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Acute Toxicity Test of Gastroretentive Mucoadhesive Granule Preparation of Earthworm Flour (*Lumbricus rubellus*)

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Abstract: Earthworms (Lumbricus rubellus) contain the compound lumbricin, which is beneficial for treatment, and its use is still simple, leading to the innovation of gastroretentive mucoadhesive granule formulations. However, its security has not yet been fully supported by research. This research aims to determine the toxic effects caused by earthworm granule preparations in terms of toxicity symptoms and the determination of the LD50 value. This research used 20 male white rats that were randomly divided into 4 groups. One control group was given Aquadest, and three treatment groups were given earthworm granules at doses of 500 mg/kgBB (P1), 2000 mg/kgBB (P2), and 5000 mg/kgBB (P3) with a single oral administration. The research results indicate that the preparation of earthworm granules (*Lumbricus rubellus*) causes toxic symptoms such as decreased locomotor activity, piloerection, and increased grooming frequency in line with the increase in dosage, but does not result in the death of the test animals. (LD50). Based on the research findings, it can be concluded that the LD50 value of the earthworm granules (*Lumbricus rubellus*) is >5000 mg/kgBB and falls into the practically non-toxic category with an LD50 range of 5–15 g/kgBB

Keywords: Acute toxicity; LD50; *Lumbricus rubellus*

Introduction

The use of traditional medicine in Indonesia is part of the nation's culture and has been practiced by the community for centuries (Herlina et al., 2024; Saija et al., 2021). Traditional medicine is advancing and developing rapidly, and this is being utilized by researchers to conduct studies ranging from plants to animals. (Hakim et al., 2020; Bestari et al., 2022).

The use of traditional medicine is widely utilized by the community, but its effectiveness and safety have not yet been fully supported by research (Gupta et al., 2018). Therefore, it is necessary to research and develop its utilization (Jabbar et al., 2020). The safety of potential toxic effects in drug research is crucial to ensuring its use. Toxicity testing is the initial step in the safety parameters of a drug before it becomes a pharmaceutical product used by humans (Musdalipah et al., 2021).

The assessment of drug safety can be conducted through toxicity testing (Darmayanti et al., 2024; Lubis et al., 2023). There are three types of toxicity security testing that need to be conducted, namely: acute, subchronic, and chronic (Dipayana et al., 2024). Acute toxicity testing is parat of preclinical testing (Tonholo et al., 2020), which aims to detect toxic effects that occur shortly after the administration of the test preparation given orally in a single dose or repeated doses administered within 24 hours (BPOM, 2022). Acute oral toxicity testing is the first phase of safety toxicology evaluation, with the median lethal dose (LD50) being the most commonly used assessment parameter (Li et al., 2024; Setiani et al., 2023). LD50 is the statistically determined amount of a substance that can cause death

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in 50% of animals when administered via a specific route as a single dose, with the animals observed over a certain period of time (Adamson, 2016). To express acute toxicity, the LD50 value is generally used. LD50 is benchmark after the administration of a single dose of a compound or substance that will cause the death of 50% of the test animals (Andriani et al., 2023).

Earthworms (*Lumbricus rubellus*) are one of the living organisms in soil biota that have many benefits for human life. Marnelis et al. (2024) as antipyretics, antispasmodics, antidiuretics, antihypertensives, and anti-allergics, and they show fibrinolytic activity (Sardi et al., 2023), treat cases of typhoid fever (Fauzi et al., 2022; Samatra et al., 2017), and demonstrate anti-inflammatory effects (Dewi & Mahendra, 2020), possess hepatoprotective properties (Lestari et al., 2019), and exhibit antibacterial activity (Ulhaq et al., 2021). According to Mendrofa et al. (2022), the nutritional content of earthworms (*Lumbricus rubellus*) is 76% high-quality amino acid protein, 17% carbohydrates, 45% fatty acids, and 1.5% ash.

