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Safety Evaluation of Dried Gedi (Abelmoschus manihot L.) Leaf Brewed Water Based on Urinalysis Parameters in Postpartum Female Rattus norvegicus

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Abstract
Gedi (Abelmoschus manihot L.) is a plant that grows a lot in the Minahasa area which is often processed for traditional medicine, especially pregnant women consume this plant a lot because it is believed to provide good results for health, especially during pregnancy and postpartum. This study aims to evaluate the effect of brewed water of dried Gedi leaves on the urine parameters of female rats (Rattus norvegicus) after giving birth. This study is an experiment using 6 female rats after giving birth divided into 2 groups: (1) control (water), (2) treatment (brewing) of Gedi leaves 1 g/100 mL. The treatment was administered orally for 34 consecutive days. Urine was observed using reagent strips with the observed parameters, namely urine volume, urine color, leukemia, protein, ketones, bilirubin, nitrite, pH, blood, specific gravity, glucose and urobilinogen. The results showed that the brewed water of dried Gedi leaves affected the urine volume more than the control. Another effect was an increase in protein levels of 5 mg/dL compared to the control. Increased urine volume due to brewed water has the potential to be a natural diuretic agent that is safe to use in supporting postpartum recovery in female rats. Other urinalysis parameters showed the same results between the control and the treatment of dried Gedi leaf brewed water, indicating that dried Gedi leaves were safe in terms of urinalysis parameters.

Keywords: Abelmoschus manihot; Brewing; Gedi; Postpartum; Rat.

1. Introduction

Traditional medicine is often used as a therapy for treatment that is carried out from generation to generation, based on ancestral prescriptions, customs, and local customs or beliefs. Based on research, traditional medicine is beneficial for health and currently its use is quite popular among the public because it is easier to reach both in terms of price and availability. Traditional medicines that are often used are leaves, roots, rhizomes, fruits, stems, and flowers (Parwata, 2017). Based on data from the World Health Organization (WHO), the population in developing countries who choose to use traditional medicine is as much as 80% and for developed countries there are 65% (Oktakarlina et al., 2018). Traditional medicinal plants in their management can be in the form of fresh ingredients (extracts or decoctions) or dry powders (Parwata, 2017).

Gedi (A. manihot L.) is a plant that grows a lot in the Minahasa area. The people of Minahasa use gedi a lot as a food ingredient and are often processed for traditional medicine. The people of Minahasa, especially pregnant women, consume this plant a lot because it is believed to provide good results for health, especially during pregnancy and postpartum. According to Ipandi (2016), gedi plants have many important compounds, such as antioxidants, steroids, phenolics, antidiabetic effects, analgesic effects, alkaloids, and flavonoids.

The development of research on Gedi plants in 2021 was once registered with BPOM as a functional drink from dried leaves, but based on BPOM reply that Gedi is its use as a traditional medicine, it turned out that it had not been registered and standardized, or there was no standard for levels that are suitable for use as traditional medicine, especially side effects on pregnancy and postpartum in other words, it still needs standardization and safety as raw materials medicine. Consequently, scientific evidence supporting its safety is still required before Gedi can be broadly recommended as a standardized herbal medicine.

Research on the effects of traditional medicines to be suitable for use by consumers must go through preclinical trials before clinical trials are carried out in humans. Preclinical trials were conducted on test animals (Hodgson et al., 2000). White rats (R. norvegicus) are often used as a test animal because this animal has a body faal system similar to humans because they are both from the mammalian class (Johnson, 2012). A series of experiments conducted on these animals included a study of the safety of a traditional medicine through resiliency testing (Arome et al., 2014). Evaluation of the safety of medicinal plants is a critical component of herbal medicine development. Although many traditional remedies have been used empirically for generations, their biological effects and potential toxicity must be scientifically verified through preclinical studies before clinical application in humans. According to Pandiangan et al. (2022b), there are many parameters for testing effectiveness, one of which is the urine examination parameter or known as urinalysis. Urinalysis is an important and non-invasive tool for evaluating the safety of medicinal plants because it provides information regarding renal function, metabolic status, and the body's response to xenobiotic compounds. Changes in urinary parameters such as protein, glucose, leukocytes, ketones, bilirubin, pH, and specific gravity may indicate physiological disturbances or toxic effects associated with the administration of herbal preparations. The results of urine tests can reflect toxicity in the tested animals because urine is the result of the excretion of toxic substances from the kidneys that have a volume of blood flow to concentrate toxins (Mangindaan et al., 2014). Since the kidneys play a central role in filtering blood and eliminating metabolic waste products, urinalysis can serve as a sensitive indicator of potential adverse effects during pregnancy and postpartum conditions (Evans, 2009). Therefore, urinalysis is considered a valuable parameter in the preclinical safety assessment of traditional medicinal products.

Research on gedi plants used as traditional medicine has been carried out by many researchers previously (Rebecca et al., 2020), but research on the effect of this plant's brewed water on pregnancy to postpartum using the examination/visualization method of urinalysis results has never been carried out. Considering the widespread traditional consumption of Gedi among postpartum women and the limited availability of standardized safety data, it is important to investigate its biological effects through preclinical evaluation (Evans, 2009). Therefore, it is necessary to conduct research on the effect of dehydration of Gedi leaves on the urine of female rats after childbirth (postpartum).

