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Safety Profile of *Abelmoschus Manihot* (L.) Medik Ethanol Leaf Extract: Acute Toxicity Study in Animal Models

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Abstract: Abelmoschus Manihot (L.) Medik, commonly known as Abelmoschus manihot, is a traditional medicinal plant from Palu, Central Sulawesi, Indonesia, widely utilized for its potential health benefits. This research aims to evaluate the acute toxicity of ethanol extract from Abelmoschus manihot leaves, focusing on determining the lethal dose (LD_{50}) in rats. The study involved extracts being administered at a range of doses of 500 mg/kg, 1000 mg/ kg, and 2000 mg/Kg of body weight (BB) in a rat population. Rats were observed for 24 hours to look for signs of toxicity and death. The LD_{50} value, which determines the dose required to cause death in 50% of the test population, is calculated based on the results. This research provides insights into the safety profile of ethanol extract from Abelmoschus manihot leaves and contributes to understanding its potential risks when used in traditional medicine. No significant deaths were observed at lower doses, but higher doses resulted in observable toxic effects, indicating the need for careful dosing in therapeutic applications. The results show that although Abelmoschus Manihot did not exhibit acute toxic effects on rats at doses of 500 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight. No significant deaths or severe toxic symptoms were observed during the 24-hour observation period up to the 14-day observation, and there were no differences in the body weight of the rats during the 14-day observation.

Keywords : Abelmoschus manihot; Animal models; Safety profile; Toxicity

Introduction

Abelmoschus manihot (L.) Medik, known locally as Abelmoschus manihot leaves, is a plant commonly found in Palu, Central Sulawesi, Indonesia. This plant has long been used in traditional medicine to address various health issues (Wulan & Indradi, 2018), including inflammation, digestive disorders, and metabolic issues (Indrawati & Setijorini, 2024; Luan et al., 2020). *Abelmoschus manihot* (L.) Medik has demonstrated protective effects against cisplatin-induced chronic kidney disease (CKD) by restoring metabolic dysregulation, particularly in tryptophan metabolism (Liao et al., 2022), the Huangkui capsule, derived from this plant, has been shown to ameliorate diabetic nephropathy by modulating gut microbiota and normalizing metabolite levels in diabetic mice (Shi et al., 2023), the total flavones of *Abelmoschus manihot* (L.) Medik exhibit significant anti-inflammatory effects, particularly in reducing lung inflammation caused by Influenza A virus (IAV) through modulation of key inflammatory pathways (Gao et al., 2022), In diabetic kidney disease models, specific flavonoids and antioxidant from the plant (Anggi, 2021; (Anggi & Adikusuma, 2019; Patala & Anggi, 2022), it have been identified as crucial in protecting podocytes from injury and inflammation (Wu & Wang, 2023), The root of *Abelmoschus manihot* (L.) Medik contains various bioactive compounds, including flavonoids, glycosides, and saponins, which contribute to its medicinal properties (Chumbhale & Khyade, 2022). Chemical composition results in *Abelmoschus manihot* (L.) Medik have a antidiabetic, antioxidant, anticancer, and

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neuroprotective activities (Abdel-Razek et al., 2023), (Anggi & Masyita, 2022; Li et al., 2016).

