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Acute Oral Toxicity of Banggai Yam Flour Extract (*Dioscorea alata*) in Mice (*Mus musculus*)

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LITERATURE REVIEW

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ABSTRACT

Banggai yam (Dioscorea alata), a local tuber cultivated in Central Sulawesi, Indonesia, is known for its rich nutritional content, including complex carbohydrates, dietary fiber, essential vitamins, minerals, and bioactive compounds such as anthocyanins and polyphenols. Although its nutritional potential has been well documented, its consumption safety, especially in processed forms such as powdered extract, requires toxicological validation. This study aimed to evaluate the acute oral toxicity of Banggai yam powdered extract using a murine model. This study had three treatment groups that received Banggai yam flour extract at different doses (1.95 g, 3.906 g, and 5.9 g) and one control group that received 1% CMC Na. Observations and measurements were analyzed using independent sample t-tests through SPSS software. The analysis did not show statistically significant differences between the treatment and control groups, with p-values of 0.55, 0.97, and 0.85, respectively. These findings indicate that Banggai yam extract does not produce acute toxic effects at the dose levels tested. In conclusion, in animal models, Banggai yam flour extract is considered non-toxic under acute exposure conditions. Further studies involving subchronic and chronic toxicity tests and evaluation in human subjects are recommended to confirm its broader safety profile and support its use as a functional food ingredient.

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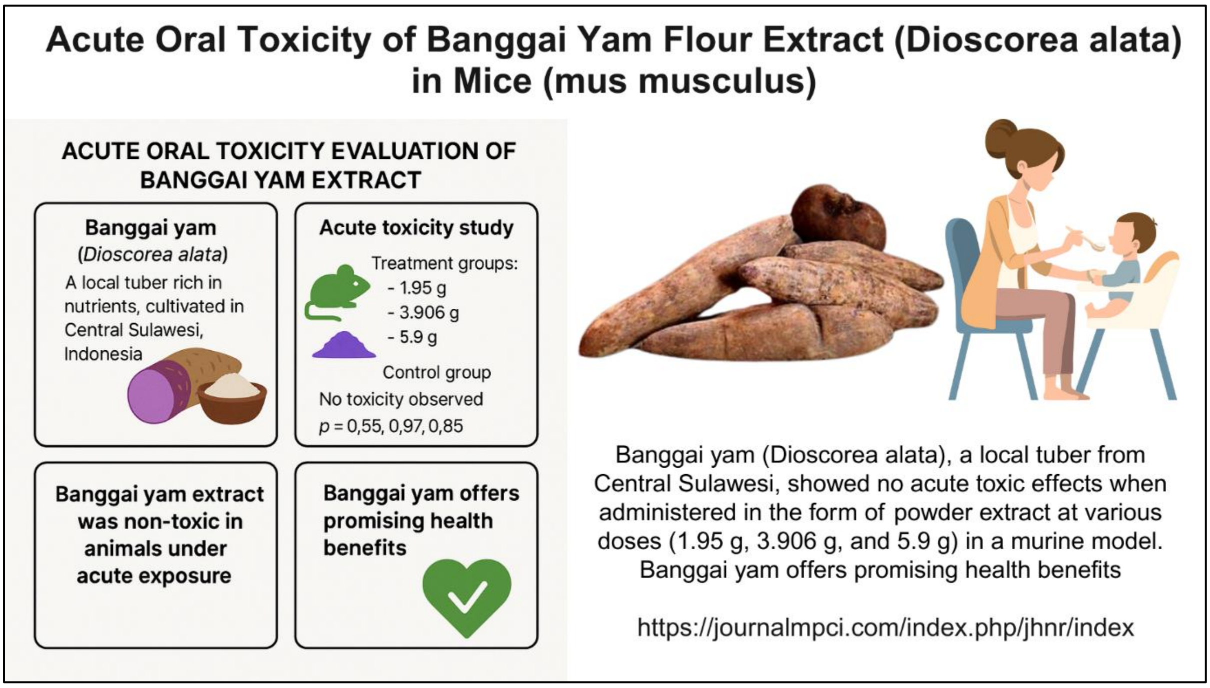
Key Messages:

- Banggai yam (*Dioscorea alata*), a local tuber from Central Sulawesi, showed no acute toxic effects when administered in the form of powder extract at various doses (1.95 g, 3.906 g, and 5.9 g) in a murine model. Banggai yam offers promising health benefits

GRAPHICAL ABSTRACT



INTRODUCTION

Complementary feeding is a critical stage in a child's growth and development, usually around six months. At this stage, breast milk alone is no longer sufficient to meet the nutritional needs of infants, and additional sources of nutrition are needed to support physical and cognitive development. However, in many low- and middle-income countries, complementary foods are often introduced too early, too late, or of poor nutritional quality, significantly contributing to the prevalence of malnutrition and stunting in young children(1) (2).

