26	26	26		
	4	28	28	28		
	5	27	27	27		
Dosage mg/kg	2.000	1	28	29	29	30.35 g
		2	31	32	32	
		3	29	30	30	
		4	31	32	32	
		5	30	31	31	

Observation of Organ Weight

In addition to body weight, organ weight was also measured as a parameter in this study. The organs examined included the liver, heart, lymph nodes, and kidneys. Organ weight is considered a highly sensitive parameter for detecting toxic effects [9]. These organs can be directly affected by toxic substances, either through direct damage to the target organ or through damage to the central nerves and blood vessels.

To observe organ weight, the test animals were dissected, and the liver, heart, lymph nodes, and kidneys were carefully removed. Each organ was then weighed using analytical scales. The obtained data were analyzed using the Minitab 2020 One-Way ANOVA test to determine any significant differences. Organ weight observation provides valuable insights into the potential toxic

effects of the tested substance on specific organs. The results of this analysis will contribute to our understanding of the effects of the ethanol extract of *Mirabilis jalapa* L. flowers on the organs of the test animals.

Table. 4. Results of Organ Weight Observations of Mice

Groups	Mice	Organ (g)				
		Heart	Liver	Lymph	Kidney	
Negative control	1	0.11	1.64	0.13	0.29	
	2	0.12	1.24	0.18	0.27	
	3	0.14	1.48	0.12	0.24	
	4	0.14	1.76	0.11	0.24	
	5	0.13	1.37	0.26	0.27	
	Average	0.128 ± 0.013	1.17 ± 0.22	0.16 ± 0.06	0.262 ± 0.01	
Dosage mg/kg	2,000	1	0.15	1.92	0.40	0.41
	2	0.17	1.76	0.16	0.41	
	3	0.14	1.68	0.18	0.36	
	4	0.15	1.85	0.22	0.41	
	5	0.15	1.77	0.30	0.38	
	Average	0.152 ± 0.01	1.796 ± 0.01	0.252 ± 0.09	0.394 ± 0.02	
Significance Value		0.014	0.019	0.115	0.00	

On the 14th day of the observation period, the organ weight of the test animals was measured. The mice were euthanized using chloroform, and their hearts, livers, kidneys, and lymph nodes were carefully extracted. The extracted organs were rinsed with normal saline and dried before weighing. In the control group, the average weight of the heart organ was found to be 0.238 g, while in the treatment group it was 0.152 g. Statistical analysis using one-way ANOVA indicated a significant difference between the control and treatment groups, with a significance value of 0.014 ($p < 0.005$). The heart is susceptible to abnormalities caused by exposure to cardiotoxic compounds, which can result in damage to the mitochondria within the cardiac muscle (Farmasi Poltekkes Kemenkes Makassar & Farmasi Universitas Pancasakti Makassar, 2018).

Regarding the liver organ, the average weight in the control group was 1.17 g, compared to 1.79 g in the treatment group. The statistical analysis using one-way ANOVA yielded a significance value of 0.019 ($p < 0.005$), indicating no significant difference between the treatment and control groups. The liver plays a crucial role in metabolizing drug compounds. Since the ethanol extract of the four o'clock flower was administered orally in this study, the test compound underwent first-pass metabolism in the liver after absorption in the gastrointestinal tract. Therefore, if any hepatotoxicity were to occur, it would directly impact the liver.

The average weight of the lymph organs in the control group was found to be 0.16 g, while in the treatment group it was 0.25 g. The statistical analysis using one-way ANOVA yielded a significance value of 0.115 ($p < 0.005$), indicating no significant difference between the control and treatment groups. The lymph organs have important functions in regulating hematopoiesis and other hematological processes. Additionally, they play a role in the immune system by synthesizing complement factors and immunoglobulins (de Matos Filho & Petroianu, 2015).

Further observations on organ weight revealed that the average weight of the kidney organ in the control group was 0.26 g, compared to 0.39 g in the treatment group. The statistical analysis using one-way ANOVA showed a significance value of 0.00 ($p < 0.005$), indicating a significant difference between the control and treatment groups. The kidneys can be significantly affected by exposure to

toxic compounds due to their role in metabolite dissolution and excretion from the body. The toxic effects can manifest as biochemical changes and cell death, leading to impaired kidney function and potential kidney failure.

The research conducted in the pharmacology laboratory of Cendikia Farma Husada Academy did not result in any deaths among the test animals during the 14-day observation period. This indicates that the LD₅₀ of the *Mirabilis jalapa* L. flower extract is higher than 2,000 mg/kg BW. According to expert consensus, if the maximum dose does not cause death in experimental animals, the LD₅₀ is expressed as a pseudo LD₅₀. Therefore, the obtained result of 2,000 mg/kg BW is not the actual LD₅₀ (Abrori et al., 2019).

In this study, a dose limitation test of 5,000 mg/kg BW was not conducted due to the observed changes in clinical symptoms in mice at a dose of 2,000 mg/kg BW. To prioritize animal welfare, testing at a dose of 5,000 mg/kg BW is only considered when there is a strong likelihood that the test results will directly contribute to the protection of animal or human health. The dose of 2,000 mg/kg BW represents the maximum dose conversion from humans to mice based on the body surface area ratio. Experts state that if the maximum dose does not cause death in experimental animals, the compound can be classified as "Practically Non-Toxic." Therefore, the maximum dose in humans is converted to 2,000 mg/kg BW in mice, where it does not cause death in any of the test animals (Abrori et al., 2019).

CONCLUSION

Based on the conducted acute toxicity test using the ethanol extract of *Mirabilis jalapa* L. flowers, there were no signs of toxicity or mortality observed in mice at a dose of 2,000 mg/kg BW. However, the administration of the ethanol extract did affect the weight of the heart, liver, and kidney organs in mice (Sig<0.05), while it had no significant effect on the weight of the lymph organs (Sig>0.05). The estimated LD₅₀ range for the ethanol extract of four o'clock flowers (*Mirabilis jalapa* L.) is greater than 2,000 mg/kg BW, indicating that the dose of 2,000 mg/kg BW is safe to use and falls under the category of "Practically Non-Toxic."

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