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Research

Toxicity Test of Sentul Fruit (*Sandoricum koetjape*) Peel Extract on Mice

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ABSTRACT

Background: Sentul fruit peel extract has an antibacterial activity since it contains saponin, flavonoid, phenol, and tannin. Hence, it can be utilized in oral infection treatment. The safety level of herbal medicines needs to be studied by using a toxicity test. The study aims to discover the toxicity of Sentul fruit peel extract.
Subjects and Method: The study was conducted in the laboratory of the Faculty of Food Technology of Warmadewa University and the laboratory of the Faculty of Medicine and Health Sciences of Warmadewa University. The independent variables of the study were the various doses of sentul peel extract administered orally. The dependent variable was an acute toxicity test. The toxicity test in mice used an experimental study design in the laboratory that was observed qualitatively and quantitatively. The animals used were 25 male white mice (Mus musculus) and were divided into 5 groups. Data were collected based on the results of examinations from the laboratory and subsequently analyzed qualitatively and descriptively.
Results: Based on the results of the LD50 Toxicity Test on Mice, sentul peel extract samples did not cause toxic symptoms in the form of decreased heart activity, convulsions, decreased movement activity, and slow breathing. The administration of sentul peel extract was categorized as non-toxic because mice do not experience abnormal symptoms and there was no death after the administration of the extract using the oral gavage method at all doses for 14 days, thus sentul peel extract was safe to use as a medicinal ingredient.
Conclusion: Sentul peel extract is categorized as non-toxic because mice do not experience abnormal symptoms and no death occurs.

Keywords: sentul peel, extraction, oral infection, mice, toxicity

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BACKGROUND

Sentul fruit is edible and also usable in traditional herbal medicines. Its root can cure diarrhea, its leaves are able to reduce fever, and its wood powder can be used as an anthelmintic (Aria et al., 2013). Several researchers have proven the efficacy of sentul plant as a medicine for leukorrhea, such as (Warsinah et al., 2011) report that sentul bark methanol extract can inhibit the growth of the fungus *Candida albicans* by 39.65%. In addition, the ethyl acetate extract of the sentul leaves also has an antibacterial activity (Swantara and Ciawi,

2009). The results of the phytochemical screening examination of sentul fruit peel simplicial powder show the presence of alkaloids, flavonoids, tannins, saponins, glycosides, anthraquinone glycosides, and steroids (Silaban, 2009). Sentul fruit peel extract also contains several phytochemical compounds such as flavonoid, saponins, and tannins which are aromatic hydroxyl groups that are antibacterial (Wirata et al., 2021). Wirata et al., 2022 also report that sentul fruit peel extract (*Sandoricum koetjape*) has the ability to inhibit Streptococcus mutans bacteria and *Staphylococcus Aureus* bacteria with a diameter of the inhibitory zone was Mean= 14.31; SD= 1.06 mm and Mean= 15.34; SD=1.81 mm respectively.

Streptococcus mutans bacteria and Staphylococcus Aureus bacteria cause oral infections. Infectious diseases are diseases caused by microorganisms. A disease will occur if bacteria cause damage to both functional and structural (Ambarwati, 2007). Staphylococcus aureus is one of the microorganisms that reside in the oral cavity. This bacterium is a normal flora in the oral cavity that can cause disease if there are predisposing factors such as changes in the number of microorganisms either increasing or unbalanced and the decrease of the host's immune system (Syahrurachman, 2010). Infection by *Staphylococcus aureus* can give rise to diseases with characteristics of inflammation, necrosis, and abscess formation (Anastasia, 2010: 10). Thus, sentul peel extract with the ability to inhibit the growth of *Streptococcos mutans* bacteria and *Staphylococcus Aureus* bacteria has the potential as a cure for infections in the oral cavity.

Based on the agreement stipulated by WHO, a material/substance used for medicinal purposes for both humans and animals must go through the test stages, namely preclinical tests and clinical trials. Regulation of the Minister of Health of the Republic of Indonesia Number 760/menkes/per/ X/1992 states that drugs derived from plants must prove their efficacy and safety. The preclinical test is a test stage that aims to find out and determine the level of safety and validity of the efficacy of a test material/substance that is still under presumption, thus toxicity tests and activity tests are carried out scientifically (Meles, 2010).