Earthworms (*Lumbricus rubellus*) contain antimicrobial peptides called lumbricin (Dharmawati et al., 2019; Mercya et al., 2024) in high concentrations, but they are easily denatured in the stomach, leading to changes in solubility and biological activity. Therefore, its application can be developed in the form of mucoadhesive gastroretentive granules (Magfirah et al., 2024). Gastroretentive Drug Delivery System (GRDDS) is a system or preparation approach designed to extend the residence time of drugs in the gastrointestinal tract. One of the mechanisms of this gastroretentive drug delivery system is mucoadhesive, where the formulation can adhere to the gastric mucosa or the epithelial cell surface, thus prolonging the residence time of the drug at the absorption site (Febryani et al., 2021). The formulation of mucoadhesive allows the material to be retained and localized in the stomach, thereby enhancing its effects in treating gastric ulcers (Nofianti, 2024). Although the health benefits of earthworms (*Lumbricus rubellus*) have received much attention, their safety has not yet been fully supported by research. Therefore, it is necessary to continue toxicity testing on test animals as scientific data for the safety of earthworm granules (*Lumbricus rubellus*) when consumed by humans. Research on the acute toxicity testing of gastroretentive mucoadhesive granules made from earthworm powder (*Lumbricus rubellus*) has not been conducted, prompting the researcher to undertake a study titled "Acute Toxicity Testing of Gastroretentive Mucoadhesive Granules from Earthworm Powder (*Lumbricus rubellus*)," focusing on toxic effects observed through clinical symptoms and the determination of LD50.

Method

This study uses an experimental method with a randomized group design, involving 20 male white rats divided into 4 groups. In this study, there is 1 control group and 3 test groups. In the control group, only Aquadest was administered, while in treatment group 1 (P1), a preparation of earthworm granules (*Lumbricus rubellus*) was given at a dose of 500 mg/kg body weight. Then, treatment group 2 (P2) received a preparation of earthworm granules (*Lumbricus rubellus*) at a dose of 2,000 mg/kg body weight, and treatment group 3 (P3) was given a preparation of earthworm granules (*Lumbricus rubellus*) at a dose of 5,000 mg/kg body weight. The test preparation was administered orally in a single dose, or repeated doses were given over a period of 24 hours (BPOM, 2022).

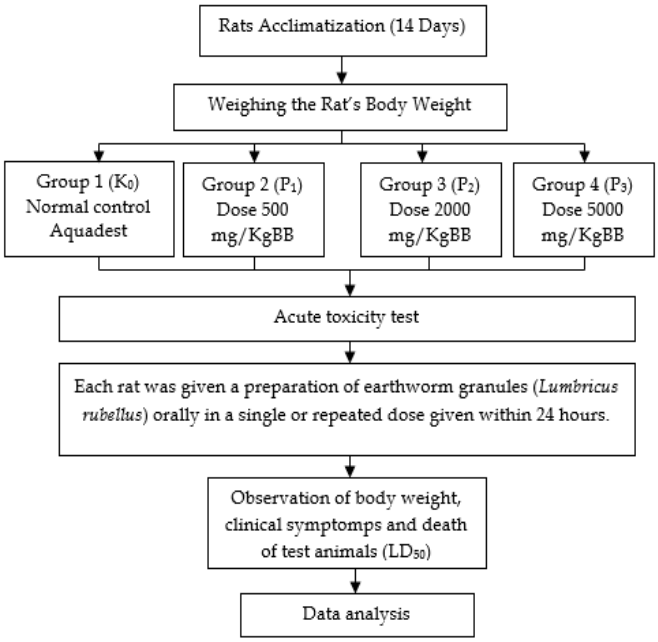

Figure 1. Research working method

Research Procedure

Preparation of Suspended Gastroretentive Mucoadhesive Granules from Earthworm Flour (Lumbricus rubellus)

After calculations were made, each suspension was prepared by weighing the earthworm granules (*Lumbricus rubellus*). Then, each preparation of earthworm granules (*Lumbricus rubellus*) with three dosage variations, namely 500 mg/kgBB, 2000 mg/kgBB, and 5000 mg/kgBB, was mixed with distilled water until the total volume reached 25 ml. The mixture was then stirred until completely homogeneous.

Preparation of Test Animals

The test animals used in this study are white rats (Rattus norvegicus) from the Wistar strain Hartika et al.

(2024) with the criteria of being 3-4 months old, weighing 150-250 grams, having white fur, being active and healthy, male, and having been adapted for 14 days. In this study, male white rats are used because they are known to be a good experimental animal model, easy to handle, and can be obtained in large quantities, with more stable research results as they are not affected by menstrual cycles and pregnancy like female white rats. The Wistar strain was chosen because Wistar rats have a relatively fast metabolic capacity, making them more sensitive when used in research (Lahamendu et al., 2019; Wardani et al., 2024). In this study, the test animals were divided into 4 test groups, with each group consisting of 5 rats. Test animals are treated according to the standards of the Institutional Animal Care and Use Committee (IAEC). Before the treatment, the rats were fasted for 16 hours and still given water ad libitum (Sukarjati et al., 2024). The treatment for the experimental group, namely the control group, was given distilled water. Then the treatment group was given a preparation of earthworm granules (*Lumbricus rubellus*). In P1, the dosage was 500 mg/kgBB, in P2, it was 2,000 mg/kgBB, and in P3, it was 5,000 mg/kgBB.

