Acute Toxicity Test of Probiotic Juice Red Guava, Lemon, Melon, and Apple With Bacteria Starter *Lactobacillus casei* on Female Balb/C Mice

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Abstract

Introduction: Probiotic drinks are drinks that have health effects and contain living microbes in them. These drinks are popular among the public, and have started to consume these drinks. Probiotic drinks contain probiotic agents made through fermentation, one of which is Lactobacillus casei. Before consumption, probiotic drinks will undergo preclinical testing, one of which is in vivo. This study aimed to determine if the purpose was to determine if there was an acute toxicity effect after being given a juice probiotic drink. **Methods:** This quantitative research type applied research applied research was a quantitative research type applying an experimental study design. The variables used are independent and dependent. This study used 15 female white mice (Balb/c) divided into five groups. The test material used was the Probiotic drink, administered orally at 5, 50,300, and 2000 mg/KgBW doses and distilled water as the control group. The test animals were observed individually for 14 days. The variables observed were clinical symptoms like seizures, saliva, tremors, limp, and walking using the stomach. **Results:** There are no toxic symptoms that occurred in mice Balb/C like seizures, saliva, tremors, limp, and walking using the stomach during 14 days of observation. **Conclusion:** There were no clinical symptoms that occurred in the toxicity test. This study concluded that probiotic drink juice with *L.casei* starter at doses of 5, 50, 300, and 2000 mg/KgBW did not cause a decrease in no toxic symptoms occurred in mice *Balb/*C.

Keywords: *Probiotic Drinks; Acute Toxicity; Fix Doses; Balb/C; Mice*

Introduction

Indonesian people already have new habits, such as consuming healthy foods that have more benefits for the body, which serves to help maintain a healthy body. Functional food is defined as food or drink that has undergone a modification and innovation process in the processing process (Rahmawati *et al*., 2021). According to the Food and Drug Supervisory Agency (2021), health supplements supplement nutritional needs, maintain health functions, and contain one or more ingredients in the form of vitamins and minerals combined with plants. Food or drink functions to be used as a supplement to replace synthetic drugs. It is necessary to carry out consumption safety tests, including selection stages, preclinical trials, standardization, and clinical trials. In efforts to carry out clinical trials, it is necessary to carry out preclinical trials first. According to Pradono *et al*. (2019), preclinical testing is a test used to collect information on toxicology and pharmacology to determine the efficacy and safety of a scientifically tested product through toxicity tests or activity tests. Drug toxicity tests are generally divided into 2: in vitro and in vivo. According to (BPOM 2022), a toxicity test is a test used to determine whether or not a substance has a toxic effect on biological systems and also to obtain data responses that are typical of the test preparation, where the data obtained will be used to provide information about the dangers of the prepared preparation and tested in case of exposure to humans to determine the user's dose for human safety. According to (BPOM 2020), toxicity tests on test animals are carried out as evidence of the safety of test preparation. The choice of the test is related to the purpose of a substance user and the possibility of a risk of exposure to humans. Research on animals can determine whether the material under study is safe or causes toxic effects on medication (Jawi, 2014).

According to Rahayu and Firman (2018), toxicity is a term used to compare a chemical substance with another. A substance will be said to be toxic (toxic) if the substance has the potential to harm the biological mechanisms of an organism. This toxic effect is determined based on the dose, the nature of the substance, and the form of the effect it causes. There are several acute toxicity tests, including subacute, acute, chronic and subchronic. *Subacute toxicity* is a test carried out to detect the presence or absence of toxic effects after administering the test preparation with repeated doses given orally to test animals for 28 days. This toxicity test aims to provide information on the toxic effects on the target organs it affects (Ritonga & Ayu, 2017). Acute toxicity is a test to detect toxic effects that appear within a short time after administering the test preparation by mouth (orally) in a single dose or repeated doses within 24 hours (Melisa *et al*., 2022). Chronic toxicity is a test to detect toxic effects that appear after administering the test preparation to test animals repeatedly for 9-12 months (BPOM, 2020). A subchronic toxicity test is a test of a compound that is given for 1-3 months with repeated doses to test animals (Wahyuni *et al*., 2017)

The probiotic drink to be developed in this study consists of several fruits, including red guava, lemon, melon, and apple, with a starter Lactobacillus casei using the fixed doses method. This research needs to carry out preclinical tests before being used by humans, one is an acute toxicity preclinical test using Balb/C strain female mice.

Methods

This research is quantitative experimental research. This research was conducted at the STIKes Mitra Keluarga, Pharmacology Laboratory, Bekasi, West Java, from January to March 2023. The samples in this research were guava fruit, apples, lemons, melons, and *Lactobacillus casei* bacterial cultures obtained from PT Agritama Sinergi Innovation and mice—balb/C females obtained from Karanganyar, Central Java.

