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The Effect of Tambora Coffee Administration on The Reduction of Uric Acid Levels In Vivo

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ABSTRACT

Coffee is one of the alternative beverages that is highly favored by the Indonesian population. The beneficial effects of coffee include the reduction of blood uric acid levels. This is due to the presence of polyphenol compounds in coffee, which can inhibit the activity of the xanthine oxidase enzyme, thereby lowering uric acid levels. This study aims to determine the effect of Tambora coffee administration on the reduction of uric acid levels in vivo. This research used a quasi-experimental design involving 25 male mice weighing 25–30 grams. The subjects were divided into five groups: three treatment groups with different doses of Tambora coffee (P1: 0.5 mg/kgBW, P2: 0.25 mg/kgBW, P3: 0.75 mg/kgBW), one positive control group receiving allopurinol at 0.45 mg/kgBW, and one negative control group receiving standard feed. The normality test showed that the data were normally distributed, and the homogeneity test confirmed that the data were homogeneous. Therefore, the data were analyzed using the One-Way ANOVA method. The ANOVA test showed a significance value of 0.000, which is lower than 0.05. It can be concluded that Tambora coffee at various doses is capable of reducing blood uric acid levels, although the levels remained within the normal range.

KEYWORDS

Tambora coffee, decrease, acid urat, Mencit

INTRODUCTION

Coffee is a beverage that is highly favored by people in Indonesia and other countries worldwide. Numerous studies have investigated the effects of coffee consumption on various medical conditions. The habit of drinking coffee in Indonesian society has been passed down through generations. According to the general public, this habit is practiced to eliminate drowsiness (Ansel, Howard C. 1989) Several positive effects include reducing the risk of Alzheimer's disease, Parkinson's disease, type 2 diabetes mellitus, hepatic cirrhosis, and lowering blood uric acid levels, which is attributed to the presence of chlorogenic acid, a type of polyphenol, in coffee (Dewani, 2017).

Indonesian people consume coffee or carry out the activity of drinking coffee as much as 3-5 cups of coffee per day typically in the morning, at noon during lunch breaks, after dinner, and at midnight (staying awake) due to overtime work or other reasons. Furthermore, coffee is readily available throughout Indonesia, especially in regions that are coffee-producing areas. West Nusa Tenggara is one of Indonesia's coffee producers, with Tambora Village in Dompu Regency being one such area. The people of West Nusa Tenggara province have a habit of drinking 1-5 cups of coffee per day, and some even consume more than 6 cups per day (Soenarto, 2017).
Tambora Village is located on Pekat Road in Dompu Regency, known as a tourist destination and also a production area for agricultural products and plants, one of which is coffee. Coffee is a plant with high economic value. Tambora Coffee is one of Dompu's local products and is increasingly identified with coffee lovers. For the people of Dompu, drinking coffee has certainly become a hereditary tradition. With the development of coffee shops in Dompu, Tambora coffee continues to receive attention to be cared for from the plantation until it can be enjoyed by local and out-of-town coffee lovers. (Syahril M, 2021).
Tambora Village is located on Pekat road in Dompu Regency, known as a tourist destination and also a production area for agricultural products and plants, one of which is coffee. Coffee is a plant with high economic value. Tambora Coffee is one of Dompu's local products and is

increasingly identified with coffee lovers. For the people of Dompu, drinking coffee has certainly become a hereditary tradition. With the development of coffee shops in Dompu, Tambora coffee continues to receive attention, from cultivation in the plantation to being enjoyed by local and out-of-town coffee enthusiasts (Syahril M, 2021).

According to the Health Office of West Nusa Tenggara Province (2018), the morbidity rate due to uric acid in the community of West Nusa Tenggara Province is 14,825 cases. Gout commonly affects the elderly (> 65 years) with 2,570 cases, but this disease also frequently occurs in people who are not yet elderly or are >25 years old, with 3,310 cases, due to unhealthy lifestyles that can increase uric acid levels.

Uric acid is the final product of purine metabolism, existing as crystals within the body. Purines are derived from nucleic acids (the core acids of cells), which belong to the amino acid group and are components of proteins. Every individual has uric acid in their body as it is produced during normal metabolic processes. Normally, this uric acid is eliminated from the body through feces and urine (Kee, Joyce LeFever. 2007). According to Dahlia (2018), caffeine has the opposite effect of polyphenols, increasing the level of the enzyme xanthine oxidase, thereby lowering blood uric acid levels. In the body, uric acid is synthesized from purine-rich foods. However, if the amount of uric acid in the blood is excessive and reaches a saturated level, it will crystallize, potentially leading to gout. This habit of coffee consumption may have a positive effect on coffee drinkers, thus warranting further research into this phenomenon.

Based on the aforementioned description and background, a study needs to be conducted with the title "The Effect of Tambora Coffee Administration on the Reduction of Uric Acid Levels In Vivo."

Based on the background described above, the research problem is formulated as follows: "Is there an effect of Tambora coffee administration on the reduction of uric acid levels in vivo?"

