Amerta Nutrition
e-ISSN: 2580-1163 (Online)
p-ISSN: 2580-9776 (Print)
Baskara et al. | Amerta Nutrition Vol. 8 Issue 3SP (December 2024). 336-343

RESEARCH STUDY
English Version
OPEN ACCESS

The Effect of Food Bars Made from Katuk Leaf and Torbangun Leaf on the Toxicity Profile of White Rats

Pengaruh Food Bar *Berbasis Daun Katuk dan Daun Torbangun pada Profil Toksisitas Tikus Putih*

Elya Sugianti[1]*, Berliana Devianti Putri[2], Annas Buanasita[3]

Shelfia Chakarita Baskara[1], Nanang Nasrulloh[1]*, Avliya Quratul Marjan[1]

[1]Program Studi Ilmu Gizi Program Sarjana, Fakultas Ilmu Kesehatan, Universitas Pembangunan Nasional "Veteran" Jakarta, Kota Depok, Indonesia

ARTICLE INFO

Received: *12-09-2024*
Accepted: *31-12-2024*
Published online: *31-12-2024*

Correspondent:
Nanang Nasrulloh
nasrullah@upnvj.ac.id

DOI:
10.20473/amnt.v8i3SP.2024.336-343

Available online at:
https://e-journal.unair.ac.id/AMNT

Keywords:
Food bar, Male white wistar rat, Toxicity symptoms, Rat body weight

ABSTRACT

Background: Various herbal plants that grow in Indonesia, such as katuk leaves and torbangun leaves, are known to have many health benefits. These two plants contain important compounds such as vitamins, minerals and antioxidants which are beneficial for human health. Based on previous research, katuk leaves and torbangun leaves have been proven to increase breast milk production. However, studies regarding the benefits and potential toxicity of using these two herbal plants in experimental animals are still very limited. Therefore, this study was conducted to comprehensively evaluate and identify the toxicity profile observed in white rats after the administration of herbal supplements based on katuk leaves and torbangun leaves. This information is important to assess the safety of using these herbal preparations.
Objectives: This study aimed to determine the level of safety and identify symptoms of toxicity in food bars containing katuk and torbangun leaf flour in male Wistar white rats.
Methods: This study used a quantitative method with a true experimental design. The study samples consisted of 32 white mice divided into two groups. The rats were given food bars with katuk and torbangun leaf flour with doses ranging from 1.75 mg/kgBW to 17.5 mg/kgBW. Observations were made for 14 days regarding symptoms of toxicity, such as skin and fur changes, seizures, tremors, coma, and death. The statistical tests used were the normality test, homogeneity test, and hypothesis testing (ANOVA).
Results: The results of the study showed that administering a food bar with katuk and torbangun leaf flour did not induce symptoms of toxicity in white rats. There was no significant difference in the mean body weight of mice between the two groups (p-value=0.109; p-value>0.05).
Conclusions: Food bars with katuk and torbangun leaf flour did not have toxic properties and is safe to consume. Symptoms of toxicity were not evident in white mice given the food bar.

INTRODUCTION

Various herbal plants are found in Indonesia, such as katuk and torbangun leaves, which are recognized for their substantial potential benefits in the health sector. These plants have been traditionally used due to their proven nutritional properties that are beneficial to human health[1]. Katuk leaves, characterized by their dark green color and creeping or upright growth, are rich in vitamins, such as vitamin C, vitamin B, vitamin A, and vitamin K, as well as important minerals such as calcium, iron, potassium, phosphorus, and magnesium[2]. In addition, katuk leaves contain higher levels of phytosterols and flavonoids compared to other tropical plants that can be consumed[3]. On the other hand, torbangun leaves, characterized by their smooth, thick texture and aromatic scent reminiscent of oregano, have long been consumed daily as an additional food or traditional medicine. Overall, both katuk leaves and torbangun leaves offer nutritional and health benefits, especially because of their rich content of vitamins, minerals and bioactive compounds.