Banggai yam (Dioscorea Alata), a local variety of Banggai yam cultivated in Central Sulawesi, Indonesia, is a promising candidate for complementary food due to its rich nutritional profile(3). Sweet potatoes are a source of complex carbohydrates, dietary fiber, essential vitamins (such as vitamins A, C, and several B vitamins), and minerals, including potassium and iron. In addition, sweet potatoes contain bioactive compounds such as anthocyanins, carotenoids, and polyphenols, which have been linked to antioxidant, anti-inflammatory, and immune-boosting properties(4)(5). These compounds support its potential role in improving infants' and young children's nutritional status and health outcomes.

Despite their nutritional advantages, the safety of new or underutilized food ingredients must be scientifically validated before they can be recommended for widespread use, especially in vulnerable populations such as infants. Toxicity refers to the potential of a substance to cause adverse effects on a biological system. In food safety evaluation, assessing the risk of acute and chronic toxicity is important to prevent harmful consequences from contaminants or intrinsic antinutritional factors(6).

Although Banggai yam are generally considered safe, some varieties may contain anti-nutritional or toxic compounds such as alkaloids, saponins, and glycosides, which may interfere with nutrient absorption or pose toxicological risks if consumed in large amounts(7). Furthermore, contamination during cultivation, processing, or storage may also introduce potentially harmful substances. Therefore, comprehensive toxicity testing, including acute oral toxicity assessment, is essential to ensure that Banggai yam is safe for infant consumption.

Little literature discusses the toxicological safety of locally sourced foods used in complementary feeding in Indonesia, including Banggai yam. Previous studies have highlighted the importance of evaluating these foods' nutritional value and potential health risks to reduce allergic reactions, gastrointestinal disorders, or long-term toxic effects in infants. Toxicological assessment using animal

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models provides an important preclinical basis for understanding safety margins and informing appropriate dose levels for human consumption(6)(8). Some research on the effect of ethanol extract of Banggai tubers (Dioscorea alata L.) on pancreatic necrosis in rats has no toxic effects, so it is safe in subchronic tests. Other studies conducted on the effects of subchronic toxicity and effective doses have toxic effects of ethanol extract of purple Banggai tubers (Dioscorea alata L.) seen from the parameters of SGOT and SGPT levels in white rats (Rattus novergicus) showed that ethanol extract of Banggai tubers has no toxic effects (9).

Considering the need for evidence-based validation of local food ingredients(10). this study aimed to evaluate the acute oral toxicity of Banggai yam flour extract using a murine model for infant nutrition. These findings are expected to contribute to the scientific basis for the safe utilization of Banggai yam as a complementary food and support broader efforts to promote local food sources as a sustainable and health-enhancing alternative in addressing malnutrition in children in Indonesia.

METHODS

This study used a laboratory experimental design with an acute and sub chronic toxicity test approach in mice (Mus musculus) as an experimental animal model. The study was conducted at the Biopharmaceutical Laboratory, Faculty of Pharmacy, Hasanuddin University, Makassar. This type of research is purely experimental, using a Completely Randomized Research Design (CRD) by administering Banggai yam extract (Dioscorea alata L.) orally in graded doses to each experimental group. The population in this study consisted of mice (Mus musculus) in one area. The sample size was determined according to the provisions, with a minimum sample size of 10 for each treatment group. Sampling was based on the following inclusion and exclusion criteria. Inclusion Criteria: Mice, Female gender, Weight 20-50 grams, Age 2-3 months, Normal behavior and activities, no anatomical abnormalities are visible. Exclusion Criteria: The mice appeared sick (less active, and their eyes were unclear).