Research ObjectiveTo determine the effect of Tambora coffee administration on the reduction of uric acid levels in vivo.Specific ObjectivesTo determine the uric acid levels before the administration of Tambora coffee at doses of 0.25, 0.5, and 0.75 mg/BW.To analyze the uric acid levels before and after the administration of Tambora coffee at doses of 0.25, 0.5, and 0.75 mg/BW.This research falls within the field of Clinical Chemistry, aiming to understand the in vivo effect of Tambora coffee on uric acid levels.

LITERATURE REVIEW

1. Tambora Coffee
   Tambora coffee is a type of plantation crop that has been cultivated for a long time and has a relatively high economic value.

Figure 1. Arabica Coffee

a. Coffee Taxonomy

Kingdom : Plantae
Division : Magnoliophyta
Sub Division : Spermatophyta
Class : Magnoliopsida
Ordo : Rubiales
Family : Rubiaceae
Genus : Coffea
Species : CoffeaarabicL.

b. Types of Coffee

The types of coffee that are widely cultivated are Arabica coffee (*Coffea arabica*) and Robusta coffee (*Coffeacanephora*).

a. Arabica Coffee

Arabica coffee is the most widely cultivated coffee in the world and in Indonesia in particular. This coffee is planted in highlands with a dry climate, around 1350-1850 meters above sea level. Meanwhile, in Indonesia, this coffee can grow in high areas up to an altitude of 1200 meters above sea level. This type of coffee tends to be susceptible to coffee leaf rust disease (*Hemilieiavastatrix*), but it has a strong aroma and flavor (Hamni, 2017).

The characteristics of Arabica coffee are as follows:

1. Its aroma is fragrant, resembling a blend of flowers and fruits.
2. It thrives in cool and cold regions.

3. It has an acidity that is not found in Robusta coffee.
4. It has a full body or a viscous feel when sipped in the mouth (Hasrianti, 2017).

b. Robusta Coffee

Robusta coffee is widely cultivated in Africa and Asia. Robusta coffee can be considered a second-class coffee due to its more bitter and slightly acidic taste, and it contains a much higher level of caffeine. Additionally, the growing range of Robusta coffee is wider than that of Arabica coffee, which requires specific altitudes to grow. This coffee can be grown in lowlands up to an altitude of 1,000 meters above sea level. This type of coffee is more resistant to pests and diseases (Hamni, 2017).

c. Coffee Content

Caffeine is one of the components found in coffee. Caffeine consumption can increase body metabolism, energy expenditure, lipid oxidation, and lipolysis. Several antioxidant compounds present in coffee include polyphenols, flavonoids, proanthocyanidins, coumarin, chlorogenic acid, trigonelline, and tocopherol. Coffee contains polyphenol compounds, which are known as antioxidants that can fight freeradicals (Hamni, 2017).

d. Benefits of Coffee

The high polyphenol content in coffee plays a significant role in preventing various diseases such as cancer, joint inflammation, rheumatism, immune systems, and in boosting body stamina. The benefits of coffee include potentially lowering the risk of Alzheimer's and dementia, reducing the risk of gallstones, decreasing the risk of Parkinson's disease, improving cognitive function, acting as an analgesic, antidiabetic, liver protectant, lowering cancer risk, being cardioprotective, acting as

an antioxidant, preventing dental cavities, and gout. Another compound found in coffee is diterpene, which can affect the increase of serum cholesterol levels. Coffee processing without filtering can lead to a higher concentration of diterpene compounds in the coffee. Cafestol and kahweol are natural diterpene extracts obtained from unfiltered coffee beans. Cafestol and kahweol can suppress LDL receptors, including reducing the binding, uptake, and degradation of LDL, thus havingan effect on increasing cholesterol levels.

2. Uric Acid

Uric acid is the end product of purine metabolism. Purines (adenine and guanine) are constituents of nucleic acids. Within the body, purine turnover occurs continuously along with the synthesis and breakdown of RNA and DNA, so even without purine intake, a substantial amount of uric acid is still formed. Uric acid is synthesized primarily in the liver, in a reaction catalyzed by the enzyme xanthine oxidase. Uric acid then flows through the blood to the kidneys, where it is filtered, partially reabsorbed, and partially excreted before finally being excreted in the urine. On a low-purine diet, daily excretion is about 0.5 grams; on a normal diet, excretion is about 1 gram per day. Meat, legumes (pod plants), and yeast are foods rich in purines (Sacher, 2017).

Uric acid is a naturally occurring substance found in the bloodstream of humans, containing purines. Purines are the primary source of uric acid. Uric acid is more commonly found in men than in women. In men, gout appears between the ages of 30 till early 40, and in women, gout appears around the age of 60 (Siswoputranto, P.S, 1993)

This disease commonly affects the elderly. A person is considered elderly if their age is greater than or equal to 60 years. The elderly often face health problems due to physical decline and organ weakness, leading to various diseases such as increased uric acid levels, which can cause conditions like kidney stones, gout, and rheumatism (Umami, 2018).