Problem formulation that based on the background of the description, the problem arises which can be formulated as follows: Is there an effect of consumption of dry Gedi brewed water on the urine of female white rats after childbirth? This study aims to find out whether there is an effect of consumption of dry Gedi brewed water on the urine of white rats postpartum females. The results of this study are expected to provide information on the safety of Gedi brewed water to be used as a functional tea or traditional medicine that can be registered with BPOM.

2. Research Methods

Time and Place of Research

This research was carried out from February to July 2024 and was further clarified June to December 2025. The research was carried out at the Biovina Herbal Laboratory, Jaga 2, Sea Mitra, Pineleng District, Minahasa, North Sulawesi Regency, and samples were taken at the Biovina Plantation in South Bolaang Mongondow.

Tools and Materials

The tools used in this study were lab coats, modified test animal cages, wood powder, feeding containers and drinking bottles, gloves, tea bags, threads, glasses, digital scales, blenders, ATK, pots, VerifyTm urinalysis reagent strips, modified urine reservoirs, tape, micropipettes and tips, durham tubes, cotton swabs, syringes. The ingredients used were 18 healthy female white rats and 6 male white rats as mating, 10 kg of fresh Gedi leaves, B553 High-Ago rat feed (pellets), and drinking water.

Working procedure

Research Design

This research design was experimental using a Complete Random Design (RAL) with two treatments where each treatment had three replicates. The treatment design is as shown in Table 1.

Table 1 Rats Maintenance Workchart During Dry Gedi Brewing Water Treatment (T) and Control (C)

Control (C)	Treatment (T)
C1	T1
C2	T2
C3	T3

Test Animal Cage Preparation

The rat cage is modified using a 45×20 cm diameter container. To prepare a clean rat cage, line the cage with fresh wood powder and arrange the feeding place and rat bottles well. Once the cage is ready, put the

rats in the cage and cover them with a cover wire, making sure the rat position is comfortable (Tagulihi et al., 2022; Pandiangan et al., 2022).

Ethical Approval

The experimental procedures in this study were approved by the Health Research Ethics Committee of Politeknik Kesehatan Kemenkes Manado, Indonesia, under ethical clearance number DP.04.03/FXXX.28/568/2025. All procedures involving laboratory animals were conducted in accordance with institutional guidelines for the care and use of laboratory animals and complied with internationally recognized standards for animal welfare (National Research Council, 2011; World Health Organization, 2011).

Acclimatization of Rats

Rats acclimatization or rat adaptation in this study follows Pasaribu et al., (2024). The test animals used in this study were White Rats obtained from the Biovina Herbal Laboratory, Jaga 2, Sea Mitra, Pineleng District, Minahasa, North Sulawesi Regency. Animals must be prepared before being tested through acclimatization, acclimatization is a process of physiological adjustment or adaptation by experimental animals to environmental climate change. Acclimatization needs to be carried out to observe that the test animals to be used are ready and in a healthy state physiologically and behaviorally. The mice selected in this study were mice that successfully adapted within 14 days, with a body weight ranging from 160-200 g. The number of rats used was 18 female rats divided into two groups, namely 9 control rats for comparison, and 9 rats treated with dry gedi. The total of all mice used for the treatment was used for three repetitions each. Each repetition uses 3 female rats with 1 male rat in 1 cage, both control and treatment, (Pasaribu at al., 2024).

Rat Maintenance

This study used white rat (R. norvegicus) test animals with a weight of 160-200 g and ± 4 months old. The rats were kept in a 45×20 cm plastic cage with a wire cover and equipped with a feeding place and bottled drinking water. Rats were grouped according to treatment, for control treatment there were 3 cages and treatment with dry Gedi water brewing 3 cages, where each cage contained 1 male and 3 females.

Making Dry Gedi Brewing Water in Tea Bags

The procedure for this study follows the modified Pandiangan et al., (2022). Fresh gedi leaves are taken as much as 10 Kg then cleaned and then air-dried. Dry the Gedi leaves under the sun until the weight is constant. The dried gedi leaves are then mashed using a blender until they become powdered. The mashed Gedi leaf powder is then put into tea bags of 1 g/tea bag each, then distributed into each glass according to the treatment group, namely 1 tea bag for brewing 1 g of gedi leaves in 100 ml of water. The filled tea bag is then brewed with boiling water like how to make tea, then let it sit until the water turns slightly brownish. The finished Gedi brew is then left to sit until it is cold enough to be put in a rat drinking bottle. The design is as shown in the next Figure 5.

Test Animal Treatment

In this study, each cage contained four mice (1 male and 3 female). Male rats are intended only to mate with female rats until they experience pregnancy. Maintenance takes 8 weeks (56 days) where the first 14

days are acclimatized, 21-22 days of mating period assuming that during this time it is expected that the rats will get pregnant then 7 days later the rats will give birth after giving birth, wait up to ± 2 weeks until the rats are ready to be separated from the offspring for the urinalysis process of 3 animals each, 1 each in each cage. The treatment of giving dry and controlled drinking water is carried out during the marriage period until the postpartum period. The administration of gedi water infusion in each cage is carried out at 16.00 consistently every day.

Urine Examination of White Rats (R. norvegicus)

This examination follows the method in Pandiangan et al., (2022b). Urine tests were carried out after 2 weeks of the rats giving birth. The ready mice were first induced with 5 ml of aqueous and then the mice were placed in a container that had been modified for urine storage. The urine examination chart is as shown in Figure 2 and 3 below.