Previous study by Lutfiani and Nasrulloh on the consumption food bars with torbangun and katuk leaf flour demonstrated an increase in breast milk production[4]. Recognizing the health benefits of katuk and torbangun leaves, one of which is to increase breast milk production, and the lack of research on their benefits in experimental animals, this study was deemed necessary. This study aimed to identify toxic symptoms in white mice. In accordance with the regulations of the Indonesian Food and Drug Supervisory Agency (BPOM RI), to ensure the safety of substances intended for

How to cite: *Baskara, S. C., Nasrulloh, N., & Marjan, A. Q. (2024). The Effect of Food Bars Made from Katuk Leaf and Torbangun Leaf on the Toxicity Profile of White Rats: Pengaruh Food Bar Berbasis Daun Katuk dan Daun Torbangun pada Profil Toksisitas Tikus Putih. Amerta Nutrition, 8(3SP), 336–343.*

humans, it is necessary to carry out pre-clinical in vivo toxicity tests. These tests aim to evaluate the cumulative effects of doses that can cause potential toxic effects in humans, such as carcinogenic effects, that can impact health[5]. Accordingly, this study aimed to identify toxicity symptoms in a group of white mice after the administration of a food bar containing a combination of katuk and torbangun leaf flour. Conducting in vivo toxicity tests on experimental animals such as white mice is an important step in evaluating the safety of a material or product before it can be applied to humans.

METHODS

This study was carried out for approximately two months on white mice that were administered a food bar containing torbangun and katuk leaf flour. This study was conducted across multiple locations, with the food bar manufacturing and formulation stages carried out at the Food Technology Laboratory, Nutrition Science Bachelor's Program, Veteran National Development University of Jakarta, and the final stage, namely product effectiveness testing, carried out at the Animal Research Facility Unit Laboratory (International Conference on Health Innovation and Technology/ICOHIT), "Veteran" National Development University of Jakarta. This study employed a quantitative method using a true experimental design and a randomized post-test control group only design, where the intervention and control groups were compared after treatment was administered to the intervention group. The study population consisted of male white Wistar rats aged between two and three months with a body weight of 150-200 grams. The research sample consisted of 32 mice divided into two groups, determined using the Federer formula. Inclusion criteria included healthy male white mice aged between two and three months with a weight of 150-200 grams, while exclusion criteria included mice that were sick, dead, or had experienced a weight loss exceeding 10%. The manufacturing stage of the food bar involved mixing ingredients such as margarine, sugar, salt, full cream milk powder, eggs, water, wheat flour, torbangun flour, and katuk flour. The mixturewas then molded and baked. The following figure illustrates the formulation stages of the food bar.

Prepare ingredients for manufacturing the food bar, namely katuk and torbangun flour, milk powder, wheat flour, salt, sugar, egg, and butter

Mix sugar and eggs in a container, then add margarine

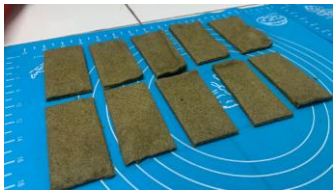
Then add wheat flour, milk powder, and salt gradually

Add katuk and torbangun leaf flour, then add water little by little and stir again until the mixture is even

Mold the food bar into a mold container

Place the dough in an electric oven at 130°C for 15 minutes

How to cite: *Baskara, S. C., Nasrulloh, N., & Marjan, A. Q. (2024). The Effect of Food Bars Made from Katuk Leaf and Torbangun Leaf on the Toxicity Profile of White Rats: Pengaruh Food Bar Berbasis Daun Katuk dan Daun Torbangun pada Profil Toksisitas Tikus Putih. Amerta Nutrition, 8(3SP), 336–343.*



Food bar swith katuk and torbangun leaf flour are ready to be served

Figure 1. Manufacturing steps of the food bar

This study used various tools and materials, including experimental animal cages, analytical scales, sound equipment, and rat food (Rat Bio)[7]. The experimental animals were given food bars containing katuk and torbangun leaf flour orally at doses ranging from 1.75 mg/kgBW to 17.5 mg/kgBW. In this study, observations focused on detecting toxicity signs in the treated white mice over a 14-day period by monitoring changes in the skin and fur condition, as well as the appearance of clinical symptoms such as seizures, tremors, coma, and death of the experimental animals. Comprehensive monitoring of these parameters is an important step in preclinical toxicity testing as such changes may indicate toxic effects associated with the doses of the test substance. The control group was only given regular food and water, while the intervention group was given additional food in the form of food bars with katuk and torbangun leaf flour. Observation results were compared between the control and intervention groups to determine the treatment effects. The parameters observed included changes in physical conditions, such as changes in fur and skin, as well as clinical symptoms, such as seizures, tremors, coma, and death of the experimental animals after the administration of food bars, which were observed for 24 hours throughout a 14-day intervention period[6]. This study received ethical approval from the KEPK of UPN "Veteran" Jakarta under a certificate number 81/III/2024/KEP on March 11, 2024.