Acute Toxicity Test

The preparation for each treatment group was administered to the test animals with a single oral dose within a 24-hour period. Observations included monitoring clinical symptoms and the mortality of the test animals (LD_{50}).

The observed clinical symptoms include changes in movement activity characterized by weakness (Musdalipah et al., 2022), Straub's sign indicated by the test animal's tail being upright and stiff (Amal, 2022), piloerection marked by the fur standing on end due to the test animal being tense (Ubang et al., 2022), ptosis characterized by abnormalities in the eyes, specifically drooping eyelids (Ubang et al., 2022), mydriasis indicated by abnormalities in the eyes, namely pupil dilation (Sulastra et al., 2020), diuresis marked by an increased frequency of urine production (Sulastra et al., 2020), defecation characterized by the excretion of waste material/emptying of the intestines and the excretion of feces. An abnormal frequency of bowel movements is referred to as diarrhea (Ubang et al., 2022), salivation marked by excessive saliva production (Ofori et al., 2021), and grooming characterized by the test animal's habit of cleaning itself, which can be observed by licking parts of its body such as the face; an increased frequency of grooming from the usual indicates that the test animal is experiencing heightened pain (Ubang et al., 2022). Observations were conducted for 30 minutes, 4 hours, 24 hours, and then once a day for 14 days.

Observation of test animal mortality for determining the LD50 value is obtained using the Thompson and Weil formula. This method is chosen for its relatively high level of confidence and is commonly used because it does not require a large number of test animals. This method also employs an LD50 calculation table to ensure that the results obtained are more accurate (Yenny Nonci et al., 2014). This calculation is performed using the value of r, which is:
The formula used is: Log LD50 =

$$Log\ D + d\ (f + 1) - 2\ d\ .\ Df \quad (1)$$

Description: D = Smallest dose used; d = multiple logarithms; F = factor obtained from the table.

Then, the body weight of the test animals was observed by weighing the test animals on day 0 before the administration of the test preparation and then on days 7 and 14 after the administration of the test preparation (Lukman & Christin, 2020). The average weight of the rats was calculated by summing the weights of the rats and then dividing by the number of rats in each group (Kuncarli & Djunarko, 2014).

Result and Discussion

The results of the weight observation of the test animals can be seen in Figure 1, which shows that the administration of the mucoadhesive gastroretentive granule preparation of earthworm flour (*Lumbricus rubellus*) led to an increase in the weight of the test animals. It can be seen from the average body weight values that there is an increase in the body weight of the test animals in each group (both normal group and test group). It states that there is no relationship between clinical symptoms and toxic effects on the body weight of rats, as there was no decrease in body weight observed during the 14 days of monitoring (Sulastra et al., 2020). Weight change is an early indicator and the easiest indicator to observe the toxic effects of test samples. Experimental animals that exhibit toxic symptoms generally experience weight loss due to a decreased appetite (Putra et al., 2023).

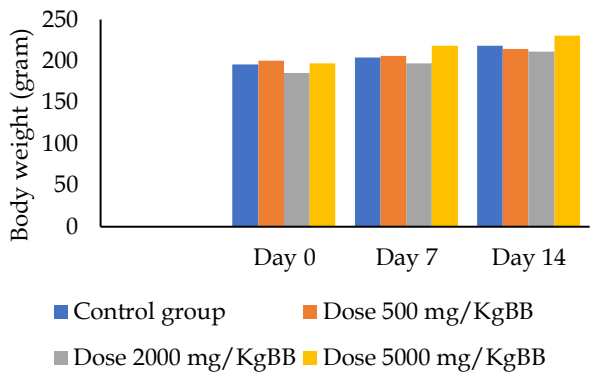

Figure 2. Body weight of rats for 14 days

The results of the observations of clinical symptoms and mortality in test animals can be seen in Table 1 and Table 2. The results of the clinical symptom observations in the treatment group indicate that in the normal control group, there were no signs of toxicity and no mortality among the test animals. At a dose of 500 mg/kgBB, the rats experienced a decrease in locomotor activity, piloerection, and grooming, with no fatalities among the test animals. At a dose of 2000 mg/kgBB, the rats also showed a decrease in locomotor activity, piloerection, and grooming, with no fatalities. At a dose of 5000 mg/kgBB, the rats exhibited a decrease in locomotor activity, piloerection, and an increased frequency of grooming, which correlated with the increase in dosage, and again, there were no fatalities among the test animals. The clinical symptoms and toxic effects increased in frequency at the 30-minute mark after the administration of the earthworm granule preparation (*Lumbricus rubellus*) and then returned to normal after 24 hours of administering the earthworm granule preparation (*Lumbricus rubellus*).