Urine Reservoir

Urine containers are usually made of plastic in this case made of 1000 ml used drinking bottles that have been cleaned then given a hole next to the bottle so that the rats can continue to breathe, the top of the drinking bottle is cut to make it easier for rats to enter and closed with tape, the most important thing is that the container must be wide-mouthed, clean, and dry. The bottle is positioned upside down and the mouth of the bottle is used as a rat shelter which is attached with a cotton-based durham tube which functions so that rat droppings do not participate in the durham tube, the durham tube is attached using tape to the mouth of the drinking bottle. After the rats were put into a urine reservoir, the rats were left for ± 4 hours until the durham tube was filled with urine.

Urine Examination

Urine that is good to observe is urine that is fresh or that is less than 4 hours after it has been removed. After the rat's urine is contained in a durham tube, then the urine is measured in volume and its color is observed. Urine was collected using a micropipette with a tip measuring 100 µL and dripped at the parameters on the label of the reagent strip. Leave the urine samples that have been dripped on the reagent strip with the time conditions that have been set for each parameter until the color on the parameter develops optimally (Figure 2). The parameters tested are as follows:

Leukocyte

Leukocyte examination, the results must be read between 60-120 seconds so that the color development is complete and produces colors ranging from beige-pink to purple. The intensity of the developed color is proportional to the number of leukocytes present in the urine specimen (Figure 2).

Protein

The color of the protein test ranges from yellow to yellow-green for negative results and green to green-blue for positive results. The reading of the protein test results was 60 seconds (Figure 2).

pH

On pH checks, the color ranges from dark orange to deep dark green. The range for normal urine specimens is pH 5. The pH check ranges for 60 seconds (Figure 2).

Specific gravity

The indicator checks the specific gravity color range from dark green to orange. Observation is carried out for 45 seconds until the color development is complete. Urine normally has a specific gravity of 1,000 (Figure 2).

Ketone

A ketone test will result in a color change ranging from pink for a negative result to purple for a positive result. The ketone examination lasted for 40 seconds (Figure 2).

Bilirubin

The bilubirin examination ranges for 30 seconds until the color develops perfectly. Positive bilirubin results are characterized by a change in the color of the strips to dark brown while positive results are light brown (Figure 2).

Glucose

The colors produced in glucose tests range from green to brown. For negative positives it is green and for positive results it is brown. The glucose test ranges for 30 seconds (Figure 2).

Blood

The resulting color ranges from yellow without spots to green accompanied by spots. Each green spot develops on the area of the reagent. The time required for a urine blood test is 60 seconds (Figure 2).

Urobilinongen

The color produced ranges from beige to pink, for negative results get a beige color and for positive results get pink. The time it takes to see the urobilinongen test results ± 60 seconds (Figure 2).

Nitrite

The color produced by the nitrite test is white for negative results and pink for positive results. The time it takes to see the nitrite test results ± 60 seconds (Figure 3.2).



Figure 2 Urinalysis Reagent Strip for testing multiple analytes in Urine VerifyTm