Material: Extracts to be given: 1.95g; 3.906g; 5.86g, Sodium carboxy methyl cellulose (Na.CMC) 1% Aquadest Permanent marker Parchment paper Hand skin Paper mask Aluminum foil. Equipment: Analytical balance (OHAUS) Hot plate stirrer (DRAGON LAB MS-H-PRO) Spatula 1 cc (One Med) Per oral cannula/sonde, porcelain container Spatula 25 mL, 50 mL, 500 mL Erlenmeyer flask, 100 mL, 250 mL, 500 mL.

Preparation of Na.CMC 1% (for 500mL)

Na. CMC weighs 5 g. Heat about 250 mL of distilled water to 80oC in an Erlenmeyer flask. Add Na.CMC is gradually heated in distilled water while stirring with a stirrer at 1000 rpm. Transfer the thoroughly mixed Na.CMC solution into a 500 mL measuring flask. Add distilled water to 500 mL, stir, and cool to room temperature. Na.CMC, which has been perfectly mixed, is used as an extract solvent and a control.

Making Banggai Yam Flour Extract Solution

Weigh 1.95 g, 3.906 g, 5.86 g of flour extract using weighing paper/parchment. Add a little Na.CMC to the cup containing the extract and stir gently with a spatula. Put the mixture of extract and Na.CMC into a 25mL measuring flask, then add Na.CMC until exactly 25ml. Do the same for the other doses with the same amount, namely 25ml.

Oral administration to experimental animals.

The experimental animals used were 40 mice (Mus musculus) grouped into 10 per group according to the dose. The experimental animals were adapted for approximately 1 week. Weigh each experimental animal (mouse) that has been adapted and marked using a permanent marker to avoid being easily lost/erased. Prepare the cannula/spoon, syringe, and extract for each dose. Correctly perform oral administration for each experimental animal (incorrect oral administration can result in death immediately after administration). A single oral administration was performed on the first day after adaptation and observed after 24 hours for acute toxicity for 7 days

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Data analysis

All data collected from the acute oral toxicity test, including the mean values and standard deviations of the measured parameters for each treatment and control group, were analyzed using the Statistical Package for the Social Sciences (SPSS). Descriptive statistics were first calculated to summarize each group's central tendency and variability. An independent sample t-test was performed to determine whether a statistically significant difference existed between the control group and each Banggai Yam treatment group.

RESEARCH ETHICS

This research has been recommended by the Ethics Commission of the Faculty of Public Health, Hasanuddin University. With the Ethics Approval Recommendation Number: 6210/Un4.14.1/Tp.01.02/2023.

RESULTS

Table 1 shows the performance of four groups, each with a sample size of 10. The three Banggai yam groups (at doses of 1.95, 3.906, and 5.9) had mean measurements ranging from 30.5 to 31.9, with standard deviations between 3.58 and 5.62, indicating relatively similar measurement values with moderate variability among subjects. In contrast, the 1% Na CMC control group had a mean measurement of 30.4 and a standard deviation of 5.56, comparable in measurement values but not in other outcomes.

Table 1. Comparison of Administration of Banggai Yam Flour Extract to the Control Group and Banggai Yam Flour Extract Group in Mice

Group	n	Means	SD	Death	Mortality rate
1.95 grams (Banggai yam)	10	31.9	5.4	1	10
3,906 grams (sweet potato)	10	30.5	5.62	0	0
5.9 grams (sweet potato)	10	30.8	3.58	0	0
1%Na CMC (control)	10	30.4	5.56	3	30

An important observation is the difference in mortality rates. The Banggai yam groups at doses 3.906 and 5.9 showed a mortality rate of 0%, while the group at dose 1.95 showed a relatively low mortality rate of 10%. However, the control group showed a much higher mortality rate of 30%. This suggests that Banggai yam treatment, regardless of dose, may have a protective effect against mortality compared to the control treatment.

Although the mean measurements across groups were quite similar, the marked differences in mortality rates imply that Banggai yam treatment may improve survival, especially when compared to the 1% Na CMC control group.