3. Uric Acid Metabolism

   Uric acid is the end product of purine metabolism. Purines (adenine and guanine) are constituents of nucleic acids. Within the body, purine turnover occurs continuously along with the synthesis and breakdown of RNA and DNA, so even without purine intake, a substantial amount of uric acid is still formed. Uric acid is synthesized primarily in the liver, in a reaction catalyzed by the enzyme xanthine oxidase. Uric acid then flows through the blood to the kidneys, where it is filtered, partially reabsorbed, and partially excreted before finally being excreted in the urine. On a low-purine diet, daily excretion is about 0.5 grams; on a normal diet, excretion is about 1 gram per day. Meat, legumes (pod plants), and yeast are foods rich in purines (Lantika, 2018).

4. Uric Acid Levels

   The average level of uric acid in the blood or serum varies. According to the WHO, the normal uric acid level is 3.5 – 7.0 mg/dL for men and 2.6 – 6.0 mg/dL for women. Before puberty, it is around 3.5 mg/dL. After puberty, in men, the level gradually increases and can reach 5.2 mg/dL. In women, uric acid levels usually remain low, only increasing to near male levels around premenopause, potentially reaching 4.7 mg/dL. If it exceeds these values, a person is said to have hyperuricemia, which is an increase in blood uric acid levels above the normal limit (Lantika, 2018).

   Uric acid levels in the body depend on the dietary intake of purine-containing foods, the degradation of endogenously formed purines, and excretion by the kidneys. Physiologically, the kidneys play an important role in hemostasis and uric acid excretion. The kidneys excrete 2/3 to 3/4 of the uric acid in the body, and the remainder is largely eliminated through the intestines. Most adults have uric acid levels in the range of 3.5 – 7.0 mg/dL for men and 2.6 – 6.0 mg/dL for women (Darmawan, 2016).

METHOD

The type of research used in this study is a quasi-experimental design. It is called a quasi-experiment because this experiment does not yet or does not have the characteristics of a true experimental design, as the variables that should be controlled or manipulated cannot be or are difficult to do. The main difference between true experimental research and quasi-experimental research lies in randomization (Notoatmodjo, 2012). This research was conducted from June to July 2023 at the Laboratory of PoliteknikMedicaFarmaHusadaMataram.

The independent variable is the variable that causes or theoretically has an impact on another variable. The independent variable in this study is Tambora Coffee. The dependent variable is the variable that, in the scientific framework, is the variable caused by changes in other variables. The dependent variable in this study is Uric Acid.

Tambora coffee is pure Arabica coffee powder that has been previously roasted for 10 minutes at a temperature of 236ºC and ground until fine. It was then made into a coffee solution at concentrations of 0.25 mg, 0.5 mg, and 0.75 mg. Uric Acid refers to the measured levels of uric acid in blood serum. Normal uric acid levels are 5.6-6.7 mg/dL. The Negative Control used Chicken Liver Juice at a dose of 0.625 ml/25 g BW (body weight). The Positive Control used Allopurinol at a dose of 0.45 g/25 g BW. In vivo refers to experiments using whole living organisms (Laboratory Animals).

The population is the entire subject of research or the object being studied (Nursalam, 2013). In this study, the population consisted of male white rats (Rattus norvegicus Wistar strain) as experimental animals.

a. Inclusion Criteria
    1) Male Wistar strain white mice
    2) Age 2-3 months
    3) Body weight 20-25 grams

4) Healthy condition (active & agile)
5) No anatomical abnormalities

b. Exclusion Criteria
Male Wistar strain white mice that died during the study.

The sample is a subset of the population that will be studied and is considered representative of the entire population that meets the criteria (Nursalam, 2002). The sample used in this study consisted of 30 male rats. The sampling technique used was random sampling, where a portion of the population was selected as the sample based on the inclusion criteria: Healthy condition (agile), and No anatomical abnormalities.

The determination of the sample size to be used in this study was based on Federer's Formula, as follows:

$(t-1)(n-1) > 15$

t = The number of groups= 5
n = The number of subjects per group.
$(t-1)(n-1) > 15$
$(5-1)(n-1) > 15$
$4n-4>15$
$4n>19$
$n>4,75$
So, the number of subjects per group is 5.

1. How to Make Tambora Coffee
Arabica tambura coffee powder. For a dose of 0.25 mg, weigh 2.5 g of coffee powder then dissolve it in 100 mL of distilled water and heat it at 90C. For a dose of 0.5 mg, weigh 5 g of coffee powder then dissolve it in 100 mL of distilled water and heat it at 90C. For a dose of

0.75 mg, weigh 7.5 g of coffee powder then dissolve it in 100 mL of distilled water and heat it at 90C.

a. Concentration 0,5 mg/kg/BB

$$\frac{0{,}5\ \text{mg} \times 25\ \text{gr}}{1000} = \frac{12{,}5\ \text{mg}}{1000\ \text{gr}} = 0{,}0125\text{mg}$$

To determine the dosage for each, which is:

a. Weigh the empty container = 13,63 (13,63 + 0,0125)
b. Weight of container + coffee= 13,6425 Rounded to13,64

$\frac{13{,}64}{13{,}63}$ - = 0,01ml Dose for one mouse.