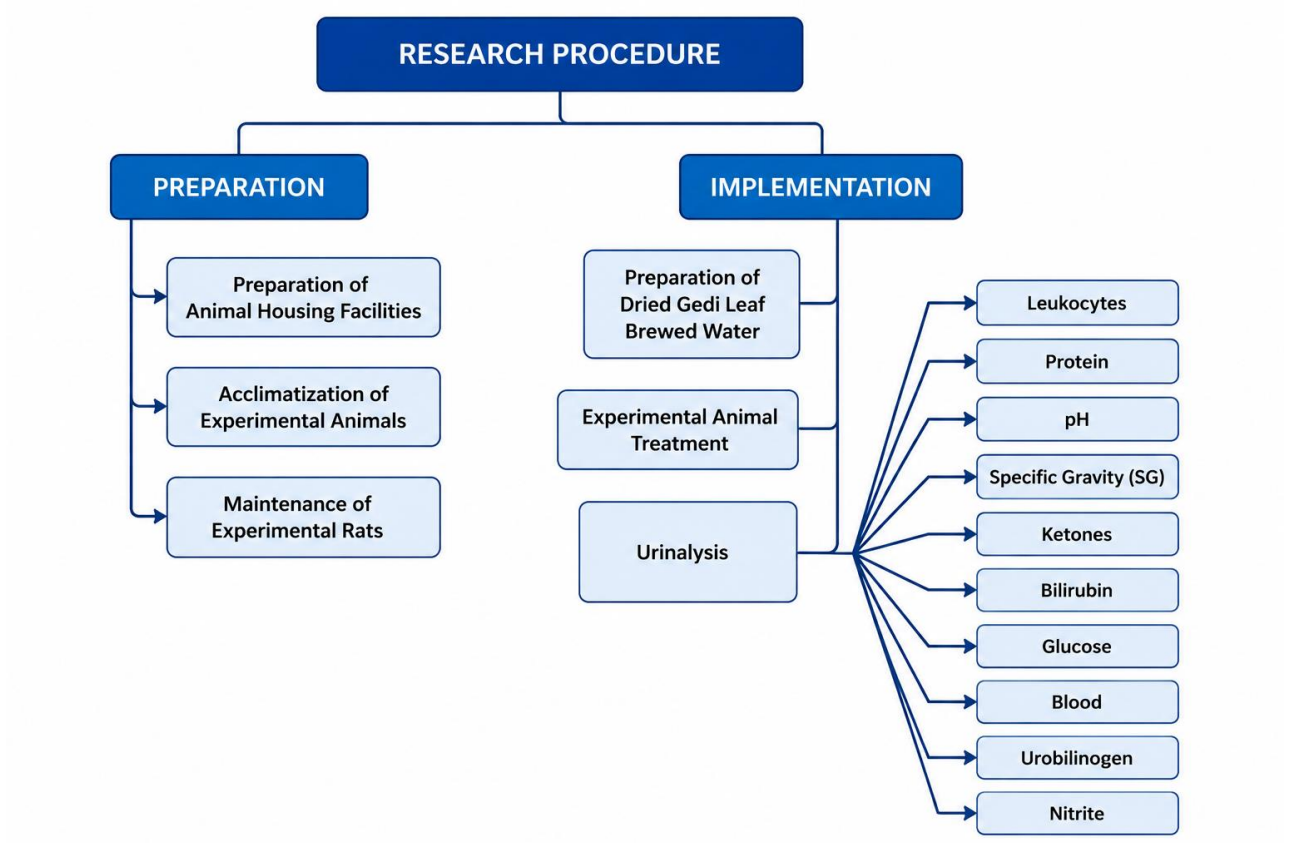


Figure 3. Urinalysis research procedure chart of the effect of Gedi (A. manihot L.) brewing water treatment In White Female Rats (R. norvegicus).

3. Data Analysis

The analysis of research data was carried out by collecting test animal data and tabulating data (in the form of tables) containing comparative control and treatment data with urinalysis parameters. It was analyzed using appropriate statistical methods, namely comparing control groups and treatments for each evaluated urinalysis parameter (Pandiangan at al., 2023).

4. Results and Discussion

1. Organoleptic

Organoleptic testing or sensory evaluation is a science used to evoke, measure, analyze, and interpret responses to products perceived by the senses of sight, smell, touch, taste, and hearing (Stone and Sidel, 1993). Organoleptic test of dry gedi leaf brewing water for its color looks yellowish-brown, liquid viscosity, has a typical smell of gedi leaves, light and fragrant, for a bitter taste.



Figure 4. Organoleptic test, brewing of dried Gedi leaves

Acclimatization of Rats (Rattus norvegicus)

Acclimatization or adaptation of rats was carried out at the Biovina Herbal Laboratory, Jaga 2, Sea Mitra, Pineleng District, Minahasa, North Sulawesi Regency. Acclimatization is carried out by providing drinking water, providing a clean place for rats, and feeding rats for 14 days while observing their physical and behavioral conditions. Rats that successfully went through acclimatization were selected as test animals.

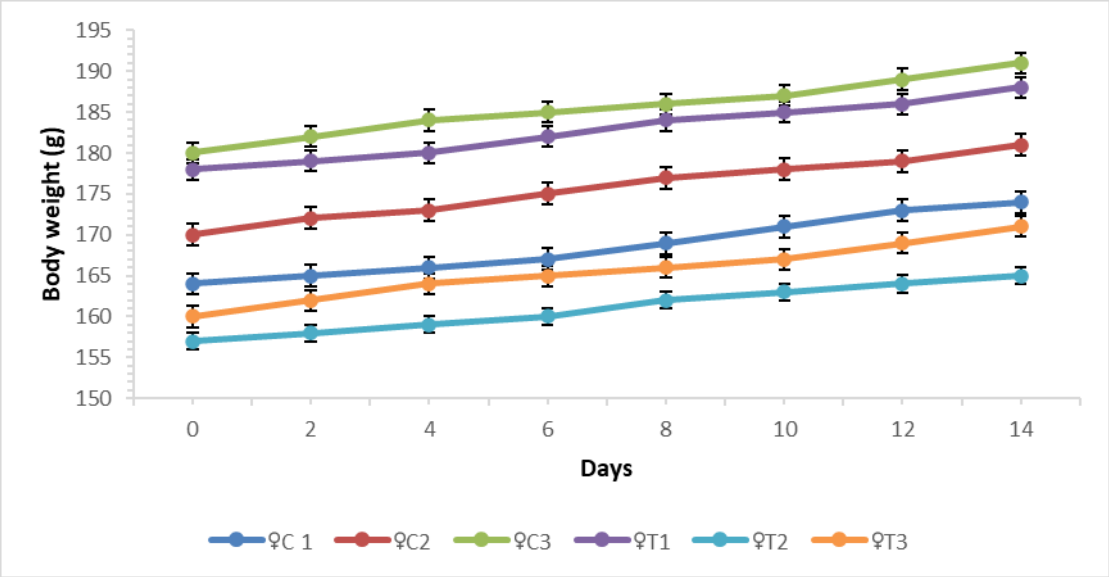


Figure 5. Body weight change (g) of rats during 14 days of acclimatization, C1 (control rat 1), C2 (control rat 2), C3 (control rat 3), T1 (treatment rat 1), T2 (treatment rat 2), T3 (treatment rat 3)

According to Pasaribu, et. al., (2024) Rats that have agile movements, often scratch their ears, normal breathing, have bright and clean eyes, white, smooth fur, and experience weight gain along time (days) acclimatization are characteristics of good and healthy rat physical conditions and behavior so that they can be used as test animals in research. The weight gain of white rats during the acclimatization process can be observed in Figure 5. The rats selected after going through the acclimatization process were 6 for one repetition and for division, 3 rats in the control group, 3 rats in the dry gedi leaf brewing water treatment group where each group was provided with 1 male rat as a mating rat. On the graph in Figure 5, It can be seen that each rat experienced normal weight growth with good physical condition and behavior.

