

Acute toxicity test of probiotic red guava, apple, lemon, melon juice with bacteria starter *Lactobacillus acidophilus* on male Balb/c. Mice

Reza Anindita^{1*}, Nurin Natasha², Nofria Rizkia Amalia Harahap³, and Ariska Deffy Anggarany⁴

^{1,2,3,4}Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga Bekasi, Indonesia

*Correspondence author : rezaanindita@gmail.com

Abstract

Introduction: Before a new product candidate for a functional drink can be produced and distributed as a supplement that is beneficial to the body, it is necessary to test its safety for consumption by humans, which includes the selection stages, namely preclinical testing, one of which is an acute in vivo toxicity test. Purpose: This study was to determine whether or not a toxic effect occurred after the administration of probiotic drinks. In doses of 5, 50, 300, and 2000 mg/KgBB. **Methods:** The study used an experimental design with a quantitative research type using the oxidation method, namely the Fixed Dose Method. Fifteen male mice (Balb/c) were used as test animals divided into 5 groups. Probiotic drinks were administered orally at 5, 50, 300, and 2000 mg/KgBW doses, and distilled water was used as the control group. The test animals were observed individually for 14 days of observation. The parameters observed were symptoms of toxicity and the number of deaths that occurred. **Results:** The results of the *Kruskal Wallis* analysis showed no significant difference in the number of deaths caused by the administration of probiotics. Toxicity symptoms occurred in the group treated with distilled aqua. (Toxic symptoms include hair loss, trembling, and walking on the stomach. **Conclusion:** From this study, giving juice probiotics with *L.acidophilus* starter and doses of 5, 50, 300, and 2000 mg/KgBW did not cause death and symptoms of toxicity were changed in male balb/c mice observed for 14 days.

Keywords : lemons;Apple; melon; red guava; *Lactobacillus acidophilus*; Probiotics, acute; toxicity

Introduction

Along with the development of technological and information advances. Many people, especially in Indonesia, are starting to pay attention to the type of food they consume. In this regard, the modern food industry is starting to compete to produce various types of functional food, namely food that provides nutrition and can also make the body healthy. One type of functional food widely produced contains probiotics (Helmalia *et al.*, 2019). Probiotic drinks can affect health if contains some live cells of 200 µl (BL (1 x 10⁷ CFU/ml), with a total consumption of probiotic drinks of 100-150 mL/day or 3-4 times a week (Wibawa, 2016). However, the current market production of probiotic drinks is still dominated by probiotic drinks that use milk-based ingredients such as yoghurt, acidophilus milk, Bulgarian milk, kefir, raisins, prima, skyr, taetee, Leben from Egypt, dahi from India, hamao from Central Asia, and bayakult from Japan, so the price relatively more expensive (Rizal *et al.*, 2016). Most consumers allergic to milk find it challenging to find healthier alternatives at more affordable prices. In addition, the fat content in cow's milk is also higher (4.52 g/100 g) compared to camel milk (2.69 g/100 g) (Mojikon *et al.*, 2022). Therefore, research needs to be conducted to develop the use of fruit as an essential ingredient in making probiotic drinks to meet market demand as an alternative to milk-based products. However, before being sold commercially, empirical tests need to be carried out to support scientific evidence so that probiotic drinks based on fruit juice can be developed and accepted for health purposes. Data can be evidence from systematically obtained study results that are safe for human consumption. Functional food products with new formulations need to undergo safety testing, which includes selection stages, namely preclinical testing, standardization and clinical testing, before being produced and distributed to consumers as supplements that are beneficial for the body (Rizal *et al.*, 2016).

Based on this background, it is crucial to carry out toxicity tests to ensure the safety of consumption of pharmaceutical preparations, one of which is functional drinks (Sandhiutami *et al.*, 2022). This test is carried out to examine the pharmacological activity of the compound after administration of a single dose or repeated doses, which can occur within a short time, namely less than 24 hours after exposure (Jumain *et al.*, 2018). As for the acute toxicity test in the study this was done by administering probiotics from red guava, lemon, melon, and apple juice at 5, 50 - 300, and 2000 mg/KgBW to male balb/C strain mice. This research is expected to be able to provide information to consumers regarding the safety of consumption from the results of preclinical beverage test studies probiotics from red guava, lemon, melon, and apple juice are good for health.

Methods

This research uses an experimental design. This research design was carried out by providing treatment or intervention to test animals using controls. Method used is a Fixed-Dose Method to test the effect of acute toxicity of the innovative probiotic drink Guava, Lemon, Melon and Apple juice with the starter bacteria *Lactobacillus acidophilus* using white male mice Strain Balb/c with various formulas F1, F2, F3, F4 (**table 1**). The sample used was the probiotic drink juice Red Guava, Lemon, Melon, and Apple with the starter bacteria *Lactobacillus acidophilus*. The test animals were 15 Balb/C strain mice aged 8-12 weeks. Ethical permission was issued for this study by the Muhammadiyah University Health research ethics commission Purwokerto with Ethical clearance number KEPK/UMP/07/II/2023.