Univariate Analysis

The results of univariate analysis were used to describe the distribution of data for the variables under investigation, including toxicity symptoms and the body weight of the experimental mice. Univariate analysis can provide basic descriptive information about a variable before conducting further analysis, such as bivariate analysis.

Bivariate Analysis

The results of bivariate test were used to examine the relationship between the two variables, namely the independent variable (food bar) and the dependent variable (toxicity symptoms). This study employed the Shapiro Wilk test. The following are the results of bivariate test for the variables under study.

Normality Test

Toxicity Symptoms

This study involved a total of 32 white mice as samples. Considering that the number of samples did not exceed 50, the Shapiro-Wilk test was selected for the normality test. The hypothesis states that if the p-value is more than 0.05, the data are normally distributed, but if the p-value is less than 0.05, the data are not normally distributed. Based on the results of the normality test, a p-value of less than 0.01 was obtained for both groups of mice with toxicity symptoms (0.000) and those without toxicity symptoms (0.000). A significance level below 0.05 indicates that the data are not normally distributed. Therefore, the next step was to carry out a non-parametric analysis using the Wilcoxon test to evaluate the differences between the groups.

Descriptive Analysis of Rat Body Weight

The analyses involved several stages of analysis, including normality tests, univariate tests, and bivariate tests. The process began with a normality test to determine whether the data were normally distributed or not. If the data were normally distributed, the process continued with the homogeneity test. Normally distributed data have a symmetrical curve, where most of the data are clustered and evenly distributed on both sides of the curve, not exceeding the average. The homogeneity test is a statistical procedure designed to assess whether two or more sample data sets originate from populations with the same variance. The aim of the homogeneity test is to evaluate the uniformity of the data. If the data were identified as homogeneous, the next step involved conducting an analysis of variance (ANOVA) test. The ANOVA test is used to evaluate the effect of the treatment or intervention given to the test sample. The following is a further explanation of the stages:

Homogeneity Test (Levene)

Levene's test is commonly used to assess the homogeneity of variance because it is more robust against deviations from normal distribution than other homogeneity tests. Conducting a homogeneity test ensures that the key assumptions underlying statistical analysis are met, thereby validating the analysis results and allowing for accurate interpretation. In this study, the Levene's homogeneity test results for body weight measurements from day 1 to day 14 indicated a significance value of more than 0.05. This finding confirmed that the data were homogeneous (equal), allowing for further parametric statistical methods, namely the ANOVA.

Hypothesis Testing (ANOVA)

ANOVA is a statistical method used to test mean differences between groups. This method is also known by various other terms, such as analysis of variance,

How to cite: *Baskara, S. C., Nasrulloh, N., & Marjan, A. Q. (2024). The Effect of Food Bars Made from Katuk Leaf and Torbangun Leaf on the Toxicity Profile of White Rats: Pengaruh Food Bar Berbasis Daun Katuk dan Daun Torbangun pada Profil Toksisitas Tikus Putih. Amerta Nutrition, 8(3SP), 336–343.*

variance analysis, analysis of variation, or the F test, which is commonly employed for drawing conclusions. ANOVA is closely related to regression analysis, contributing to its widespread application in various fields, from laboratory experiments to social research[14]. In this study, the calculated F count was less than the F table. As a result, H0 was accepted, indicating that the differences in rat body weight were not significant. Additionally, the ANOVA test yielded a p-value of 0.109 (p-value≥0.05), indicating no significant differences in rat body weight between the control and treatment groups. Rat body weight is one of the parameters measured in toxicity evaluation as changes in body weight can provide supporting data for analyzing the toxic effects of an intervention. According to Herlina's theory, changes in body weight, either decreases or increases, of less than 10% both in the control and treatment groups suggested that the intervention had no significant impact on the body of the animals. Therefore, data on changes in rat body weight can be used as an additional indicator to support the results of toxicity analysis, where body weight fluctuations that do not exceed 10% indicate that the intervention given does not have a significant toxic effect on the animals[15].