b. Concentration 0,25 mg/kg/BB

$$\frac{0{,}25\ \text{mg} \times 25\ \text{gr}}{1000} = \frac{6{,}25\ \text{mg}}{1000\ \text{gr}} = 0{,}00625\text{mg}$$

To determine the dosage for each mouse, which is:

a. Weigh the empty container = 13,63 (13,63 + 0,00625)
b. Weight of container + coffee= 13,63625 Rounded to13,64

$\frac{13{,}64}{13{,}63}$ - = 0,01ml Dose for one mouse.

c. Concentration 0,75 mg/kg/BB

$$\frac{0{,}75\ \text{mg} \times 25\ \text{gr}}{1000\ \text{gr}} = \frac{18{,}75\ \text{mg}}{1000\ \text{gr}} = 0{,}01875$$

To determine the dosage for each mouse:

a. Weigh the container= 13,63 (13,63 + 0,01875)
b. Weight of container + coffee= 13,64875 Rounded to13,65

13,65 - = 0,02 ml Dose for one mouse.
13,63

2. Preparation of Chicken Liver Juice
   50 grams of chicken liver and 40 ml of distilled water were blended until smooth and the volume was adjusted to 50 ml (ratio 1:1) with a dose of 0.5 (Rahayu et al, 2016).
   a. Concentration 0,5 mg/kg/BB

      0,5 mgx25 gr / 1000 = 12,5 mg / 1000 gr = 0,0125mg

      To determine the dosage for each mouse, which is:
      a. Weigh the empty container = 13,63 (13,63 + 0,0125)
      b. Weight of container + chicken liver juice= 13,6425 Rounded to13,64
         13,64 - = 0,01ml Dose for one mouse.
         13,63

3. Calculation of Allopurinol Dosage for Test Animals
   The usual human dose of allopurinol is 25 mg daily. If we measure something by human standards, the equivalent value in mice is 0.018 times smaller.
   . The conversion of the human dose (70 kg) to white mice (20 grams) is 0.018. The calculation of the allopurinol dose for mice weighing 25 grams is as follows:
   0.018×25 mg=0.45 mg/20 BB
   Therefore, the allopurinol dose becomes 0.45 mg/25 g BB.

   a. Preparation of Allopurinol Suspension
      20 mg of tablet powder was weighed and placed in a mortar, then ground and mixed with distilled water. The homogeneous solution was poured into a 10 mL volumetricflask until it reached the mark.

b. Calculation of Allopurinol Concentration

1. Concentration 0,45 mg/kg/BB

$$\frac{0{,}45\ \text{mg} \times 25\ \text{gr}}{1000\ \text{gr}} = \frac{11{,}25\ \text{mg}}{1000\ \text{gr}} = 0{,}01125\ \text{mg}$$

To determine the dosage for each mouse, which is:

a. Weigh the empty container = 13,63 (13,63 + 0,01125)
b. Weight of container + coffee = 13,64125 Rounded to13,64

$\frac{13{,}64}{13{,}63}$ - = 0,01ml Dose for one mouse.

The tools and materials used to obtain data in this research are as follows:

1. Tools:
   Individual mouse cages and their accessories, OHAUS animal scale, analytical balance, oral gavage needle, Coleman spectrophotometer with accessories, centrifuge, pipettes, test tubes, Eppendorf tubes, ice bath, common glassware.
2. Materials:
   10-week-old male Wistar strain rats, weighing 20 grams, standard rat food, Tambora coffee powder, materials for uric acid level examination.

The acclimatized test animals (for 7 days) were then induced with high uric acid levels (except for the negative control group) using a high-purine diet, namely chicken liver juice. Chicken liver juice was administered orally at a dose of 5 mL/20 g BW daily for 7 days using an oral gavage needle to the positive control group and all treatment groups. After 7 days of chicken liver juice administration (day 8 to day 14), blood uric acid levels were measured on day 15. Blood uric acid levels were measured for all animal groups by collecting blood from the tip of the lateral tail vein, which was then dropped onto Autocheck uric acid strips. The blood uric acid level was

automatically measured and displayed on the Autocheck blood uric acid meter. The results of the blood uric acid level measurements were recorded as "pre-test" results. Oral treatment with Tambora coffee bean (Coffea arabica L.) infusion was administered orally using an oral gavage needle at doses of 0.5, 0.25, and 0.75 mg/g BW according to their respective treatment groups for 7 days. On day 22, On the 22nd day, blood uric acid levels were measured. Previously, the mice were fasted for 6 hours before blood was taken. Blood uric acid levels were measured by collecting blood from the tip of the lateral tail vein of the test animals, which was then dropped onto Autocheck uric acid strips. The blood uric acid level was automatically measured and displayed on the screen of the Autocheck blood uric acid meter. The results of the blood uric acid level measurements were recorded as "post-test" results.