2. Amount of Water Rats Drink Treatment and Control

The comparison of the number of dried gedi leaves that have been blended into powder with well-cooked drinking water, namely 1 g of dried gedi leaves per 100 mL of drinking water. Table 2 shows the control group C1 (♀1) with a body weight of 242 g drinking an average of 90.53 ml of water. C2 rats (♀2) with a body weight of 191 g drank an average of 89.05 ml of water per day. C1 (♀3) with a body weight of 200 g drinks an average of 89.69 ml of water per day.

Table 2. Average amount that control group rats drank during the treatment period

Rats	Body weight (g)	x̄ amount drunk
C1 (♀1)	242	90,53 ml
C2 (♀2)	191	89,05 ml
C3 (♀3)	200	89,69 ml

Table 3. Average amount of dry gedi group rats drinking during the treatment period

Rats	Body weight (g)	x̄ amount drunk
T1 (♀1)	252	51,34 ml
T2 (♀2)	241	50,87 ml
T3 (♀3)	246	51,12 ml

In Table 3, it can be seen that the control group T1 (♀1) with a body weight of 252 g drank an average of 51.34 ml of water. T2 (♀2) rats with a body weight of 241 g drank an average of 50.87 ml of water per day. T3 rats (♀3) with a body weight of 246 g drank an average of 51.12 ml of water per day.
Rats with the control treatment had the highest average water consumption compared to rats treated with dry gedi, this is because the dry gedi brewed water has a bitter taste and is also bitter (Pasaribu et al., 2024). During the mating period, there was a change in appetite in the treated rats, which during the acclimatization period normal appetite and drinking became less or less than in the control group. Changes in the body's metabolic rate can affect the absorption and use of nutrients in the body. The decrease in appetite and the small amount of average drinking volume of treated rats caused the condition of the rats that often looked weak so that the metabolism of the treatment rats decreased, the observation of the physical condition and behavior of the rats in this study obtained similar results as in the study of Pasaribu et al., (2024) it was found that the physical condition and behavior of rats brewed with dried gedi leaves showed paralysis, rat fur that turns yellowish, muzzle is watery, abnormal movements and there is death in the mother and young of the rat.

3. Amount of Urine Volume and Color

The results of urine volume measurement from control mice and dry Gedi brewed water treatment had significant differences. For repeat rats 1, control rats produced a urine volume of 0.8 mL while for rats treated with dry Gedi brewed water obtained 1 mL, the result of the treatment urine volume was higher than the results obtained in control mice, this indicates that the treatment by giving dry Gedi leaves incubated water increased the urine volume by 0.2 mL. Repeat rats 2, control mice obtained a urine volume of 4 mL while for rats treated with dry Gedi brewed water obtained 5.5 mL, the results of the treated urine

volume were higher than the results obtained in control mice, this indicates that the treatment by giving dry gedi leaves incubated water increased the urine volume by 1.5 mL. Repeat rats 3, control mice obtained a urine volume of 4 mL while for rats treated with dry Gedi brewed water obtained 5 mL, the result of the treatment urine volume was higher than the results obtained in control mice, this indicates that the treatment by giving dry gedi leaves distilled water increased the urine volume by 1 mL.

Table 4. Total Urine Volume (mL) of Rats (R. norvegicus) Treated with Dried Gedi (A. manihot) Brewing Water

Deuteronomy	Control			Treatment		
	Volume (mL)	Average (mL)	Urine Color	Volume (mL)	Average (mL)	Urine Color
1	0,8	2,93	Clear	1	3,83	Clear
2	4		Cloudy Clear	5,5		Clear
3	4		Clear	5		Clear

The results from Table 4 above show that the urine volume in treated rats given dry gedi brewed water was higher when compared to the urine results of control rats. Rats treated with dry Gedi brewed water had an average volume of 3.83 mL while the control had 2.93 mL. This can happen because gedi leaves are known to have bioactive content such as flavonoids that have the potential to be diuretic agents (Lenny, 2006). Diuretics themselves are substances that can increase urinary output (diuresis) through direct action on the kidneys (Tjay and Rahardja, 2002). The mechanism of action of flavonoids as diuretic agents is by inhibiting the reabsorption of Na+, K+, and Cl- so that there is an increase in electrolytes in the tubules so that diuresis occurs. With increased excretion, it will also increase water excretion and cause urine volume to increase (Nessa, 2013).

4. Urine Analysis

a. Leukocyte

The leukocyte examination between treatment and control obtained the same results, namely trace (15 leu/μ), trace results indicated the presence of leukocytes in the urine with a small amount. Leukocytes themselves play a role in responding to the body's immunity when a foreign body enters the body, which causes an increase in the number of leukocytes. This increase is a response to overcome or destroy foreign objects that can interfere with bodily functions (Kataranovski et al., 2009). Factors that can contribute to an increase in urinary leukocytes are dehydration, stress, changes in leukocyte mobility and impaired glomerular membrane permeability (Agustina et al., 2023).