RESULTS AND DISCUSSIONS

This study builds upon previous research which focused on the characteristics of food bars containing katuk and torbangun leaves, with this research focusing on interventions, especially on experimental animals[8]. The earlier findings, which identified a food bar composition that can increase breast milk production, had not been supported by clinical food safety tests on experimental animals. The initial step was carried out by determining the test animal to be used, namely the Wistar strain male white rats which have physical characteristics similar to humans, with 98% of human genes being homologous to rat genes. The test animals were acclimatized for seven days during which they given food in the form of Bio Rat pellets and other care such as replacing the husks and washing the cages. The test materials consisted of food bars made from katuk and torbangun leaf flour which was given in the form of liquid food or sonde to ensure that the dose received met the requirements. The dose given adhered to BPOM standards with daily observations carried out individually for 14 days. Toxicity symptoms observed included physical condition, behavior, central nervous system, respiratory system, and death. Body weight of the test animals was also recorded as an important parameter for assessing signs of toxicity. After 14 days of the observation period, the collected data were analyzed according to the initial objectives. Additionally, this study adhered to ethical guidelines for handling and maintaining the health of test mice. After treatment, the test mice were euthanized according to procedures designed to minimize pain and stress.

Before conducting the experiment, the test animals were acclimatized for seven days to familiarize them with the research environment and conditions. During this stage, the test animals were given standard feed in the form of pellets (Rat Bio) containing 60% carbohydrates, 20% protein, 4% fat, 4% crude fiber, 12% calcium, and 0.7% phosphorus. In addition, the test animals received other necessary care during the adaptation period. This acclimatization process is important to ensure that the test animals are in an appropriate condition and ready to respond to the treatment that will be given during the research. The treatment in question consisted of replacing the husks once every three days and washing the cages once every week. To keep white mice active and reduce stress levels, a pipe was placed in the drum as a toy for mice to encourage mouse movement. The test materials used in this study was food bars made from were katuk and torbangun leaf flour. To ensure ease of consumption, the food bars were provided in the form of liquid food or sonde. This approach was adopted to address potential issues with the test animals not fully consuming the solid food bars. Prior to administration the food bars were to ensure that they met the safety standards for ingestion by the test animals.

In this study, the test mice were observed individually for at least the first 30 minutes after administering the test preparation. Furthermore, observations were carried out every four hours for the first 24 hours. Subsequently, observations were carried out once a day for 14 days. To differentiate each sample, special markings, namely Roman numerals, were applied to the tails of the test mice with different colored markers to distinguish the control group from the treatment group. This method was crucial to prevent confusion during the monitoring process. Signs of toxicity symptoms observed included physical condition, behavior, central nervous system, respiratory system, and death. The observed symptoms were systematically recorded in individual notes for each test mouse. These records indicated any toxic effects from the food bars being tested on the animals.

Monitoring the body weight of the test animals served as an indirect parameter for evaluating the effects of the test preparations and ensuring the health of the test animals during the experiment. This is an important part of standard procedures in toxicity testing. After 14 days of the experiment and the research data were collected, the next stage involved processing the data in accordance with the initial research objectives. This study adhered to ethical guidelines for handling and maintaining the health of the test mice. After treatment, the test mice were euthanized according to procedures designed to minimize pain and stress. Euthanasia was performed to eliminate the animals' lives through methods that reduced consciousness[16]. One method of euthanasia in test animals is by dislocating the neck, namely severing the spinal cord by pressing the mouse's head with the thumb and forefinger while simultaneously pulling the tail with a sudden and strong motion[17]. This approach is considered to be one of the most effective ways to minimize pain experienced by experimental animals. For this reason, the test mice in this study were euthanized following the established protocols, ensuring that they did not experience pain and distress during the process.

Toxicity Symptoms

How to cite: *Baskara, S. C., Nasrulloh, N., & Marjan, A. Q. (2024). The Effect of Food Bars Made from Katuk Leaf and Torbangun Leaf on the Toxicity Profile of White Rats: Pengaruh Food Bar Berbasis Daun Katuk dan Daun Torbangun pada Profil Toksisitas Tikus Putih. Amerta Nutrition, 8(3SP), 336–343.*

The observations conducted in this study aimed to identify signs of toxicity to assess the toxic effects in the test animal groups. Toxic symptoms serve as early indicators of toxicity or poisoning. According to Kurniawidjaja, toxicity refers to the intrinsic ability of a toxicant to produce harmful effects on an organism[9]. These symptoms may appear gradually in test animals. A summary of the recorded observations is presented in Table 1.