The pharmacological potential of this plant has attracted the attention of researchers, especially due to its content of active compounds, such as flavonoids and polysaccharides (Hou et al., 2020; Winata et al., 2024), that has anti-inflammatory, antioxidant properties, and protection against various degenerative diseases (Selvaraj et al., 2020). Abelmoschus manihot L. Medik has garnered attention for its diverse medicinal properties, particularly in managing diabetes, renal health, and inflammatory conditions (Shi et al., 2023). Research highlights its potential as a therapeutic agent through various mechanisms as a reproductive performance (Chang et al., 2022). The research paper explores phytochemical compounds and antioxidant capacities of Abelmoschus manihot leaf extracts using different solvents, providing valuable insights into its potential medicinal properties (Winata et al., 2024). The phytochemical compounds and antioxidant capacities of Abelmoschus manihot leaf extracts using different solvents , providing valuable insights into its potential medicinal properties (Li et al., 2016). Abelmoschus manihot flower extracts have shown significant inhibitory effects on enzymes related to carbohydrate metabolism, such as α-glucosidase and lipase, while enhancing α-amylase activity (Prasetiyo, 2023). These extracts also demonstrated antioxidative properties, reducing reactive oxygen species (ROS) in hyperglycemic conditions, indicating potential for diabetes management (Wu & Wang, 2023). The total extracts of A. manihot have been found to alleviate radiation-induced cardiomyocyte ferroptosis, suggesting a protective role against radiation-induced heart disease through modulation of oxidative stress pathways (Hou et al., 2023). The seeds of A. manihot exhibit anti-rheumatoid arthritis effects by modulating the JAK2/STAT3 signaling pathway, reducing inflammation markers, and improving joint health in collagen-induced arthritis models (Zhang et al., 2022) . Ethanol extracts of A. manihot have shown promise in mitigating contrast-induced nephropathy by reducing oxidative stress and apoptosis in renal cells, highlighting its potential as a therapeutic agent for kidney protection (Tandi et al., 2017) . Gedi leaf extracts contain various phytochemicals with pharmacological activities and show multiple myeloma activity and low toxicity levels (Hou et al., 2020).

Although the health benefits of Abelmoschus manihot have been widely reported in the ethnopharmacological literature, information regarding its safety profile, particularly in terms of acute toxicity, remains limited. One important step in the development of medicinal plants into modern therapies is ensuring their safety through evaluation toxicology, including the determination of safe doses that can be used in treatment. The acute toxicity test aims to determine the lethal dose threshold and the potential side effects that may occur in the short term, thereby providing an initial overview of its safety for use. In this concept, research is conducted to evaluate the acute toxicity of ethanol extract from Abelmoschus manihot leaves on laboratory rats. This study aims to determine the lethal dose 50 (LD50), which is the dose of the extract that can cause death in 50% of the test population. By conducting tests at various doses (500 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight), this study aims to provide insights into the safety profile of this extract and offer guidance for its safe use in therapeutic applications, both in traditional medicine and in the development of plant-based pharmaceutical products. The results of this research will provide significant contributions to understanding the potential risks of using Abelmoschus manihot, particularly at high doses, as well as determining a safe dosage for short-term use. Furthermore, this study will serve as a foundation for further research to evaluate the long-term toxicity and potential therapeutic benefits of this plant in the treatment of various diseases.

Method

Time and Place

This research was conducted from June to July 2024 at the STIFA Pelita Mas Palu laboratory. The research was carried out in three stages, which include: the preparation stage, the implementation stage, and the evaluation stage.

Tools

The tools used in the research include a stirring rod (Herma), measuring cylinder (pyrex®), beaker (pyrex®), mouse cage, oven (Memmert), dropper, rotary evaporator (heidolph®), maserator, analytical balance (kern®), blender (Miyako), water bath (Memmert), gas stove (Rinnai), test tubes (pyrex®), test tube rack, evaporating dish, measuring pipette, filter paper, oral probe, and syringe.

Materials

The materials used in this research are aquades (WaterOne), 96% ethanol (Merck), male Wistar strain white rats (Rattus norwegicus) obtained from Tadulako University, Abelmoschus manihot leaves (Abelmoschus manihot L. Medik) collected from Palu City, Central Sulawesi, and Na. CMC.

Preparation of Simplisia Abelmoschus manihot Leaf

The plant Abelmoschus manihot identification was carried out at the UPT. Biodiversity Resource Center of Tadulako University with identification number:

No.0083/UN28.23/AL.04/2024. The sample used was 3 kg, followed by wet sorting to separate dirt that was still attached to the plants. It was then washed thoroughly with running water, drained, cut into small pieces, and dried in an oven at 70°C for 20 days. After that, dry sorting was conducted to separate foreign objects such as unwanted plant parts and any remaining dirt on the simplicia. The dried sample is weighed, then the drying loss is calculated, and afterward, it is ground using a blender (Arsyad et al., 2023).