This research began with selecting the sample used, namely the Jamale probiotic drink with doses of F1, F2, F3, and F4, with the dosage for each formula being as follows:

Fruits	Formula I (5 mg/KgBW)	Formula II (50 mg/KgBW)	Formula III (300 mg/KgBW)	Formula IV (2000 mg/KgBW)
Apple	6,5 mg	65 mg	390 mg	2700 mg
Red Guava	6,5 mg	65 mg	390 mg	2700 mg
Melon	6,5 mg	65 mg	390 mg	2700 mg
Lemon	6,5 mg	65 mg	390 mg	2700 mg
Sugar	1,3 mg	13 mg	78 mg	120 mg

The following method is the selection of test animals, where the test animals used are 15 healthy-bodied Balb/C female mice with a body weight of around 20-30 grams. Previously, mice would be acclimatized for 7 days, where this was intended, so that the animals could adapt to the atmosphere in the laboratory and the test animals could adapt to the new environment. This study used the Fixed Doses Method based on BPOM in 2022 using test animals of the same sex with doses in stages of 5, 50, 300 and 2000 mg/kgBB. Previously, the mice would be fasted for 3-4 hours, and drinking was allowed. Mice were divided into 5 treatment groups, where each group consisted of 3 female mice, which were determined randomly. Group 1 was the control group, and groups F1 5 mg, F2 50 mg, F3 300 mg, and F4 2000 mg were the treatment group.

Observations will be made after and before the dose is given. The behaviour of the mice was observed for 30 minutes after the dose was given, and observations of clinical symptoms were carried out every 4 hours once a day for 14 days. The observed toxic symptoms of mice were seizures, tremors, salivation, weakness, and walking using the stomach. For test animals that have been exposed to toxic effects after being given the appropriate dose of probiotic drink or found dead, the time of death of the mice must be recorded according to the date of death.

Results and Discussions

This research uses the Fixed Doses method, which uses test materials with a moderate degree of toxicity, and the dose used does not cause death, irritation, or corrosiveness (BPOM RI, 2022). The principle of the Fixed Dose method is to use dose levels of 5, 50, 300 and 2000 mg/kgBB. Before the primary test is carried out, a preliminary test is carried out to determine the dose that causes symptoms of toxicity (Lestari *et al*., 2022). Table 2 shows the results of this study, which showed toxic symptoms that occurred after administration of the probiotic drink.

Table 2. toxic symptoms that occurred after administration of the probiotic drink.

Doses	**Mice number**	**toxic symptoms**				
		Seizures	**Saliva**	**Tremors**	**Limp**	**Walking using stomatch**
Negatif control	25	-	-	-	-	-
	12	-	-	-	-	-
	1	-	-	-	-	-
5 mg/KgBW Doses	2	-	-	-	-	
	4	-	-	-	-	-
	5	-	-	-	-	-
50 mg/KgBW Doses	6	-	-	-	-	-
	9	-	-	-	-	-
	13	-	-	-	-	-
300 mg/KgBW Doses	14	-	-	-	-	-
	17	-	-	-	-	-

	18	-	-	+	+	-
2000 mg/KgBW Doses	19	-	-	-	-	-
	20	-	-	-	-	-
	22	-	-	+	+	-

Description: + (symptoms occur), - (no symptoms occur)

Table 2 shows that 2 mice showed toxic symptoms after being given juice at doses of 5 mg/KgBW, 50 mg/KgBW, 300 mg/KgBW, and 2000 Kg/BW, which had been observed for 14 days. It was found that mice number 18 at a dose of 5 mg/KgBW and 22 at a dose of 2000 mg/KgBB showed similar toxic symptoms in the form of a vibrating and passive stomach and hair loss in mice number 18 for 3 days. Clinical symptoms in mice number 18 and 22 were caused by stress because previously, the mice were stuck in the nets in the mouse cages, resulting in toxic symptoms that occurred during the observation. This differs from observations during acclimatization, where no mice had toxic symptoms.

The toxic symptoms or behaviour of mice were observed every day for 14 days. These toxic symptoms are observed through visualization or with the naked eye. To find out the symptoms that arise, they must be compared with the habits of the test animals during acclimatization and with the negative control group (Fithria *et al*., 2018). Factors that can make mice uncomfortable include air circulation, lighting and cages such as layout, materials and size (Mutiarahmi *et al*., 2021). Parameters observed for signs of toxicity include gait using the stomach, shaking, convulsions, salivation, and weakness. These toxic symptoms are observed visually, so they must be compared with the habits of the test animals during the acclimatization period and with the negative control group for each test group. The results of observations of the administration of Jamale probiotics are shown in Table 2, such as hair loss, body trembling, decreased appetite, and passivity on days 11 to 13, found in mouse number 18, body shaking, decreased appetite, and passivity. In mouse number 22 on day 2. It is suspected that the mice were pinched by the cage net, causing them to become stressed.