Table 1. Observation of toxicity symptoms

Observation	30 M	240 M	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hyperactivation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Description:
30m-240m: 30 minutes to 240 minutes
D2-D14: day 2 to 14
(-): not observed

Table 1 outlines the observed signs of toxicity including piloerection (changes in the mice's fur), convulsions (seizures), tremors (shaking), hyperactivity (excessive reactions), and mortality (death). The results shown in Table 1 indicated no signs of toxicity symptoms in all test animals following the administration of food bars with katuk and torbangun leaf flour. Toxicity symptoms were observed directly for 30 minutes after the administration of the test material. Subsequently, the observations were continued for 14 days. An overview of the distribution of the presence or absence of toxicity symptoms before and after treatment is provided in Table 2.

Table 2. Description of toxicity symptoms before and after treatment

Signs of Toxicity Symptoms After Treatment	Research Group		Total
	Control (n) (%)	Treatment (n) (%)	
No symptoms	16 (100%)	16 (100%)	32 (100%)
Symptomatic	0 (0.00%)	0 (0.00%)	0 (0.00%)
Total	16 (100%)	16 (100%)	32 (100%)

Table 2 shows the distribution of signs of toxicity symptoms. Table 2 indicated no toxicity symptoms in both the control group and the intervention group, with a total of 16 mice per group, resulting in a 100% of toxicity symptoms. In addition, none of the test animals exhibited changes in behavior. Therefore, observations carried out for 14 days did not reveal any toxicity symptoms in all of the test animals.

Rat Body Weight After Treatment

Changes in body weight are indicators that can be observed easily and are an early sign of the toxic effects from the test preparations given[6]. Body weight of mice in this study were recorded over a period of 14 days during the intervention period. The results showed that 24 test mice (75%) experienced an increase in body weight, while eight (12.5%) did not experience an increase. The majority of those who gained weight were in the group that was given treatment. Body weight measurements were taken from the first day to the final day of the intervention. The test mice were given standard food (Rat Bio) which contained 60% carbohydrates, 20% protein, 4% fat, 4% crude fiber, 12% calcium, and 0.7% phosphorus. The increase in body weight experienced by the test mice is consistent with Adnyana's study which showed that a high-carbohydrate and high-fat diet can induce visceral fat accumulation, which is accompanied by visible side effects, namely an increase in body weight in test animals[10]. This is proven by the composition of the rat feed given, namely 60% carbohydrates added to the test preparation. Therefore, the increase in rat body weight was not completely attributed to the composition of the test preparation, namely food bars with torbangun and katuk leaf flour.

Rat Body Weight

According to Ghozali, the normality test is a statistical test used to determine whether the data obtained from a study or experiment is normally distributed or not[11]. The normality test is carried out to test whether the standardized residual values in the regression model are normally distributed. Conducting a normality test is important before proceeding with subsequent tests as the assumption of normality is one of the prerequisites that must be met in several parametric statistical methods. Therefore, the normality test is an important initial stage in the data analysis process, to ensure that the data to be analyzed further meet the assumption of normality, which influences the selection of the appropriate analytical method to draw valid

How to cite: *Baskara, S. C., Nasrulloh, N., & Marjan, A. Q. (2024). The Effect of Food Bars Made from Katuk Leaf and Torbangun Leaf on the Toxicity Profile of White Rats: Pengaruh Food Bar Berbasis Daun Katuk dan Daun Torbangun pada Profil Toksisitas Tikus Putih. Amerta Nutrition, 8(3SP), 336–343.*

conclusions from the results of research or experiments. This test is part of the prerequisite tests for carrying out parametric statistical analysis such as analysis of variance (ANOVA) and linear regression. With the normality test, the risk bias in drawing conclusions from the results of data analysis can be avoided if the assumption of normality is not met. In this study, the results of the Shapiro-Wilk normality test indicated a significance value for body weight from day 1 to day 14 of more than 0.05, suggesting that the data were normally distributed. If the two groups of data obtained meet the assumption of normality, the analysis can be continued with the homogeneity of variance test. The results of the variance homogeneity test will determine the appropriate type of statistical test to use. If the variances are homogeneous, the ANOVA can be used. However, if the variance is not homogeneous, continuing with a non-parametric test such as Kruskal-Wallis or Mann-Whitney may be more appropriate.