Preparation of ethanol extract from Abelmoschus Manihot

The preparation of ethanol extract from Abelmoschus manihot leaves is carried out using the maceration method first before extraction. A total of 574 grams of Abelmoschus manihot leaf powder is weighed and then placed into a maceration container, followed by extraction using 2.5 liters of 96% ethanol for 3 times 24 hours, protected from light while being stirred occasionally. The extract is then filtered using filter paper. The resulting filtrate is concentrated using a Rotary Vacuum Evaporator at a temperature of 60^{o}C, followed by evaporation using a water bath until a thick extract is obtained. The yield of the extract is then calculated.

Preparation of Na CMC Suspension 0.5%

Sodium carboxymethyl cellulose (Na CMC) weighing 0.5 grams is placed in a mortar containing 10 ml of heated distilled water, allowed to sit for 15 minutes until a clear mass is obtained, and then stirred until homogeneous to form a uniform suspension. The Na CMC suspension is transferred to a 100 ml volumetric flask and the volume is adjusted with distilled water to 100 ml.

Preparation of Na CMC 0.5% Suspension of Abelmoschus manihot leaves ethanol extract

The Abelmoschus manihot leaf extract is weighed to prepare a suspension with each 1 gram (dose of 500 mg/kg body weight), 2 grams (dose of 1,000 mg/kg body weight), and 4 grams (dose of 2,000 mg/kg body weight). Subsequently, each extract is added to 0.5% Na CMC and the volume is adjusted to 25 ml, then shaken until homogeneous.

Acute Toxicity Testing

Preparation of Test Animals

This study has received ethical approval from the animal ethics committee of the Faculty of Medicine, Tadulako University, with ethics committee number 471/UN 28.1.30/KL/2024. The test animals used were 20 male Wistar white rats (Rattus Norvegicus) weighing between 180-210 grams. The rats are divided into 4 groups, where each group consists of 5 rats. Group 1 is the control group, while groups II to IV are the treatment groups.

Treatment of Test Animals

Firstly, the rats were fasted for 14-20 hours, but water was still provided. After fasting, the animals are weighed and given the test preparation. The test preparation is provided with a single-dose concept using a probe. The group division is as follows: Group I, as the control group, is given a 0.5% Na.CMC suspension; Group 2 is given a suspension of ethanol extract of Abelmoschus manihot leaves at a dose of 500mg/Kg body weight; Group 3 is given a suspension of ethanol extract of Abelmoschus manihot leaves at a dose of 1000mg/Kg body weight; and Group 4 is given a suspension of ethanol extract of Abelmoschus manihot leaves at a dose of 2000mg/Kg body weight.

Observation of Test Animals

Acute toxicity testing observations were conducted over a period of 24 hours to 14 days. Observations include: motor activity (increased, normal, decreased, stationary), strabismus, piloerection, ptosis, pineal reflex, corneal reflex, lacrimation, catalepsy, body posture (normal, abnormal), hanging, retablisment, flexion, hafner, mortality, grooming, defecation, urination, respiration (rapid, normal, and labored), salivation, vocalization, tremors, seizures, and writhing. Observations were recorded from the onset to the disappearance of toxic symptoms, as well as the weight measurements of the rats before and after the treatment (BPOM, 2022; BPOM RI, 2022).

Data Analysis

The data collected in this study is primary data obtained from observations of test animals, both from the control group and the treatment group. The data obtained consists of qualitative and quantitative data. Qualitative data refers to information about objects or subjects that can be observed but cannot be measured with numbers. The qualitative data to be obtained includes clinical appearances, morphology, and toxic effects. Quantitative data, on the other hand, is data that can be measured and counted directly in the form of numbers or diagrams. The quantitative data to be obtained includes the number of test animals that died. The LD50 data is taken from the number of mice that died and those that survived in each group. Next, the LD50 value was calculated using the Thomson and Weil method. Then, statistical analysis was performed using SPSS 26.

Results and Discussion

Results of Animal Testing

The results of the acute toxicity test observations were conducted over a period of 24 hours up to 14 days. Observations include: motor activity (up, normal, down, still), straub, piloerection, ptosis, pineal reflex, corneal reflex, lacrimation, catalepsy, body posture (normal, abnormal), hanging, reestablishment, flexion, hafner, mortality, self-care, bowel movements, urination, respiration (rapid, normal, shortness of breath), saliva volume, body weight and number of deaths rats which can be seen in Tables 1, 2 and 3.