Table 2. Independent t-Test of Banggai Yam Flour Extract and Control in Mice

Comparison	Banggai yam Mean (SD)	Control Mean (SD)	t value	p-value
Sweet potato 1.95 gr vs control	31.9 (5.40)	30.4 (5.56)	0.612	0.55
Banggai yam 3,906 gr vs control	30.5 (5.62)	30.4.(5.56)	0.04	0.97
Sweet potato 5.9 gr vs control	30.8(3.58)	30.4 (5.56)	0.192	0.85

Table 2 showed no statistically significant difference between the Banggai Yam treatment groups and the control group (1% Na CMC). The mean measurements for the Banggai Yam group ranged from 30.5 to 31.9, while the control group had a mean of 30.4. The corresponding t-values for these comparisons were 0.612, 0.040, and 0.192, with p-values of approximately 0.55, 0.97, and 0.85, respectively. These p-values were significantly greater than the 0.05 significance level, indicating that any observed differences in means were likely due to chance rather than an actual effect of the treatment. In summary, the similarity in central tendency and variability between the treatment and control groups, along with the high p-value

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of the t-test, indicated that Banggai Yam treatment at doses of 1.95 g, 3.906 g, and 5.9 g did not produce statistically significant changes in the measured outcomes when compared to the control. Based on the available data, this implies that the Banggai Yam application did not significantly affect the studied parameters compared to the 1% Na CMC control.

DISCUSSION

Complementary feeding is an important component of infant nutrition, usually starting at around six months of age, as breast milk alone can no longer meet the growing nutritional needs of infants, according to the World Health Organization(11). Timely and adequate complementary feeding supports optimal growth, cognitive development, and immunity. However, complementary foods' nutritional quality and safety remains challenging, especially in low- and middle-income countries where locally available food sources may be under-researched or lack appropriate safety evaluation.

Banggai yam (Dioscorea Alata), a local variety of Banggai yam from Central Sulawesi, Indonesia, has attracted attention as a promising ingredient for complementary feeding due to its high content of complex carbohydrates, dietary fiber, and essential micronutrients, including vitamins A, C, and B-complex, as well as potassium and iron. In addition, Banggai yam contains various bioactive compounds, especially anthocyanins, carotenoids, and polyphenols, which are known for their antioxidant, anti-inflammatory, and immune-supporting properties(12)(13). These nutritional and functional benefits support its potential as a local food that promotes health and is suitable for infants and young children.

Despite their beneficial profile, introducing new or underutilized foods into infant diets requires rigorous safety assessment. Toxicological evaluation is important in identifying potential risks posed by antinutrients or natural contaminants introduced during cultivation, processing, or storage. Antinutritional factors such as alkaloids, saponins, oxalates, and glycosides have been found in various plant foods. They are known to affect nutrient absorption and, in some cases, to cause cytotoxic effects when consumed in excess(14). This compound may pose particular risks to vulnerable populations, such as infants with developing metabolic systems.

Several recent studies emphasize the importance of toxicological testing of traditional and local food products before they are included in complementary food formulations. Evaluated the phytochemical content and toxicological safety of Banggai purple sweet potato leaf extract administered orally to male Sprague-Dawley rats(15). This study found no adverse effects at doses up to 2,000 mg/kg/day, indicating a wide safety margin for human consumption. Similarly, the acute and subchronic oral toxicity of Banggai purple yam yogurt in rats. Their findings showed no signs of toxicity at doses up to 5 g/kg (acute) and 40 g/kg (subchronic), supporting the hypothesis that Ipomoea batatas is a safe food source, provided it is properly processed and administered in appropriate amounts (16).

The results of this study indicate that administration of Banggai yam (Dioscorea alata) flour extract at doses of 1.95 g, 3.906 g, and 5.9 g did not produce statistically significant changes in the measured biological parameters when compared to the control group receiving 1% Na CMC. The p-values obtained from the t-test (0.55, 0.97, and 0.85, respectively) were well above the standard threshold of 0.05, indicating that any observed variations were likely due to random chance rather than treatment effects. These findings support the conclusion that Banggai yam extract is not toxic under acute exposure conditions and can be considered safe within the dose range tested.

These results are consistent with previous studies on Dioscorea alata, which reported minimal toxic effects when consumed appropriately. In a study of administering Dioscorea alata extract at high doses, no deaths were found in mice. The idea that Dioscorea alata has a wide margin of safety(17). In addition, the traditional use of Banggai yam in local foods further demonstrates its general safety when properly prepared. However, it is important to note that antinutritional compounds such as oxalates and saponins may be present in unprocessed sweet potatoes, although these compounds are usually reduced through cooking and other preparation methods. Another study used an in silico approach to evaluate the toxicity of 64 bioactive compounds in Dioscorea alata. The results showed that most compounds had low toxicity, with diosgenin and catechins classified as non-toxic. However, several compounds showed hepatotoxic, immunotoxic, or mutagenic potential when used in isolation(18).