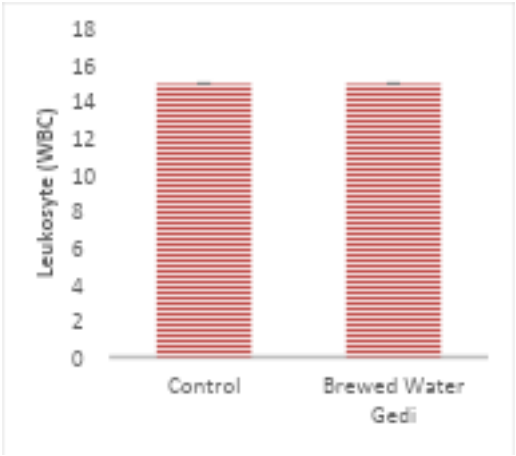


Figure 6. Effect of dried Gedi (A. manihot) Leaf Brewing Water Treatment against leukocyte content in the urine of rats (R. norvegicus)

b. Protein

In the protein examination, the measurement results in the dry gedi treatment group were greater than the control group with a control protein amount of 20 mg/dL and a protein amount of 25 mg/dL treatment. This shows that the administration of dry gedi leaf infusion affects the protein value but the amount is still within normal limits. Protein in the urine of mice themselves normally < 30 mg/dL (Harkness et al, 2010). Proteinuria is a condition in which proteins are found in the urine. Proteinuria is a marker of the presence of lesions in the glomerular membrane so that protein molecules pass into the urine (Kee, 2007). Urine containing protein indicates that the filtration carried out by the kidneys is not perfect (Wilmar, 2000).

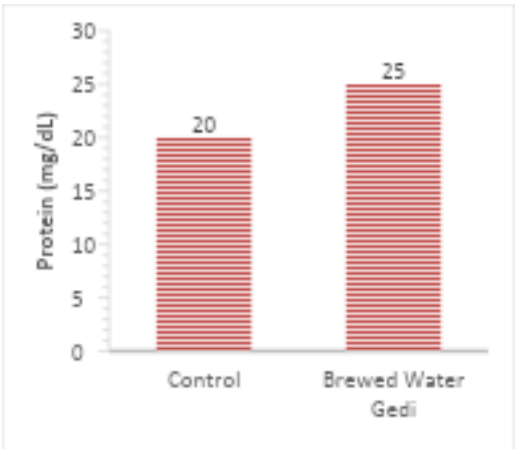


Figure 7. Effect of dry Gedi (A. manihot) leaf brewing water treatment on protein content in rat (R. norvegicus) urine

c. pH

The results of urine pH examination in the control group and treatment in this study showed abnormal results, namely pH 9 which is alkaline where the normal urine pH in mice ranges from 5-7 (Quesenberry & Carpenter, 2012). The pH of urine depends on the eating habits and metabolic balance of each individual, diseases as well as medications. The abnormal results were caused by the condition of the rats who experienced hunger and ketosis so that the alkaline of the urine increased. (Hukins, 2005).

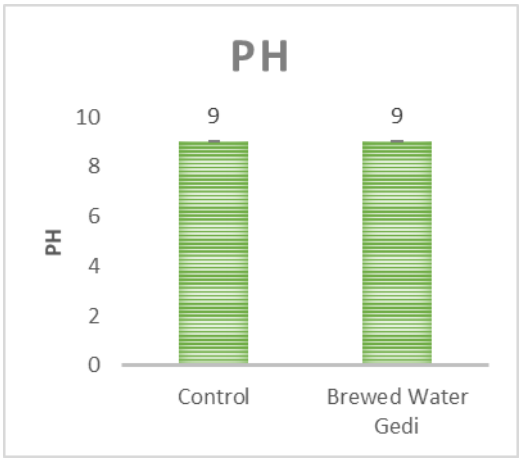


Figure 8. Effect of dry Gedi (A. manihot) leaf brewing water treatment on the pH content in rat (R. norvegicus) urine.

d. Ketone

Studies show the same results for control and treatment at 160 mg/dL. This value indicates the presence of a very high ketone content in the urine of rats, both necessary and controlled. Normally ketones are not found in urine (negative) (Quesenberry & Carpenter, 2012). Ketones are the product of the breakdown of fatty acids. The presence of ketones in urine indicates that the body uses fat as energy due to carbohydrate depletion (Devkota BP, 2015). At a time when the body is starving where the amount of carbohydrates is insufficient as energy, fatty acids will be converted into ketone bodies, a state in which the amount of ketone bodies produced exceeds the normal amount is referred to as ketonuria (Gupta et al., 2009). Because ketones are acidic, the body will produce bases as a buffer to maintain the body's acid-base balance. The presence of ketones in the body can cause fatigue and loss of strength because the body's muscles begin to be damaged (Aksu et al., 2018). If the body constantly uses ketones as energy, one day the body's base reserves will be depleted and this will cause an emergency, namely ketoacidosis which can be life-threatening. In some cases it can lead to the death of the fetus or even the death of the mother rat.