The oral administration of earthworm granules (*Lumbricus rubellus*) causes the active substances contained within to be absorbed into the digestive tract. The active substance then undergoes distribution and metabolism processes. The toxic metabolic products act as enzyme inhibitors for the subsequent stages of metabolism, and the reaction between the active substance and receptors in the effector organs leads to the emergence of poisoning symptoms. Each sample used will provide a different response to a specific dose. The differences in response are caused by the varying levels of sensitivity of each sample to that particular dose (Jumain et al., 2018).

The results of the observation of the LD50 value in white rats that were given gastroretentive mucoadhesive granule preparations of earthworm flour (*Lumbricus rubellus*) showed clinical signs of toxicity, but there were no fatalities at the three dosage levels administered. The absence of test animal deaths indicates that factor f, which is derived from the Thomson and Weil table, was not obtained, thus the LD50 value cannot be calculated. This is based on the criteria for acute toxicity testing conducted to assess LD50 based on expert consensus. If the maximum dose does not result in the death of the test animals, then LD50 is stated as the maximum "apparent" LD50 (Hafid & Medicine, 2022). From the test results, the LD50 value is >5000 mg/kg body weight. Based on the criteria, this value falls into the classification of toxicity level category 5, which means "practically non-toxic," with an LD50 range of 5–15 g/kgBB (Hidayati et al., 2024). The toxicity test using the Thomson and Weil method is a toxicity test with LD50 as a benchmark for calculating toxicity values. This method is one that requires fewer test animals and has a high level of accuracy (Komarudin et al., 2023).

Table 1. Analysis of Clinical Symptoms

Clinical symptoms	Dosage (mg/KgBB)	30 Minutes	4 Hours	24 Hours
Movement activities	500	+	-	-
	2000	+	-	-
	5000	+	+	
Straub	500	-	-	-
	2000	-	-	-
	5000	-	-	-
Piloerection	500	+	-	-
	2000	+	-	-
	5000	+	-	-
Ptosis	500	-	-	-
	2000	-	-	-
	5000	-	-	-
Mydriasis	500	-	-	-
	2000	-	-	-
	5000	-	-	-
Diuresis	500	-	-	-
	2000	-	-	-
	5000	-	-	-
Defecation	500	-	-	-
	2000	-	-	-
	5000	-	-	-
Salivation	500	-	-	-
	2000	-	-	-
	5000	-	-	-
Grooming	500	+	-	-
	2000	+	-	-
	5000	+	+	-

Table 2. Number of Test Animals that Died

Group	Number of wistar rats (tails)	Number of dead wistar rats (tails)
Control	5	0
500 mg/KgBB	5	0
2000 mg/KgBB	5	0
5000 mg/KgBB	5	0

The LD50 test is not the only test used to assess the toxicity of substances or drugs. Further research is still needed to explore the potential toxicity studies for sub-chronic and chronic levels to determine the actual toxicity potential of the gastroretentive mucoadhesive granules made from earthworm flour (*Lumbricus rubellus*) in order to strengthen the toxicity analysis of a substance or medicinal material (Syachriyani et al., 2023).

Conclusion

This research was conducted to determine the acute toxicity effects in the form of toxic symptoms and to establish the LD50 value in Wistar rats over a period of

14 days. The preparation of earthworm granules (*Lumbricus rubellus*) at doses of 500 mg/kg body weight, 2000 mg/kg body weight, and 5000 mg/kg body weight caused toxic symptoms after 30 minutes of administering the test preparation, but did not result in the death of the test animals. Thus, the LD50 value of the gastroretentive mucoadhesive granule preparation from earthworm powder (*Lumbricus rubellus*) is >5000 mg/kg body weight and falls into the practically non-toxic category with an LD50 range of 5-15 g/kg body weight.

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Author Contributions
Conceptualization, NM, and Magfirah; methodology, NM, Magfirah, and UI; validation, Magfirah; data analysis, Magfirah, and UI; investigation, NM; resources, JT; data curation, Magfirah, and UI; writing—preparation of the original draft, NM; writing—review and editing, NM, Magfirah, and UI; supervision, Magfirah All authors have read and approved the published version of the manuscript.

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Conflicts of Interest
The authors declare there is no conflict of interest.

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