A total of 5 groups, with each group consisting of 5 mice, were treated as follows:

a. Group 1 (negative control): Mice were only given standard feed.
b. Group 2 (positive control): Mice were induced with 0.5 ml/25 g BW of chicken liver juice and allopurinol at a concentration of 0.45 mg/25g BW.
c. Group 3 (treatment 1): Mice were given 0.5 ml of chicken liver juice and Tambora coffee solution at a dose of 0.25 mg/g BW.
d. Group 4 (treatment 2): Mice were given 0.5 ml of chicken liver juice and Tambora coffee solution at a dose of 0.5 mg/g BW.
e. Group 5 (treatment 3): Mice were given 0.5 ml of chicken liver juice and Tambora coffee solution at a dose of 0.75 mg/g BW.

Blood uric acid levels were measured twice. The first measurement was taken on the 8th day after the administration of chicken liver juice to the positive control group and all treatment groups. The first measurement was taken one hour after administration on the 22nd day for all animal test groups. In the second measurement, mice were fasted for 6 hours before blood collection. Both the first and second blood uric acid measurements were performed by collecting blood from the tip of the lateral tail vein of the test animals, which was then dropped

onto Autocheck uric acid strips. The blood uric acid level was automatically measured and displayed on the screen of the Autocheck blood uric acid meter.

The collected data will be processed and analyzed using the Statistical Product and Service Solution for Windows (SPSS) software. The obtained data will undergo a Kolmogorov-Smirnov normality test. This test is used to assess the normality of the data distribution, determining whether the acquired data is normally distributed or not. Normally distributed data has a p-value > 0.05, while non-normally distributed data has a p-value < 0.05. Subsequently, a Levene's homogeneity test will be performed to determine the variance of the data, are the variances homogeneous or not. If the data is homogeneous, a one-way analysis of variance (ANOVA) can be conducted. This test is performed to determine if there is a relationship between the variables being tested.

RESULT AND DISCUSSION

1. Research Results
   1) Results of Tambora Coffee Preparation

      The preparation of Tambora coffee used 105 grams of powder.

      Table 1 Results of Tambora Coffee Preparation

Plant Name	Amount of Tambora Coffee Powder Used	Amount of Coffee
Tambora Coffee	45 grams	945 ml

Based on Table 1 above, the amount of Tambora coffee powder used was 45 grams, and495 ml of coffee was produced.

2) Administration of Tambora Coffee to Mice

The administration of Tambora coffee to the mice was done once daily for 1 week. The coffee was administered using a gavage needle via the oral route. The mice used in this study were male mice weighing 25-30 grams. There were 5 treatment groups in this study with different dosages for each treatment: 0.5 mg/kg BW, 0.25 mg/kg BW, and 0.75 mg/kg BW, as well as two control groups: a positive control using Allopurinol at a dose of 0.45mg/kg BW and a negative control using standard feed.

Table 2 Uric acid levels before coffee administration

Repetition	**P1**	**P2**	**P3**	**K+**	**K-**
1	3,7	5,4	4,1	3,9	1,7
2	5,0	6,7	4,4	4,0	2,2
3	4,2	6,1	5,4	3,9	2,4
4	3,6	4,8	4,7	3,8	2,0
5	3,1	3,8	4,9	4,3	1,8
Average	**3,92**	**5,36**	**4,7**	**3,98**	**2,02**

From Table 2. it is observed that the uric acid levels before the administration of Tambora coffee to 5 mice yielded varying results across 3 treatments and 2 controls (positive and negative). The average results for each group were: P1 3.92, P2 5.36, P3 4.7,K+ 3.98, and K- 2.02.

Table 3 Uric Acid Levels After Coffee Administration

Repetition	**P1**	**P2**	**P3**	**K+**	**K-**
1	3,0	2,1	3,0	1,9	1,9
2	3,3	3,2	3,1	2,4	2,7
3	2,7	3,0	3,4	2,9	2,1
4	1,8	2,0	1,8	2,8	2,1
5	2,5	3,1	2,3	2,3	1,5
Average	**2,66**	**2,68**	**2,72**	**2,46**	**1,64**

Based on the results of Tambora coffee administration to mice regarding the reduction of uric acid levels, it was found that the treatments using Tambora coffee at doses of 0.5 mg/kg BW, 0.25 mg/kg BW, and 0.75 mg/kg BW consecutively yielded average values of P1 2.66, P2 2.68, and P3 2.72. Meanwhile, the average for the positive control was 2.46, and the average for the negative control was 1.64. From the results of the coffee administration, it is evident that all treatments showed a decrease in uric acid levels afterthe mice were given Tambora coffee. The positive control also showed a decrease.

3) Data Analysis

In this study, tests were conducted using statistical methods. Two tests will be performed: the paired samples t-test and the One-Way ANOVA test. Previously, a data

normality assumption test was conducted. If the assumption is met, the paired samples t-test and One-Way ANOVA will be used. If the assumption is not met, the Wilcoxon test and the Kruskal-Wallis test will be used as alternatives. The results of the data normality assumption test are as follows.

a) Data Normality Test

There are 5 data points for each treatment (P1, P2, P3, positive control, and negative control). Thus, each treatment group has 5 data points. Due to the small sample size, the Shapiro-Wilk test was used for the data normality test.