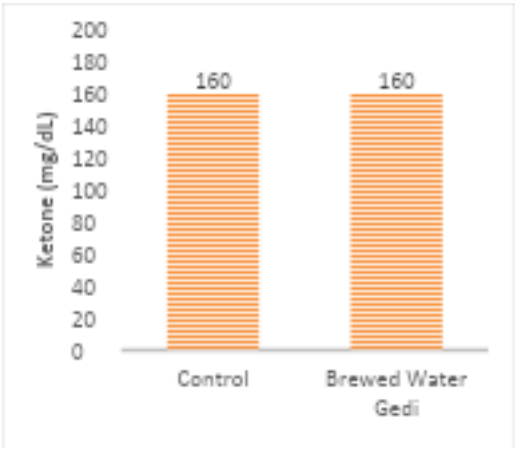


Figure 9. Effect of dry Gedi (A. manihot) leaf brewing water treatment on against ketone content in rat (R. norvegicus) urine

e. Specific Gravity

The results of the urine specific gravity examination showed an average control specific gravity of 1000 and the treatment specific gravity had an average of 1001. Urine specific gravity is a barometer to evaluate the kidneys' ability to store or excrete water. Urine specific gravity (BJU) is used to measure hydration status. BJU is categorized into four, namely good hydration status if the BJU value is <1,010, mild dehydration if the BJU value is 1,010-1,020 and moderate dehydration if the BJU value is 1,021-1,030, severe dehydration if the BJU value is >1,030 (Austin and Seebohar).

The specific gravity results obtained in both control and treatment in this study were less than 1010 because before urine analysis was carried out, the rats were first induced with 5 ml of aqueduct where based on (Pearce, 2006) the kidneys at the time of diluting urine, for example, after drinking water, the specific gravity was less than 1010 and if the kidneys thickened the urine, the specific gravity of the urine would rise above 1010.

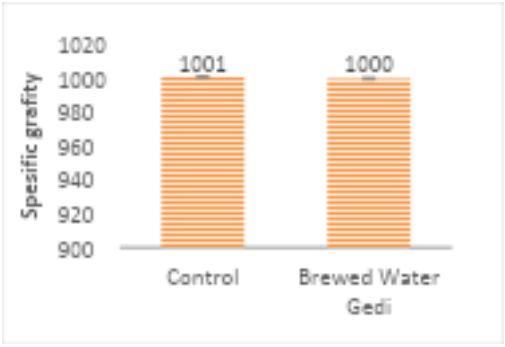


Figure 10. Effect of dry Gedi (A. manihot) leaf brewing water treatment against Urine specific gravity (BJU) content in the urine of rats (R. norvegicus).

f. Bilirubin

Bilirubin tests for control and treatment obtained the same results, namely 1 mg/dL. Bilirubin is normally not found in the urine of rats (negative) (Quesenberry & Carpenter, 2012). Bilirubin is a bile pigment produced by liver cells (Gandasoebrata, 2013). Bilirubin can be found in urine called bilirubinuria.

Hemoglobin that forms in urine bilirubin is caused by red blood cells that break down, and are subsequently released to distributed to the liver and then excreted through bile in the process of urine formation. The yellow pigment in the urine comes from the breakdown of hemoglobin in the liver. Bilirubinuria is an indication of damage to the liver organs (Nila et al. 2018).

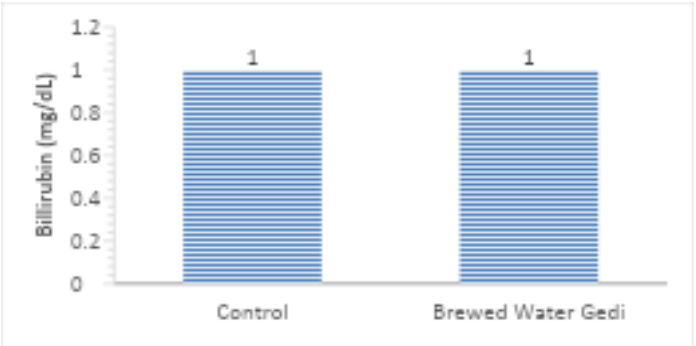


Figure 11. Effect of dry Gedi (A. manihot) leaf brewing water treatment against on Bilirubin content in the urine of rats (R. norvegicus)

g. Glucose

In this study, no glucose was found in the urine of normal rats, which is in accordance with (Price and Wilson, 2005) which stated that in the examination of glucose in normal rat urine, no glucose was found because in the renal tubules the process of reabsorption of glucose molecules will be carried out to re-enter the blood circulation. Glucosuria is a condition in which glucose is found in the urine. Glucose excretion in urine occurs when glucose levels in the blood increase and cannot be reabsorbed (Sherwood, 2014).

h. Blood

Hematuria is a symptom characterized by the presence of red blood cells or blood in the urine. Clinically, hematuria is grouped as follows: macroscopic hematuria is a condition in which urine mixed with blood is seen with the eye without an assistive device. This happens when 1 liter of urine is mixed with 1ml of blood. Microscopic hematuria is hematuri, which can only be confirmed using chemical tests or microscopically (Desen W, 2008).

i. Urobilinogen

The results of the normal positive urobilinogen dip tear test ranged from 0.2 – 1 mg/dL of urine (1 mg = about 1 EU). If the test results are negative or below normal values, it shows the presence of an abnormality, namely the presence of obstructive jaundice. If a positive result above normal shows an abnormality, it is also due to the presence of hepatocellular jaundice. Urobinogen was not found in observed rat samples. Normally it is not found in urine up to 0.1 mg/dL (Abebayehu, 2023)

Urobilinogen appears in the urine when there is an abnormality in bilirubin metabolism or liver function. The concentration of urobilinogen reported in various test strips ranged from 0.1-12 mg/dL (normal). The lowest concentration of urobilinogen detected by a common urine test strip is 0.1mg/dL. This test filters out urobilinogen in the urine. The results were considered together with urine bilirubin (Abebayehu, 2023).

j. Nitrite

Urine nitrite tests can be used to determine the presence or absence of bacteriuria. Urinary nitrites are the result of the metabolism of bacteria of the enterobacteriaceae group. Urine nitrites are the result of nitrates whose source comes from food. The conversion of nitrate to nitrite only occurs when the urine contains bacteria that can produce an enzyme called reductase nitrate. It is this nitrate reductase enzyme that converts the nitrates present in the urine into nitrites (Belay et al., 2019).