In this study, it was found that mice died at a dose of 2000 mg/KgBW in the Jamale probiotic drink at 08.00 after 5 days of observation. Death is the worst effect that can occur due to the toxic effects of an extract. Toxic doses can cause damage to the body's physiological functions, especially vital organs and body chemical functions, resulting in death. The results of this test are the effects that occur in mice when given juice using 4 doses with 1 control to compare the effects in the treatment group. In this study, 1 dead mouse was found, namely number 22. This is thought to be because the mouse was stressed after administering the drug. The mouse got caught in the lid of the mouse cage. It can be seen from the changes in the following days that toxic symptoms occur, such as body shaking (tremors), decreased appetite, hair loss, and inactivity. As a result, the mice died on the fifth day at 08.00 a.m. after being given juice. These characteristics are the same as mouse number 18, which was also caught on the 11th day (Asriningtyas and Daryanti, 2022). They observed that several factors, such as physical or psychological stimulation or both, caused the stressful conditions of mice. Physical stress is caused by exposure to a stressor that is harmful to the body, such as exposure to a cold environment, infection, and injury. It can be seen from the changes that occurred the following day, such as body shaking, decreased appetite, lack of activity, and hair loss for 3 days. However, mouse number 18 died on the 14th day of observation.

Conclusions

The probiotic juice drink given to female Balb/C mice at doses of 5 mg/KgBW, 50 mg/KgBW, 50 mg/KgBW, and 2000 mg/KgBW showed that there were no symptoms of toxicity which were observed during 14 days of observation. One mouse died at a dose of 2000 mg/KgBW, allegedly because it got caught in the cage when the mouse was actively moving, causing stress and death on the fifth day.

References

Asriningtyas, D. Z., & Daryanti, E. P. (2022). Efektivitas Terapi Murrotal Al-Quran Terhadap Stres Mencit (*Mus musculus*) Akibat Penggunaan Hewan Coba Pada Praktikum Farmakologi. 165–172.

BPOM RI. (2020). Peraturan Badan Pengawas Obat Dan Makanan Nomor 10 Tahun 2022 Tentang Pedoman Uji Toksisitas Praklinik Secara In Vivo. *Badan Pengawas Obat Dan Makanan Republik Indonesia*.

BPOM RI. (2022). Peraturan Badan Pengawas Obat Dan Makanan Nomor 10 Tahun 2022 Tentang Pedoman Uji Toksisitas Praklinik Secara In Vivo. *Badan Pengawas Obat Dan Makanan Republik Indonesia*, 1–220.

Fithria, R. F., Wulandari, R. L., Hidayati, D. N., & Rejeki, L. (2018). Toksisitas Akut Infusa Kulit Ari Kacang Tanah (*Arachis hypogea* l.) Pada Mencit *Balb*/C. *JIFFK : Jurnal Ilmu Farmasi Dan Farmasi Klinik*, *15*(2), 62.

Jawi, I. M. (2014). Peran Prosedur Uji Praklinik dan Uji Klinik Dalam Pemanfaatan Obat Herbal. *Universitas Airlangga*, 4.

Lestari, A. A., Herlina, Amriani, A., & Wijaya, D. P. (2022). *Accute toxicity of extract from melinjo (Gnetum gnemon* L*) leaf with fixed dose procedure method*. *Indonesian Journal of Pharmaceutical Science and Technology*, *9*(3), 140–148.

Mutiarahmi, C. N., Hartady, T., & Lesmana, R. (2021). *Use of Mice As Experimental Animals in Laboratories That Refer To the Principles of Animal Welfare: a Literature Review*. *Indonesia Medicus Veterinus*, *10*(1), 134–145.

Pradono, J., Sampurno, O. D., Halim, F. X. S., Widowati, L., Imaningsih, N., Handayani, S., Isnawati, A., Delima, Lestari, C. W., Rooslamiati, I., Karyana, M., Raharni, Dewi, R. M., Lisdawati, V., & Setyawati, V. (2019). Bunga Rampai Uji Klinik. *Lembaga Penerbit BALITBANGKES*, *1(2-5)*, 1–188.

Rahmawati, F. D. N., Swasti, Y. R., & Purwijantiningsih, E. (2021). Kajian Pustaka: Kualitas Minuman Probiotik Berbahan Dasar Nabati dengan Variasi Sukrosa dan Bakteri Asam Laktat. *Jurnal Ilmu Pangan Dan Hasil Pertanian*, *4*(2), 112–128.

Solihat, M. R. dan M. F. (2018). *Toksikologi Klinik*.