Toxicity Symptoms

The relationship between the two variables was assessed using the Wilcoxon signed-rank test. This test was carried out on two paired samples to evaluate whether the administration of the food bar had any effect on signs of toxicity. Based on the Wilcoxon test results, the p-value was 1.000. Since the p-value is greater than 0.05, it can be concluded that H0 is accepted, indicating that the administration of food bars with katuk and turbangun leaf flour had no effect on signs of toxicity.

In research involving test animals such as white mice, one important aspect that needs to be considered is the evaluation of potential toxicity or adverse side effects[12]. However, a more in-depth analysis needs to be carried out to ensure the safety and effectiveness of a compound or treatment under study. In toxicology research, several parameters are measured, such as piloerection (changes in the rat's fur), convulsions (seizures), tremors (shaking), hyperactivity (excessive reactions), and mortality (death). BPOM stated that the absence of significant changes in these parameters compared to the control group, serves as an initial indication that the compound or treatment being tested does not cause real toxic effects[13]. A 14-day observation period is considered sufficient to detect acute toxic effects (within a few days) and sub-chronic toxic effects (within a few weeks) of a compound or test substance. Individual observations of test animals were carried out within the first 30 minutes after the administration of the test preparation, and every four hours periodically during the first 24 hours and once a day for 14 days.

Average Increase in Body Weight of Rats

The results of body weight measurements of the mice aimed to assess the conditions of this study and to determine whether there were any differences or not in the effect of the administration of food bars on the mice's body weight. The measurements were carried out every day for a period of 14 days after the test material was administered. The results of body weight measurements on the mice administered with food bar are presented in Figure 2.

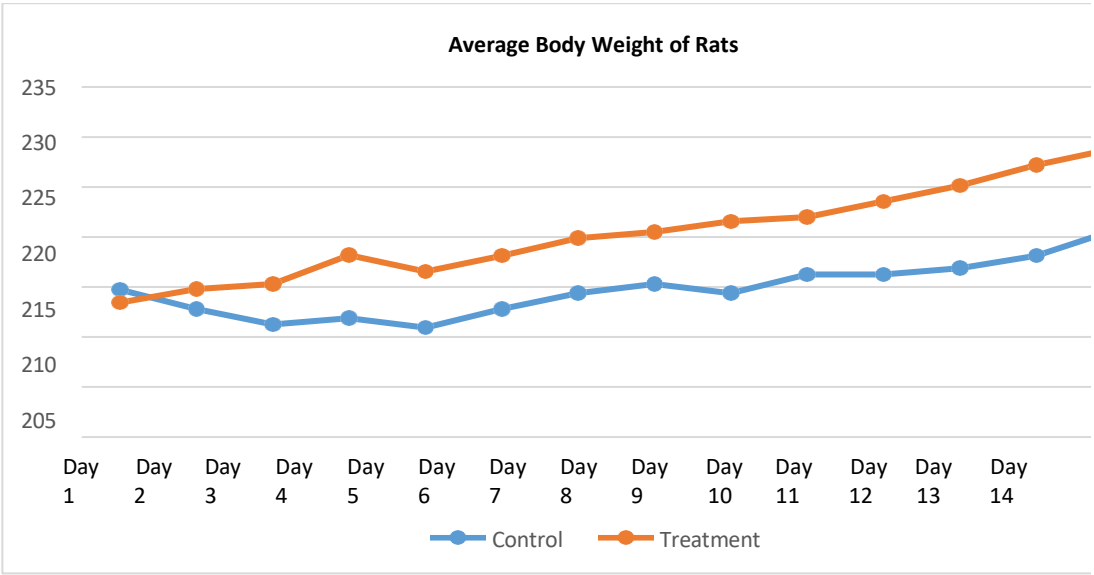


Figure 2. Mean weight of rats in control and treatment groups

The mean body weight of the mice based on Figure 2 indicated that the treatment group had the lowest body weight on day 1, namely 213.44 grams, and the highest body weight on day 14, namely 228.81 grams. Meanwhile, the control group had the lowest body weight on day 5, namely 210.94 grams, and the highest body weight on day 14, namely 220.63 grams. These findings suggested an increase in the average body weight of both the control group and the treatment group. The increase in body weight is consistent with Adnyana's study which found that a high-carbohydrate and high-fat diet can induce visceral fat accumulation, accompanied by a directly visible side effect, namely an increase in body weight that occurs in test animals[10]. In