Tabel 1. Fixed Dose per 10 mL Probiotik

Fruits	Formula I (5 mg/KgBB/ 27,3 mg)	Formula II (50 mg/KgBB/ 273 mg)	Formula III (300 mg/KgBB/ 1.638 mg)	Formula IV (2000 mg/KgBB/ 10.920 mg)
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

This research uses the *Fixed Dose Method*. Administration of test preparations should be at most 24 hours. The feed may be given again 1-2 hours after treatment. Mice were divided into 5 treatment groups, where each group consisted of 3 male mice that were determined randomly. Group 1 was the control group with distilled water, and groups 2-5 were the treatment group, so 15 mice were used. The oral route of administration is administered with a syringe compatible with a needle/sonde. Design the trial of administering the probiotic drink can be seen in **Table 2**

Tabel 2. Experimental design

Groups	Treatments
Negative control	Aquades ad libitum was 0.5 mL
Treatment group (F1)	Probiotic juice with dose of 5 mg/kgBB is 0.5 mL
Treatment group (F2)	Juice Probiotic with dose of 50 mg/kgBB is 0.5 mL
Treatment group (F3)	Juice Probiotic with dose of 300 mg/kgBB is 0.5 mL
Treatment group (F4)	juice probiotic drink with dose of 2000 mg/kgBB is 0.5 mL

Observations were made after giving the probiotic juice drink starts at 09.00 am until 16.00. Observations were made in the first 30 minutes after administration of test preparations, periodically every 3 hours for 8 hours a day and every day for 14 days to see the effects the postponed. Observe symptoms of toxicity that may arise, among others namely seizures, body tremors, decreased movement activity, loss gripping power, grooming, walking using the stomach, walking withdrawal, diarrhoea and salivation after administering the test preparation compared to controls (Jumain *et al.*, 2018). Data on symptoms of toxicity (convulsions, tremors, walking backwards, walking using the stomach, body shaking, diarrhea and salivation) was carried out using descriptive tests.

Results and Discussions

The results of the average number of deaths that occurred in acute toxicity testing regarding the effects of administering the probiotic juice with the starter bacteria *Lactobacillus acidophilus* to the group without treatment observed for 14 days are shown in **Table 3**.

Table 3. The average number of deaths of test animals

Treatment	Repetition	Number of deaths
Negative control	3	0
Dose 5 mg/KgBW	3	0
Dose 50 mg/KgBW	3	0
Dose 300 mg/KgBW	3	0
Dose 2000 mg/KgBW	3	0

Table 3. shows the results of the average number of deaths after being treated with the test substance aqua distillate, the probiotic juice at doses of 5 mg/KgBB, 50 mg/KgBB, 300 mg/KgBB and 2000 mg/KgBB. These results show that no death occurred in all treatment groups. The results of the group treated with probiotics juice at doses of 5 mg/KgBW, 50 mg/KgBW, 300 mg/KgBW and 2000 mg/KgBW showed that no deaths occurred in male balb/C mice observed for 14 days. These results indicate that administering the japelemo probiotic drink at a dose 5 mg/KgBW, 50 mg/KgBW, 300 mg/KgBW, and 2000 mg/KgBW did not affect the mortality in male balb/C strain mice observed for 14 days.

The cause of death is thought to be because the mice experienced a stressful condition during observation, characterized by symptoms of decreased body weight and hair loss. The group given the Japelemo probiotic drink showed normal clinical symptoms. It is known that the gut microbiota contained in the Japelemo probiotic drink can affect the CNS through the immune system and ENS. The enteric nervous system (ENS) can regulate gastrointestinal behaviour independently of the central nervous system (CNS). An intact ENS is crucial for life and is often associated with digestive disorders. This is related to stress disorders in stressful conditions. Probiotics can balance intestinal microbiota, increase immunity, improve digestive function, fight the overgrowth of harmful bacteria, and help achieve mental health and mood stability (psychobiotics) as well as fight psychological conditions, such as anxiety and depression, so that mice treated with the probiotic drink Japelemo show normal behaviour and stress levels tend to be low so that death due to stress can be minimized (Rao *et al.*, 2016).

These results are supported by research by Diah *et al.* (2022), who found that consumption of probiotics causes lower plasma cortisol, which can be interpreted as a measure indicating a decrease in anxiety during stressful situations. These results were also proven in weaning mice that experienced septic peritonitis given treatment using probiotics with different strains, *Lactobacillus rhamnosus* and bifidobacterium, providing a significantly increased survival benefit of 7 days survival compared to septic mice that were not given probiotic therapy (Khailova *et al.*, 2017).