The safety level of a drug can be studied by determining its toxicity. The toxicity of a substance is the ability of a substance to inflict damage on a living organism. All new substances, materials, and chemical preparations that will be used in humans, animals, and their environment need to be tested for safety when there is a possibility of being harmful to health (DiPasquale and Hayes, 2001). One of the toxicity tests that can be conducted is the acute toxicity test. Acute toxicity is those detrimental effects that arise immediately after the administration of a single dose of a substance or repeated dose within 24 hours. There are several methods of administration for acute toxicity testing, namely oral, parenteral, inhalational, skin, and eyes. An index to define acute toxicity is known as the Lethal Dose 50 (LD50). The most commonly used quantitative benchmark to express the range of toxic doses is the lethal dose (LD50). LD50 is defined as a single dose of a substance that is expected to kill 50% of experimental animals. Determination of LD50 values is the initial stage to determine the level of toxicity. Observations are made during the first 24 hours from the time the treatment is given until the 14th day. It aims to find out the changes in symptoms that occur after being treated. Observation criteria are made on toxic symptoms, weight changes, and the number of dead animals in each test group.

Currently, there are no reports on the level of safety in the use of sentul fruit peel, therefore it is important to test the toxicity of sentul fruit peel. This is because there is a substance of sentul fruit peel extract that is likely to be harmful to humans if consumed at unrecommended doses and in long-term use. The Thompson-Weil method using the LD50 calculation list is a method often used in determining the toxicity level of a compound. This method was chosen because it has a fairly high level of confidence, and accurate results, and does not require a large number of experimental animals. The importance of studying the degree of efficiency, safety, and various kinds of effects caused by the use of sentul fruit peel extract because it can provide information and as a reference to consider the use of clove plants as medicinal ingredients so that later it can improve its status as a standardized herbal medicine and so on. Specifically, there are no scientific journals that discuss the acute toxicity of sentul fruit peel extract.

SUBJECTS AND METHOD

1. Study Design

The study was conducted in the laboratory of the Faculty of Food Technology of Warmadewa University and the laboratory of the Faculty of Medicine and Health Sciences of Warmadewa University. The LD50 toxicity test of sentul fruit peel extract on mice was tested qualitatively and quantitatively using an experimental study design in the laboratory.

2. Population and Sample

The overall sample size used in this study was 25 mice. The 25 mice were divided into 5 test groups, each of which consisted of 5 mice. The control group was given a 0.5% Na-CMC suspension. Treatment group I was given a sample of 1000 mg/kg BW. Treatment group II was given a sample of 2000 mg/kg BW. The III treatment group was given a sample of 3000 mg/kg BW and the IV treatment group was given a sample of 4000 mg/kg BW. Data is collected based on the results of examinations from the laboratory and then analyzed qualitatively and descriptively.

3. Study Variables

The independent variable in this study was the various doses of *Sentul* peel extract given orally. The dependent variable was the acute toxicity test.

4. Operational definition of variables

Sentul fruit peel extract is the result of an extraction process with 96% ethanol maceration for 24 hours.

The acute toxicity test was the number of deaths of experimental animals and clinical symptoms within 24 hours duration. The controlled variable was 3 month-old male white mice (Mus musculus) weighing 25-35 g.

LD50 is a statistically derived unit, to express a single dose of a compound that can be lethal or cause significant toxic effects in 50% of experimental animals after treatment.

5. Study Instruments

The study instrument used in this study is an observation sheet.

6. Data analysis

Differences in anxiety levels were measured before and after treatment and then analyzed with a t-test on the SPSS program.

RESULTS

1. Sample Characteristics

Based on the sample characteristics in table 1, the type of mice used in the study were 25 white mice (Mus musculus), male, 3 months old, with a body weight of 25-35 g.

2. Toxicity Test

Based on the results of the LD50 Toxicity Test on Mice, sentul peel extract samples were categorized as non-toxic because mice did not experience abnormal symptoms

after the administration of the extract through oral gavage method. The result of the LD50 Toxicity Test on Mice was said to be abnormal if there are tremors, salivation, diarrhea, weakness, walking backward, or walking on the stomach. During a 14 days observation, no mice died, therefore LD50 could not be calculated. The results of the toxicity test of sentul peel extract on the mice with the oral gavage method can be seen in Table 2.