How to cite: *Baskara, S. C., Nasrulloh, N., & Marjan, A. Q. (2024). The Effect of Food Bars Made from Katuk Leaf and Torbangun Leaf on the Toxicity Profile of White Rats: Pengaruh Food Bar Berbasis Daun Katuk dan Daun Torbangun pada Profil Toksisitas Tikus Putih. Amerta Nutrition, 8(3SP), 336–343.*

this study, the test mice did not only consume the test preparation but also standard food which tends to be high in carbohydrates. Regarding food consumption factors in the test mice, it appears that a lack of physical activity, such as reduced movement and minimal activity, likely contributed to the observed weight gain, as this would result in fewer calories being burned. Overall, although the results of the data analysis showed no toxicity symptoms in mice, which is a positive outcome, it only represents one aspect of the broader safety evaluation process. To guarantee the food safety of a product, further research is needed, including chronic toxicology studies, studies on other species, and clinical trials on humans before it can be widely used and certified safe by the Food and Drug Supervisory Agency (BPOM).

The advantages of this study include the finding that administering a food bar made from katuk and torbangun leaf four did not cause signs of toxicity symptoms in male newborn white rats. No toxicity symptoms including piloerection (changes in the mouse's fur), convulsions (seizures), tremors (shaking), hyperactivity (excessive reactions), and mortality (death) were observed, and body weight in the mice administered with food bars did not differ significantly from the non-administered control group. Meanwhile, the research limitations include that the results obtained in mice cannot be extrapolated directly to humans, and further product safety tests are required to obtain food safety certification from BPOM. Furthermore, standard feeding in this study did not use standard calculations due to a lack of environmental control at the time of the research. Finally, clinical trials or further studies on humans are needed to ensure the safety and effectiveness of the food bar.

CONCLUSIONS

The administration of a food bar with katuk and torbangun leaf flour did not cause any signs of toxicity to male Wistar white rats. Symptoms of toxicity such as piloerection (changes in the mouse's fur), convulsions (seizures), tremors (shaking), hyperactivity (excessive reactions), and mortality (death) were not observed. Other supporting parameters related to symptoms of toxicity, such as changes in body weight in mice administered with the food bars, were not significantly different from the control group that were not given these food bars. The average body weight of the test mice showed an increase in both the control and treatment groups. However, this increase was not enough to prove the effect of the administration of katuk leaf and torbangun leaf food bars because the mice also consumed standard feed which tended to be high in carbohydrates. In other words, this study concluded that food bars made from katuk and torbangun leaf flour do not have toxic properties and are safe to consume.

ACKNOWLEDGEMENT

The authors would like to thank the Animal Research Laboratory at UPN Veteran Jakarta, and all parties who have contributed to this study.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

This study was privately funded. The authors declare no conflict of interest regarding this study.

AUTHOR CONTRIBUTIONS

SCB: writing-original draft, methodology, conceptualization, writing-review and editing; NN: methodology, writing-original draft, supervision, formal analysis, resources, and editing; AQM: methodology, formal analysis, supervision, and resources.