Table 1. One Way ANOVA Test of Average Weight Change in Rats

Treatment	Average Weight Change in Rats (gram) ± SD				*Value P*
	Normal Group	Dose of 500 mg/ kgBB	Dose of 1000 mg/ kgBB	Dose of 2000 mg/ kgBB	
Before	186.6 ± 16.817	199.6 ± 20.132	188.6 ± 18.488	204.8 ± 20.266	0.394
After	187.6 ± 16.817	201.6 ± 19.476	189.8 ± 18.458	205.6 ± 21.291	0.392

Table 2. Observation of Signs of Toxicity

Symptoms	Normal Group					Dose of 500 mg/kgBB					Dose of 1000 mg/kgBB					Dose of 2000 mg/kgBB				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Decreased Motoric Activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	-	-	+	-	-		+	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	-	-	-
Pineal Reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
Corneal Reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalepsy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Posture	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hanging	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Retablismen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flexion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hafner	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortalitily	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+
Defecation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+
Urination	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Breathing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vokalization	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremor	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-
Seizure	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Writhing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Information :
(-) = No Symptoms
(+) = Symptoms Occur

Table 3. Rat Mortality Data

Group	Number of Rats	Dose mg/ kgBB	Number of Deaths
Normal Control	5	Supension Na CMC 0,5%	0
Test Group I	5	500 mg/Kg BB	0
Test Group II	5	1000 mg/Kg BB	0
Test Group III	5	2000 mg/Kg BB	0

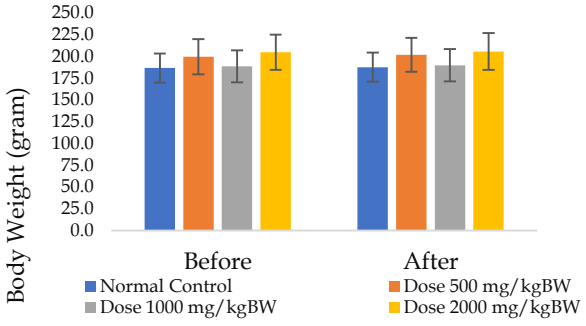


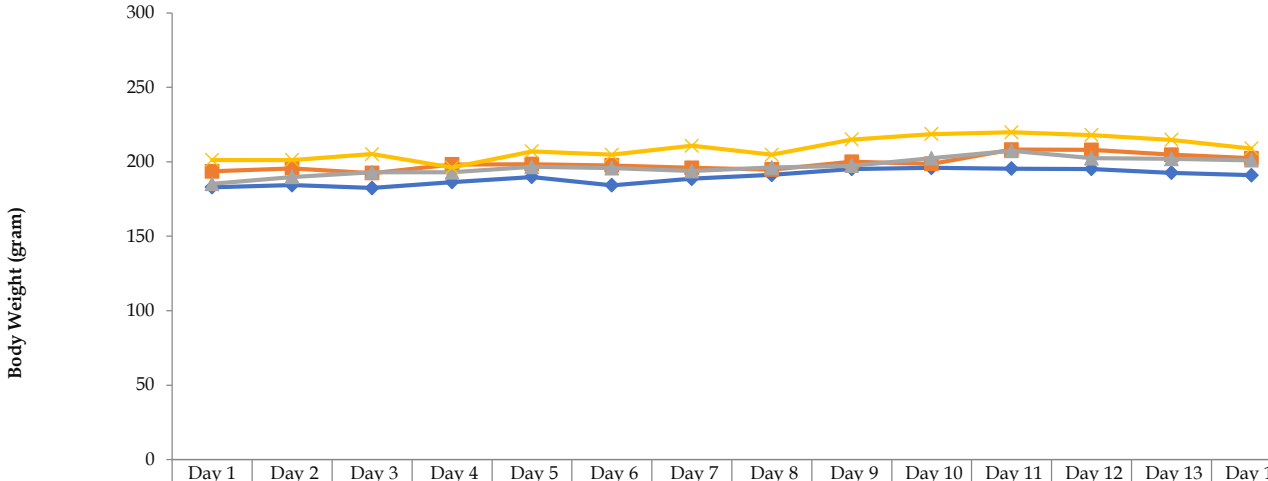
Figure 1. Graph Before and After Regarding the Body Weight of Test Animals

The parameters observed in the acute toxicity test are data on the death of mice after the administration of ethanol extract for 14 days from the first to the last day. The results of the observations can be seen in Table 2 and 3.