The criteria for meeting the normality assumption test are if the P-value (or Sig in the SPSS results) of the Shapiro-Wilk test is greater than the 5% error value, and vice versa. Theresults of the Shapiro-Wilk test can be seen in Table 4.4 below:

Table 4. Results of Data Normality Test using the Shapiro-Wilk Test

Treatment	P-Value (Sig) *Shapiro-Wilk*	Error Value (5%)	Description
P1	0,994	0,05	Assumption Met
P2	0,434	0,05	Assumption Met
P3	0,848	0,05	Assumption Met
K+	0,203	0,05	Assumption Met
K-	0,985	0,05	Assumption Met

Source: Results of SPSS Software Analysis in the Appendix

Based on Table 4. it can be seen that for the data of all treatments, the Shapiro-Wilk p-value for all treatments is greater than the 5% error value. Therefore, it is concludedthat all treatment data in the measurements follow a normal distribution pattern.

b) Data Homogeneity Test

There are 5 data points for each treatment (P1, P2, P3, positive control, and negative control). Thus, each treatment group has 5 data points. The Levene's test was used for the data homogeneity test.

The criteria for meeting the normality assumption test are if the P-value (or Sig in the SPSS results) of Levene's test is greater than the 5% error value, and vice versa. The results of Levene's test can be seen in Table 4.5 below:

Table 5. Results of Data Homogeneity Test using Levene's Test

Treatment	P-Value (Sig) *Levene*	Error Value (5%)	Description
P1	0,994	0,05	Homogeneous
P2	0,434	0,05	Homogeneous
P3	0,848	0,05	Homogeneous
K+	0,203	0,05	Homogeneous
K-	0,985	0,05	Homogeneous

Source: Results of SPSS Software Analysis in the Appendix

Based on Table 5. it can be seen that for the data of all treatments, Levene's p-value for all treatments is greater than the 5% error value. Therefore, it is concluded that all treatment data in the measurements are homogeneous. Because the data meets the normality and homogeneity assumption tests, the analysis stage will proceed using the paired samples t-test and the One-Way ANOVA test.

c) Paired Samples t-test

In proving the results of the Paired Samples t-test, it is also necessary to define the hypothesis tests as follows:

It seems there might be a slight repetition in your request. Here's the combined and translated version of the hypothesis statements:

For Tambora Coffee Administration at a dose of 0.25 mg/kg BW:

H0: The administration of Tambora coffee at a dose of 0.25 mg/kg BW does not decrease uric acid levels in mice.

H1: The administration of Tambora coffee at a dose of 0.25 mg/kg BW decreases uric acid levels in mice.

For Tambora Coffee Administration at a dose of 0.5 mg/kg BW:

H0: The administration of Tambora coffee at a dose of 0.5 mg/kg BW does not decrease uric acid levels in mice.

H1: The administration of Tambora coffee at a dose of 0.5 mg/kg BW decreases uric acid levels in mice.

For Tambora Coffee Administration at a dose of 0.75 mg/kg BW:

H0: The administration of Tambora coffee at a dose of 0.75 mg/kg BW does not decrease uric acid levels in mice.

H1: The administration of Tambora coffee at a dose of 0.75 mg/kg BW decreases uric acid levels in mice.

For Allopurinol Administration at a dose of 0.45 mg/kg BW (K+):

H0: The administration of Allopurinol at a dose of 0.45 mg/kg BW does not decrease uric acid levels in mice.

H1: The administration of Allopurinol at a dose of 0.45 mg/kg BW decreases uric acid levels in mice.

For Standard Feed Administration (K-):

H0: The administration of standard feed does not decrease uric acid levels in mice.

H1: The administration of standard feed decreases uric acid levels in mice.

The acceptance criteria for H1 in the paired samples t-test can be seen from the p-value (Sig.). by comparing the p-value with the 5% error value. If the p-value is less than the 5% significance level, then there is an effect of the treatment, and vice versa. The results can be seen in bellow.

Table 6. Results of the paired samples t-test

Treatment	P-value	Error Value (5%)	Description
P1 Pre – P1 Post	0,008	0,05	affects P1
P2 Pre – P2 Post	0,006	0,05	affects P2
P3 Pre – P3 Post	0,005	0,05	affects P3
K+ Pre – K+ Post	0,002	0,05	affects K+
K- Pre – K- Post	0,807	0,05	no effect K-

As seen in Table 4.6, the p-value for the treatment of Tambora coffee at a dose of 0.25 mg/kg BW (P1) is 0.008, which is less than the 5% error level. Therefore, the decision is that the administration of Tambora coffee at a dose of 0.25 mg/kg BW reduces uric acid levels in mice. Similarly, the treatment of Tambora coffee at a dose of 0.5 mg/kg BW (P2) has a p-value of 0.006, which is also less than the 5% error level, leading to the conclusion that the administration of Tambora coffee at a dose of 0.5 mg/kg BW reduces uric acid levels in mice. The treatment of Tambora coffee at a dose of 0.75 mg/kg BW (P3) shows the same result as P1 and P2, with a p-value of

0.005, which is less than the 5% error level. Thus, the decision made is that the administration of Tambora coffee at a dose of 0.75 mg/kg BW reduces uric acid levels in mice. For the treatment of Allopurinol at a dose of 0.45 mg/kg BW (K+), as expected, it significantly reduces uric acid levels in mice (with a p-value of 0.002). Likewise, the treatment of standard feed (K-) cannot reduce uric acid levels in mice because its p-value of 0.807 is greater than the 5% error level.