Table 1. Sample characteristics

Characteristics	Category	Frequency	Percentage
Type of Mouse	White mice (*Mus musculus*)	25	100%
Gender	Male	25	100%
Age	3 months	25	100%
Weight	25-35 g	25	100%

Table 2. Toxicity Test Results of Sentul Peel Extract on Mice

Day	Test Results				
	Negative Control	Dose I (1,000mg/kg BW)	Dose II (2,000mg/kg BW)	Dose III (3,000mg/kg BW)	Dose IV (4,000 mg/kg BW)
1	Normal	Normal	Normal	Normal	Normal
2	Normal	Normal	Normal	Normal	Normal
3	Normal	Normal	Normal	Normal	Normal
4	Normal	Normal	Normal	Normal	Normal
5	Normal	Normal	Normal	Normal	Normal
6	Normal	Normal	Normal	Normal	Normal
7	Normal	Normal	Normal	Normal	Normal
8	Normal	Normal	Normal	Normal	Normal
9	Normal	Normal	Normal	Normal	Normal
10	Normal	Normal	Normal	Normal	Normal
11	Normal	Normal	Normal	Normal	Normal
12	Normal	Normal	Normal	Normal	Normal
13	Normal	Normal	Normal	Normal	Normal
14	Normal	Normal	Normal	Normal	Normal

DISCUSSION

Sentul fruit peel extract has been proven to contain several phytochemical compounds such as flavonoids, saponins, and tannins which are aromatic hydroxyl groups that are antibacterial against S. aureus and *S. Mutans* bacteria (Wirata et al., 2021). Therefore, sentul fruit extract can be used as an herbal medicine. According to Marlinda et al., 2012, most of the active compounds found in medicinal plants are toxic when given in high doses. All poisonings occur as a result of reactions between toxic substances and receptors in the body (Katzung, 2002). Oral administration of medicinal plant extracts causes the active substances contained in the extracts to be absorbed in the digestive tract and then go through a distribution and metabolic process. A toxic metabolic product works as an enzyme inhibitor for the next stage of metabolism. The reaction between the active substance and the recep-

tors in the effector organ leads to the onset of symptoms of poisoning.

Any chemical substance or material intended as a medicine must be studied for its toxic properties before it is allowed to be widely used so that traditional medicine can be accepted among the community. Empirical efficacy should also be supported by scientific evidence of clinical benefits in the prevention or treatment of diseases and not cause serious side effects in the sense that it is safe for humans to use it as a medicine. Therefore, it is necessary to test the toxicity of sentul peel extract to see whether there are toxic effects on experimental animals (Gani, 1995). The toxicity test can be calculated from the LD50 value which is the Thompson and Weil method, with the observed parameters are all dead animals, both that died by themselves or died in a moribund state combined in number for the calculation of the LD50 value (BPOM,2014).

The experimental animals used were white male mice. The mice used were previously acclimatized for 10 days at room temperature so that the mice could adapt to their new environment. Every day the mouse cage was cleaned and the chaff was changed. The criteria for mice used were adult mice with an age range of 3 months and a weight of 25-35 grams.

The test results showed that sentul peel extract was not toxic because the experimental animals used did not experience symptoms such as tremors, salivation, diarrhea, weakness, walking backward, walking on the stomach, and even death. All experimental animals that obtained sentul peel extract at a dose of 1000 mg/kg BW, 2000 mg-/kg BW, 3000 mg/kg BW and 4000 mg/kg BW showed no abnormal symptoms within 14 days. The administration of sentul peel extract in mice was conducted orally through gastric gavage and was only given once on the first day. The test materials given were in accordance with the dosage of each group. Observation of toxic symptoms and dead mice was continued for up to 14 days. During the 14-day observation, the mice did not experience toxic symptoms at all doses of the extract administered. This shows that sentul peel extract does not contain toxic compounds. Compounds are said to be toxic when they cause abnormal symptoms to experimental animals and even cause the death of experimental animals. There are many mechanisms of toxic effects on the body, including interaction with enzyme systems, inhibition in oxygen transport due to hemoglobin disorders, interaction with immune cell function, disturbances in DNA and RNA synthesis, teratogenic action, direct chemical irritation of tissues, and toxicity in tissues (Nonci, et al. 2014). Chemicals that enter the body can cause toxic effects in 2 (two) ways, namely interacting directly (intracellularly toxic) and indirectly (extracellularly toxic). Intracellular toxicity is the toxicity that begins with the direct interaction of a chemical substance or metabolite with its receptors, while extracellular toxicity occurs indirectly by affecting the target cell environment however it can affect the target cells (Priyanto, 2010).

Based on the results of the LD50 Toxicity Test on Mice, *sentul* peel extract samples are categorized as non-toxic because mice do not experience abnormal symptoms and there are no deaths of experimental animals after the administration of the extract through oral gavage method at all doses for 14 days, therefore, sentul peel extract is safe to use as a medicinal ingredient.

AUTHOR CONTRIBUTIONS

I Nyoman Wirata contributed to making the study designs, conducting the study in laboratories, and making study reports. Anak Agung Gede Agung contributed to

collecting study materials and conducting the study in laboratories. Ni Wayan Arini contributed to data analysis and making study reports.

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CONFLICT OF INTEREST

There was no conflict of interest.

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