Information:

Before the treatment, all groups showed a normal and homogeneous distribution of body weight data. The One Way ANOVA test statistics yielded a p-value of 0.394 ($p > 0.05$), indicating that there is no significant difference in body weight among the groups before the treatment. This shows that the initial body weight of the rats was uniform across all groups.

After treatment, the normal weight data showed homogeneity in each group. The results of the One Way ANOVA test show a p-value of 0.392 ($p > 0.05$), indicating that the treatment did not have a significant effect on the body weight of the rats. Thus, there was no significant change in the body weight of the rats after the treatment was administered.



	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Normal	183.0	184.4	182.4	186.4	189.8	184.2	188.6	191.2	195.2	196.0	195.4	195.2	192.6	191.0
500 mg/kgBB	193.6	195.6	192.4	198.2	198.4	197.6	196.0	194.8	200.0	198.6	208.2	208.0	204.8	202.4
1000 mg/kgBB	185.2	189.8	193.0	193.0	196.6	195.8	193.8	196.2	197.4	202.4	207.4	202.4	202.0	201.0
2000 mg/kgBB	201.2	201.2	205.2	196.2	207.0	204.8	210.8	204.8	215.0	218.6	219.8	218.0	214.8	209.0

Figure 2. Graph Average of Body Weight From Day 1 - 14

Discussion

Research on acute toxicity of ethanol extract from the leaves of Abelmoschus manihot (L.) Medik, or red gedi, originating from Palu, Central Sulawesi, provides important insights into the potential safety of using this plant, particularly in traditional medicine applications in Indonesia. Red gedi leaves, which have long been used as a medicinal plant in the Palu region of Central Sulawesi, are known to have various health benefits, particularly in the treatment of inflammatory diseases, managing diabetes, and metabolic disorders (Kim et al., 2018; Park et al., 2023; Tangka et al., 2022). However, before this plant can be further developed as a clinical therapy, it is crucial to understand its toxicological aspects, especially the safe dosage that can be used by humans. In the research on Acute Toxicity testing, where acute toxicity is one of the fundamental parameters in the safety study of compounds or herbal extracts (Zakari, 2018). In this study, the ethanol extract of red gedi leaves was tested with various doses (500 mg/kg, 1000 mg/kg, and 2000 mg/kg) on a population of rats with the aim of observing and measuring the LD50 or lethal dose that causes death in 50% of the test population. This test is usually conducted to evaluate the potential hazards that may occur shortly after the administration of a substance. The importance of determining LD50 is as a preliminary guideline to understand the safety limits of a substance before further testing such as subchronic and chronic toxicity (Priyanka & Elumalai, 2019).

In the acute toxicity testing, during the observation period of 24 hours to 14 days, no deaths or severe clinical toxicity symptoms were found in rats given extracts at doses of 500 mg/kg and 1000 mg/kg body weight. At a higher dose of 2000 mg/kg, some signs of toxicity such as decreased physical activity, dull fur, or reduced appetite began to appear, although they did not lead to death. This indicates the potential for side effects at high doses, but it still shows that at this dosage, the extract is considered safe for short-term use (Li et al., 2024). The absence of deaths at all tested doses indicates that the LD50 of the ethanol extract of red gedi leaves is higher than 2000 mg/kg, so this extract can be considered relatively safe within that dose range. In the test