Of the five treatments administered to the mice, only the standard feed treatment did not lower uric acid levels. The other treatments, involving Tambora coffee at doses of 0.25 mg/kg BW, 0.5 mg/kg BW, 0.75 mg/kg BW, and Allopurinol, were all effective in reducing uric acid levels in the mice. A One-Way ANOVA test will be conducted next to determine which of the effective treatments, specifically the Tambora coffee treatment, can reduce uric acid levels to a similar extent as Allopurinol (K+).

d) One-Way ANOVA Test

Similar to the paired samples t-test, hypotheses were also formulated for the One-Way ANOVA test, as follows:

H0: There is no significant difference in uric acid reduction among the different Tambora coffee treatments.

H1: There is a significant difference in uric acid reduction among the different Tambora coffee treatments.

Table 7 Results of One-Way ANOVA Test for Differences in Tambora Coffee Treatments

Source	Sig.	Significance Level5%	Decision
Uric Acid Reduction	0,000	0,05	H1 Accepted

Source: Results of SPSS Software Analysis in the Appendix

From Table 7. it can be seen that the results of the One-Way ANOVA test support the previously stated research hypotheses. The value in the Sig. Column indicates the significance level for the uric acid reduction data. The significance value from the One-Way ANOVA test is 0.000, which is less than the 5% error level. Therefore, the decision is to accept H1, indicating that there is a significant difference in uric acid reduction among the different Tambora coffee treatments.

Next, a post-hoc Tukey's HSD test was conducted to determine which specific Tambora coffee treatments differed significantly. The results are as follows:

Table 8 Results of Tukey's HSD Test

Treatment	Group	
	1	2
P2 (Tambora coffee 0,5mg/kgBB)	-2.6800	
P3 (Tambora coffee 0,75mg/kgBB)	-1.9800	
K+ (Allporinol 0,45mg/kgBB)	-1.5200	
P1 (Tambora coffee 0,25mg/kgBB)		-1.2600

The Tukey's HSD test divided the treatments into 2 distinct groups. The positive control group (K+, Allopurinol 0.45 mg/kg BW) clustered in the same group as Treatment 3 (Tambora coffee 0.75 mg/kg BW) and Treatment 2 (Tambora coffee 0.5 mg/kg BW). This grouping indicates that the uric acid-lowering effect of Tambora coffee at doses of 0.5 mg/kg BW and 0.75 mg/kg BW was statistically comparable to that of Allopurinol. Furthermore, the average reduction in uric acid levels observed in these two Tambora coffee treatment groups was numerically greater than that of the Allopurinol positive control, suggesting a potentially more potent effect. Therefore, the findings suggest that Tambora coffee at doses of 0.5 mg/kg BW and 0.75 mg/kg BW are the most effective treatments among those tested for lowering uric acid levels in this study.

2. Discussion

The results of this study on the administration of Tambora coffee to mice, once daily for 1 week via oral gavage, showed its effect on uric acid levels. The male mice used weighed 25-30 grams and were divided into 5 treatment groups with varying doses of Tambora coffee (0.25 mg/kg BW, 0.5 mg/kg BW, and 0.75 mg/kg BW), a positive control group receiving Allopurinol at 0.45 mg/kg BW, and a negative control group receiving standard feed.

The baseline uric acid levels before coffee administration varied among the 5 mice in each group. The average uric acid levels for each group before treatment were: P1 (0.25 mg/kg BW) 3.92, P2 (0.5 mg/kg BW) 5.36, P3 (0.75 mg/kg BW) 4.7, K+ (Allopurinol) 3.98, and K- (Standard Feed) 2.02. After the administration of Tambora coffee, a decrease in uric acid levels was observed in all treatment groups. The average uric acid levels after treatment were: P1 2.66, P2 2.68, and P3 2.72. The positive control group also showed a decrease with an average of 2.46, while the negative control group's average was 1.64. This initial observation suggests that Tambora coffee has a potential effect in lowering uric acid levels in mice.

The statistical analysis using the Paired Samples t-test revealed significant decreases in uric acid levels within each treatment group that received Tambora coffee. The p-value for the 0.25 mg/kg BW Tambora coffee group (P1) was 0.008, which is less than the 5% error level, leading to the conclusion that this dose significantly reduced uric acid levels in mice. Similarly, the 0.5 mg/kg BW Tambora coffee group (P2) showed a significant reduction with a p-value of 0.006, also less than 5%. The 0.75 mg/kg BW Tambora coffee group (P3) exhibited the same outcome, with a p-value of 0.005, indicating a significant decrease in uric acid levels. As expected, the positive control group receiving Allopurinol at 0.45 mg/kg BW (K+) also showed a significant decrease in uric acid levels (p-value 0.002). Conversely, the negative control group receiving standard feed (K-) did not show a significant decrease in uric acid levels, as indicated by the p-value of 0.807, which is greater than the 5% error level.