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Despite its positive safety profile in this acute toxicity setting, the current study has limitations. It did not evaluate the effects of long-term or repeated exposure, nor did it include biochemical or histopathological assessments that could provide more insight into subtle toxic effects. Therefore, future studies are needed to assess subchronic and chronic toxicity, especially if Banggai yam is to be developed as a food ingredient for routine consumption. Such studies will ensure a comprehensive safety evaluation and help establish appropriate dosage guidelines for various population groups, including children. In addition, the low toxicity of Banggai yam can be explained by its natural antioxidant components. Anthocyanins and carotenoids, abundant in Banggai yam, have been shown to protect cells from oxidative stress, improve liver function, and promote gut health, all of which contribute to the overall tolerability of the food(19). These compounds reduce the potential for toxicity and offer functional benefits when used in infant nutrition.

However, it is important to acknowledge the limitations of acute toxicity studies. While these studies offer valuable initial insights into the safety of new food ingredients, they are inadequate to evaluate long-term risks such as cumulative toxicity, organ damage, or delayed adverse effects. Chronic or subchronic studies over weeks or months are needed to assess the full spectrum of toxicological responses, especially in ingredients intended for repeated or long-term consumption, such as complementary foods. In addition, variations in processing techniques, such as drying temperature, particle size, or storage conditions, can affect the chemical composition and safety profile of Banggai yam flour. Previous studies have shown that thermal processing can reduce the levels of certain anti-nutrients in Banggai yam. However, they can reduce beneficial compounds such as vitamin C and anthocyanins if not optimized(12)(20). Therefore, standardization of processing methods is essential to ensure product consistency and safety. From a public health perspective, using Banggai yam in complementary foods offers a promising strategy to improve nutritional intake among Indonesian children, especially in areas with limited access to fortified or imported complementary foods. A local food-based approach also supports food sovereignty, reduces dependence on commercial products, and promotes sustainable use of native biodiversity. However, such interventions must be based on robust scientific evidence, including safety evaluations and nutritional efficacy trials.

Although this study provides valuable insights into the acute oral toxicity of Banggai Yam (Dioscorea alata) flour extract, several limitations must be acknowledged. First, the sample size used in the animal testing phase was relatively small. Although sufficient for initial toxicological screening, a larger group would have increased the statistical power and reliability of the results, potentially revealing subtle effects that may not have been apparent in the small-scale study. Second, this study only focused on acute toxicity with a single administration and a short observation window. As a result, this study did not take into account the potential for subchronic or chronic toxic effects that may arise from prolonged or repeated consumption of Banggai Yam. Long-term exposure studies are needed to evaluate the safety of continued use, especially considering the proposed application in complementary feeding, where infants may consume the product regularly for several months. Lastly, the current study did not assess the full range of physiological and biochemical parameters, such as liver and kidney function markers or histopathological evaluation, which are essential for a comprehensive toxicological profile. In addition, antinutritional compounds such as oxalate and saponin were not measured in this study. Future studies should include a wider range of toxicity markers, different doses, and processing variations to ensure the efficacy and safety of Banggai Yam in infant nutrition.

Although the acute oral toxicity profile of Banggai Yam appears favorable, further studies are strongly recommended to validate its safety for long-term use. Future studies should focus on sub chronic and chronic toxicity assessment, biochemical analysis, and histopathological evaluation to detect delayed or cumulative adverse effects. In addition, studies on nutrient bioavailability, anti-nutrient content, and processing methods are essential to maximize the safety and nutritional value of Banggai Yam. Finally, clinical trials in human populations, particularly infants and young children, will be needed before the product can be officially recommended as a complementary food.

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CONCLUSION

Based on the results of this study, it can be concluded that Sweet Potato (Dioscorea alata) flour extract given at doses of 1.95 g, 3.906 g, and 5.9 g did not cause statistically significant side effects when compared to the control group (1% Na CMC). The absence of significant differences in the measured results and the high p-value obtained from the t-test indicate that Sweet Potato flour extract is likely safe for oral consumption within the tested range. These results support the potential of Sweet Potato as a viable ingredient for complementary foods due to its rich nutritional profile and the absence of acute toxicity in rat models.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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