preparation that was administered once on day 0, observations were conducted over the first 24 hours with intensive care for 4 hours. If no death occurs, an observation will be conducted to monitor delayed toxicity for 14 days as seen in Table 2 and 3. The observation will focus on the toxic symptoms that arise, namely motoric activity (increased, normal, decreased, immobile), Straub, piloerection, ptosis, pineal reflex, corneal reflex, lacrimation, catalepsy, body posture (normal, abnormal), hanging, retablismen, flexion, hafner, mortality, grooming, defecation, urination, respiration (rapid, normal, and labored), salivation, vocalization, tremors, seizures, and writhing. Additionally, daily weight measurements will be taken (Erhirhie et al., 2018). In the observation of body weight as seen in Table 1 and Figure 1 and 2, prior to the treatment, all groups showed a normal and homogeneous distribution of body weight data. The One Way ANOVA test produced a p-value of 0.394 ($p > 0.05$), indicating that there were no significant differences in body weight among the groups before the treatment. This suggests that the initial body weight of the rats was uniform across all groups, and similarly, after the treatment, the body weight data remained normal and homogeneous in each group. The results of the One Way ANOVA test showed a p-value of 0.392 ($p > 0.05$), indicating that the treatment did not have a significant effect on the body weight of the rats. Thus, there was no significant change in the body weight of the rats after the treatment was administered. The significance of the results for traditional and clinical use in relation to this research is highly relevant in the context of the traditional use of the Abelmoschus manihot plant, particularly among the people of Palu City, who have long utilized red gedi leaves as a treatment for various ailments. The absence of heavy toxicity at low to medium doses provides scientific support that this plant is safe when used in reasonable doses. It also offers scientific validation for traditional practices that have existed, helping to reassure traditional users that this plant is relatively safe. However, it is important to emphasize that although this research shows safety at doses of 500 mg/kg to 2000 mg/kg, higher doses may still cause toxic effects that might not be immediately apparent in acute tests.

In clinical applications, high doses used continuously have the potential to cause the accumulation of active compounds in the body, which can trigger more serious toxic effects. Therefore, these results emphasize the importance of regulating the doses used in traditional medicine and the need for further monitoring if used in high doses or over a long period. Comparison with Previous Studies, where this research aligns with earlier findings that report the safety of Abelmoschus manihot L. Medik in a pharmacological context. Several other studies have revealed the anti-inflammatory, antioxidant, and cardiovascular protective effects of this plant extract (Xu et al., 2022) . However, the low acute toxicity of this extract is an added value as it opens up opportunities for broader use in clinical therapy. Previously, although some studies have explored the pharmacological effects of this plant, its toxicological data remains limited. Therefore, this research provides an important contribution to understanding the basic toxicology profile of Abelmoschus manihot, which is crucial before proceeding to the clinical research stage in humans.

The Potential of Applications in Modern Therapy, with a solid safety background from this research, the extract of Abelmoschus manihot has great potential to be developed as a modern therapy, particularly in traditional medicine. This plant has long been known to contain active compounds such as flavonoids and polysaccharides that act as antioxidants and anti-inflammatories (Gurav & Vithoba, 2016; Wang et al., 2022). Therefore, the validation of its safety provides a strong impetus for the development of phytopharmaceutical products based on red gedi leaves from Palu, Central Sulawesi. Nevertheless, the next step that needs to be taken is long-term toxicity testing to evaluate the potential side effects if this extract is used continuously. Chronic and subchronic toxicity tests will help in understanding whether prolonged repeated use can lead to side effects, such as organ damage or a decline in physiological function (Kharchoufa et al., 2020). In addition, clinical trials on humans are essential to determine the optimal dose that can provide maximum therapeutic effects with minimal risk.

Conclusion

Based on the results of the acute toxicity test, it can be concluded that the ethanol extract of Abelmoschus manihot leaves (Abelmoschus manihot L. Medik) does not show any acute toxic effects on rats at doses of 500 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight. No significant deaths or mild to severe toxic symptoms were observed during the observation period of 24 hours up to 14 days.

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Author Contributions

Conceptualization, V.A; methodology, V.A.; validation, M.B.P. and I.K.U.; formal analysis, M.B.P.; investigation, V.A., and I.K.U.; resources, V.A. and V. A.; data curation, M.B.P.: writing—original draft preparation, M. B. P and V.A.; writing—review and editing, Y.R.: visualization

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Conflicts of Interest
The authors declare no conflict of interest of reported research results.

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