The subsequent One-Way ANOVA test results supported the previously stated research hypotheses. The p-value (Sig. column) for the uric acid reduction data was 0.000, which is less than the 5% error level. Therefore, the decision was to accept H1, indicating a significant difference in uric acid reduction among the various Tambora coffee treatments.

Following the ANOVA, a post-hoc Tukey's HSD test was conducted to identify which specific Tambora coffee treatments differed significantly. The Tukey's test grouped the treatments into two categories. Notably, Treatment 3 (Tambora coffee 0.75 mg/kg BW) and Treatment 2 (Tambora coffee 0.5 mg/kg BW) were grouped with the positive control (Allopurinol 0.45 mg/kg BW). This suggests that the administration of Tambora coffee at doses of 0.5 mg/kg BW and 0.75 mg/kg BW resulted in the most effective reduction of uric acid levels, comparable to and even better than the positive control treatment with Allopurinol.

Previous research on the administration of Sidikalang coffee (*Coffeacanephora* var. *robusta*) to mice as experimental animals showed that the coffee infusion optimally reduced uric acid levels by 64.93%, with a significant difference between groups (sig. = 0.000) as determined

by One-Way ANOVA. This finding is consistent with the research conducted by Anna Maria Dewajanti et al., which investigated free radical levels in rats by measuring malondialdehyde (MDA). Elevated uric acid levels due to a high-purine diet can lead to a twenty-fold increase in xanthine oxidase enzyme oxidation compared to normal. This results in increased free radicals in the body, which can damage cell membranes in organs such as the liver and kidneys. The researchers used a 0.25 mg infusion of Gayo Arabica coffee (*Coffea arabica* L.) seeds administered to hyperuricemic rats, demonstrating that the Gayo Arabica coffee seed infusion could reduce free radical levels. Another study involving Robusta and Arabica coffee given to individuals with hyperuricemia in Teguhan Village, GrobоganSubdistrict, Grobogan Regency, showed that Robusta coffee normalized uric acid levels in 70% of the samples, and Arabica coffee normalized levels in 90% of the hyperuricemic samples. The average reduction was 1.23 mg/dL for Robusta coffee and 2.37 mg/dL for Arabica coffee.

Coffee contains compounds that can lower blood uric acid levels, primarily polyphenol compounds. The main polyphenol in coffee is chlorogenic acid. Chlorogenic acid is a water-soluble phenolic compound. This compound can inhibit the activity of the enzyme xanthine oxidase, thereby lowering uric acid levels, and it also acts as an antioxidant, protecting cells from free radicals. Chlorogenic acid's antioxidant properties enable it to inhibit xanthine oxidase activity in the formation of uric acid. The enzyme xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and further to uric acid. During the oxidation of hypoxanthine and xanthine, oxygen acts as an electron acceptor, producing superoxide radicals and hydrogen peroxide, which are Reactive Oxygen Species (ROS). The more purine molecules catabolized in the body, the higher the uric acid levels and the greater the resulting oxidative stress. By inhibiting xanthine oxidase, both uric acid levels and the production of Reactive Oxygen Species (ROS) can be reduced, thus producing an anti-hyperuricemic effect.

The research conducted by Yulsyafrida (2022) showed that all four treatment groups experienced a decrease in blood uric acid levels, with the average difference in reduction for each treatment visible in Table 4.1. The highest reduction in blood uric acid levels was observed in the second treatment group, which received Sidikalang Robusta coffee (*Coffeacanephora* var. *robusta*) seed infusion at a dose of 0.5 mg/g BW. The lowest reduction was in the third treatment group, which received Gayo Arabica coffee (*Coffea arabica* L.) seed infusion at a dose of 0.25 mg/g BW. The difference in blood uric acid reduction between the coffee types is influenced by the chlorogenic acid content in each type of coffee bean. The chlorogenic acid content in Arabica coffee beans ranges from 6-7%, while in Robusta coffee beans, it ranges from 7-11%. This indicates that a higher chlorogenic acid content in the coffee bean type and a higher administered dose of coffee will lead to a greater reduction in blood uric acid levels.

Out of the five treatments given to the mice, only the standard feed treatment did not reduce uric acid levels. The other treatments, including Tambora coffee at doses of 0.25 mg/kg BW, 0.5 mg/kg BW, 0.75 mg/kg BW, and Allopurinol, were all effective in lowering uric acid levels in the mice. Further analysis using the One-Way ANOVA test will be conducted to determine which of the effective treatments, specifically the Tambora coffee treatment, can reduce uric acid levels to a similar extent as Allopurinol (K+).

CONCLUSION

Based on the results of the research that has been conducted using the One-Way ANOVA test, it can be concluded that the One-Way ANOVA test results support the previously stated research hypothesis. The value in the Sig. The column shows the significant value for the uric acid reduction data. The significant value in the One-Way ANOVA test results is 0.000, which is smaller than the 5% error value. Therefore, the decision made is to accept H1, indicating that there is a significant difference in the effect of Tambora coffee administration in lowering uric acid levels in mice.

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