REFERENCES

1. Oktiningrum, M., Harjanti, A. I., Nurhidayah, A., Dewi, I. & Ayu, L. Literatur Review : Pemanfaatan Bahan Alam Guna Memperlancar ASI pada Ibu Menyusui. *Pros. Semin. Nas. dan Call Pap. Kebidanan Univ. Ngudi Waluyo* **2**, 138–146 (2023). p-ISSN: 2961-7340 dan e-ISSN: 2962-2913.
2. Pusat Kajian Hortikultura Tropika (Lembaga Penelitian dan Pengabdian kepada Masyarakat). KATUK (Sauropus androgynus (L.) Merr.). *IPB* https://pkht.ipb.ac.id/index.php/2018/05/17/katuk- sauropus-androgynus-l-merr/ (2018).
3. Subekti, S., Piliang, W., Manalu, W. & Jitv, T. M. Penggunaan tepung daun katuk dan ekstrak daun katuk (Sauropus androgynus L. Merr) sebagai substitusi ransum yang dapat menghasilkan produk puyuh. *Oaji.Net* 254–259 (2006).
4. Lutfiani, L. & Nasrulloh, N. Total Flavonoid and Antioxidant Activity of Food Bar Torbangun – Katuk on The Effectiveness of Breast Milk Production. *Amerta Nutr.* **7**, 88–97 (2023). https://doi.org/10.20473/amnt.v7i1.2023.88-97.
5. BPOM RI. Uji Toksisitas Praklinik secara In Vivo. *Berita Negara Republik Indonesia* (2022).
6. Lukman, M. & Christin, V. Analisis Profil Bobot Badan Tikus dan Gejala Toksis Pada Pemberian Ekstrak Etanol Daun Parang Romang (Boehmeria virgata) Terhadap Tikus Putih (Rattus novergicus). *J. Farm. Galen. (Galenika J. Pharmacy)* **6**, 1–6 (2020). https://doi.org/10.22487/j24428744.2020.v6.i1.13928.
7. Saputra, A., Junaidi, Supriyanto, A. & Surtono, A. Desain dan Realisasi Alat Ukur Massa (Neraca Digital) Menggunakan Sensor Load Cell Berbasis Arduino. *J. Teor. dan Apl. Fis.* **10**, (2023).
8. Luthfiah, L., Setyati, D. & Arimurti, S. Antibacterial Activity of Liverworts of Dumortiera hirsute (Sw.) Nees Ethyl Acetate Extract Against Pathogenic Bacteria. *Berk. Sainstek* **9**, 75 (2021). ISSN: 2339-0669 https://doi.org/10.19184/bst.v9i2.22645.
9. Kurniawidjaja, L. M. *Konsep Dasar Toksikologi Industri.* (FKM UI, Jakarta, 2021). https://doi.org/10.21109/kesmas.v1i6.284.
10. Adnyana, I. K., Sukandar, E. Y., Yuniarto, A. & Finna, S. Anti-obesity effect of the pomegranate leaves ethanol extract (Punicagranatuml.) in high-fat diet induced mice. *Int. J. Pharm. Pharm. Sci.* **6**, 626–631 (2014). https://doi.org/10.2991/smichs-17.2017.43.
11. Ghozali, I. *Aplikasi Analisis Multivariate Dengan Program SPSS 23.* (Undip Press, Semarang, 2018).

How to cite: *Baskara, S. C., Nasrulloh, N., & Marjan, A. Q. (2024). The Effect of Food Bars Made from Katuk Leaf and Torbangun Leaf on the Toxicity Profile of White Rats: Pengaruh Food Bar Berbasis Daun Katuk dan Daun Torbangun pada Profil Toksisitas Tikus Putih. Amerta Nutrition, 8(3SP), 336–343.*

12. Riana, E., Ischak, N., Ayudia, E., Khairani, I., Prabandari, A., Miftahurrahmah, M. & Mulyana, J. I. *Toksikologi Dasar*. *Angewandte Chemie International Edition.* **6,** 951–952. (1967). https://doi.org/10.1051/bioconf/202410102005
13. BPOM. Panduan penyusunan protokol uji praklinik uji toksisitas akut. 1–3 (2022).
14. Meimaharani, R. & Listyorini, T. Analisis Varian (Anova) Untuk Mengetahui Statistik Tingkat Kemajuan Prestasi Karate di Kabupaten Kudus. *Pros. Semin. Nas. Ilmu Komput.* (2013). https://doi.org/10.24176/simet.v5i1.127.
15. Herlina & Wijaya, D. P. Accute Toxicity Of Extract From Melinjo (Gnetum Gnemon L) Leaf With Fixed Dose Procedure Method. *Indones. J. Pharm. Sci. Technol.* **9**, 140 (2022). https://doi.org/10.24198/ijpst.v9i3.33683.
16. N.G. Kostomitsopoulos & S.F. Durašević. *The ethical justification for the use of animals in biomedical research*, *Archives of Biological Sciences*, 62(3), pp. 781–787. (2010). https://doi.org/10.2298/ABS1003781K.
17. Setiawan, H., Maliza, R., and Wulandari, S.W. Petunjuk Praktikum Fisiologi Hewan Eksperimental (2021). http://eprints.uad.ac.id/id/eprint/36604.

How to cite: *Baskara, S. C., Nasrulloh, N., & Marjan, A. Q. (2024). The Effect of Food Bars Made from Katuk Leaf and Torbangun Leaf on the Toxicity Profile of White Rats: Pengaruh Food Bar Berbasis Daun Katuk dan Daun Torbangun pada Profil Toksisitas Tikus Putih. Amerta Nutrition, 8(3SP), 336–343.*