Symptoms of toxicity are the first indication that can be seen directly when a toxic effect occurs after administering the test substance. **Table 4** shows the results of observations of symptoms of toxicity in the treatment group given aqua distillate as a negative control and the probiotic drink japelemo at doses of 5, 50, 300, and 2000 mg/KgBW from the start of treatment until 14 days of observation.

Table 4 Results of behavioural observations of test animals

Dosage		Symptoms of Toxicity for 14 days						
		Seizures	Trembling	Walking backwards	Running with stomach	Salivation	Grooming	Diarrhoea
Control	P1	-	-	-	-	-	-	-
	P2	-	-	-	-	-	-	-
Negative	P3	-	-	-	-	-	-	-
	P1	-	-	-	-	-	-	-
Dose 5	P2	-	-	-	-	-	-	-
	P3	-	-	-	-	-	-	-
mg/KgBB	P1	-	-	-	-	-	-	-
	P2	-	-	-	-	-	-	-
Dose 50	P3	-	-	-	-	-	-	-
	P1	-	-	-	-	-	-	-
mg/KgBB	P2	-	-	-	-	-	-	-
	P3	-	-	-	-	-	-	-
Dose 300	P1	-	-	-	-	-	-	-
	P2	-	-	-	-	-	-	-
mg/KgBB	P3	-	-	-	-	-	-	-

Table 4. The treatment group given the test ingredient probiotic juice at 5, 50, 300, and 2000 mg/KgBB showed normal symptoms. The symptoms of toxicity observed did not indicate any toxic symptoms due to the administration of probiotic juice. Behavioural testing aims to see whether there are symptoms of toxicity and the influence of changes in clinical symptoms in test animals after administering the probiotic drink Japelemo. Behavioural test observations include seizures, tremors, salivation, walking on the stomach, walking backwards, and grooming or loss of test feathers, which were observed for 14 days. These toxic symptoms are observed through visualization or with the naked eye and

tend to be subjective to see whether or not toxic symptoms are caused to find out the symptoms that are caused. They must be compared with the habits of the test animals during the acclimatization period or with the negative control group for each test group. External factors greatly influence changes in the behaviour of test animals, including temperature, humidity, lighting and everything about the cage where they live (Abdillah *et al.*, 2020). In this study, cages were placed in a particular room for test animals to avoid noise, which could trigger stress in the test animals. Indoor cages are supported by adequate ventilation and air circulation equipment to provide adequate air quality and oxygen supply (Mutiarahmi *et al.*, 2021). These results are similar to research by Mojikon *et al.* (2022), which revealed that mice given probiotics could reduce depression compared to mice not given probiotics. These results are also supported by McGinn's (2019) research on rhesus macaque monkeys with Self-injurious behaviour (SIB) given orange-flavoured chewable tablets containing the probiotic *Lactobacillus reuteri* can reduce biting behaviour and improve the monkey's sleep quality.

Conclusions

The administration of japelemo probiotic drinks at doses of 5, 50, 300, and 2000 mg/KgBB did not cause any toxicity symptoms that appeared after oral administration of japelemo probiotic drinks observed during 14 days of observation of test animals with the administration of test materials showed normal and healthy behaviour during observation.

References

- Abdillah, R., Permatasari, D., Badriyya, E., Rachmaini, F., & Lailaturrahmi. (2020). Penuntun Praktikum Farmakologi. *Universitas Andalas*, 2(1), 1–12.
- Adejumo, I., Fageyinbo, M., Obiekwe, P., Ibe-uba, F., & Agbaje, E. (2022). *Preliminary study : Pharmacotherapeutic effect of the aqueous stem cell extract of Swiss apple { Malus domestica (Borkh)} of the Uttwiler Sp " atlauber specie in rats.* 1–16.
- Aeni, Q., Aini, S. R., & Pratama, I. S. (2022). Kajian pustaka toksisitas tanaman nanas (*Ananas comosus* [L.] Merr). *Sasambo Journal of Pharmacy*, 3(1), 49– 62.
- Abdillah, R., Permatasari, D., Badriyya, E., Rachmaini, F., & Lailaturrahmi. (2020). Pharmacology Practicum Manual. Andalas University, 2(1), 1-12.
- McGinn, P. (2019). Does *Lactobacillus reuteri* Probiotic Treatment Improve Sleep Quality in Rhesus Macaques (*Macaca mulatta*) Displaying the Self Injurious Phenotype? March.
- Mirza, D. M. (2019). In Silico and In Vitro Study of Antineuroinflammatory Activity of 96% Ethanol Extract of *Marsilea crenata* C Presl Leaves. Thesis, State Islamic University Maulana Malik Ibrahim Malang, 1-134.
- Mojikon, F. D., Kasimin, M. E., Molujin, A. M., Gansau, J. A., & Jawan, R. (2022). Probiotication of Nutritious Fruit and Vegetable Juices: An Alternative to Dairy- Based Probiotic Functional Products.