Table 5. Summary of urinalysis parameters and normal reference ranges in postpartum female white rats (Rattus norvegicus) following administration of dried Gedi (Abelmoschus manihot L.) leaf brewing water.

Parameter	Control	Treatment	Unit	Standard Normal Value	Reference
Leukocyte	15	15	WBC/µL	0–15 WBC/µL	Vilano (2012, as cited in Pandiangan et al., 2022)

Protein	20	25	mg/dL	Negative–Trace (<30 mg/dL)	Vilano (2012, as cited in Pandiangan et al., 2022)
pH	9	9	–	7.5–9.0	Han et al., 2010
Ketone	160	160	mg/dL	Negative (0–5 mg/dL)	Han et al., 2010
Specific Gravity	1.001	1.000	–	1.015–1.050	Han et al., 2010
Bilirubin	1	1	mg/dL	Negative (0 mg/dL)	Vilano (2012, as cited in Pandiangan et al., 2022)
Glucose	0	0	mg/dL	Negative (0 mg/dL)	Vilano (2012, as cited in Pandiangan et al., 2022)
Blood	0	0	RBC/µL	Negative	Vilano (2012, as cited in Pandiangan et al., 2022)
Urobilinogen	0	0	mg/dL	0–1 mg/dL	Han et al., 2010
Nitrite	0	0	mg/dL	Negative	Han et al., 2010

Table 5, summarizes all urinalysis parameters observed in postpartum female white rats following administration of Gedi (A. manihot L.) leaf brewing water. Overall, the urinalysis profile of the treatment group was comparable to that of the control group, with no marked differences observed in the evaluated parameters. Although a slight increase in urinary protein concentration was observed in the treatment group (25 mg/dL) compared with the control group (20 mg/dL), both values remained within the normal reference range (<30 mg/dL) reported by Vilano (2012, as cited in Pandiangan et al., 2022). In addition, leukocyte, glucose, blood, bilirubin, urobilinogen, and nitrite values showed no apparent differences between groups.

Urinalysis is widely used as a non-invasive tool for evaluating renal function, urinary tract integrity, hydration status, and metabolic alterations in laboratory animals (Fox et al., 2015; Suckow et al., 2019). Parameters such as urinary protein, glucose, blood, leukocytes, and bilirubin are commonly assessed because alterations in these indicators may reflect renal injury, urinary tract disorders, or systemic metabolic disturbances (Hall, 2016; Stockham & Scott, 2008). The comparable values observed between the control and treatment groups suggest that administration of Gedi leaf decoction was not associated with measurable changes in these urinary indicators during the study period.

The most notable findings were the elevated ketone concentrations and low urine specific gravity values observed in both groups. Urinary ketone concentrations reached 160 mg/dL in both control and treatment animals, exceeding the cited reference range. Under normal physiological conditions, ketones are absent or present only at trace levels in urine, whereas increased ketone excretion may occur during fasting, reduced feed intake, negative energy balance, or increased metabolic demands that promote lipid mobilization and ketogenesis (Gupta et al., 2009; Hall, 2016). The identical ketone concentrations

observed in both groups indicate that this condition was not unique to the treatment group and may have been influenced by physiological conditions common to all animals, including the increased metabolic demands associated with the postpartum period and the reduced feed and water consumption observed during the experiment (Bell, 1995; Neville et al., 2012).

Similarly, urine specific gravity values were low in both the control (1.001) and treatment (1.000) groups compared with the cited reference range. Urine specific gravity reflects the kidney's ability to regulate water balance through urine concentration and dilution and is influenced by hydration status, water intake, and renal tubular function (Hall, 2016; Stockham & Scott, 2008). In the present study, the low specific gravity values were not accompanied by increases in urinary protein, blood, glucose, or leukocytes, findings that might otherwise suggest renal dysfunction. Furthermore, water administration prior to urine collection may have contributed to urine dilution, resulting in lower specific gravity values (Pearce, 2006).

Previous observations reported several morphological and behavioral abnormalities in rats receiving Gedi leaf decoction, including paralysis, yellowish fur coloration around the muzzle, watery snouts, and abnormal movements (Pasaribu et al., 2024). However, these manifestations were not accompanied by corresponding alterations in the urinalysis profile. This finding suggests that the observed external signs were not reflected in measurable changes in urinary parameters and highlights the importance of integrating urinalysis results with other physiological, hematological, biochemical, and histopathological evaluations to obtain a comprehensive assessment of the biological effects and safety profile of Gedi leaf brewing.

Conclusion

Administration of Gedi (Abelmoschus manihot L.) leaf brewing in postpartum female white rats produced urinalysis profiles comparable to those of the control group. Most evaluated parameters, including leukocytes, protein, glucose, blood, bilirubin, urobilinogen, and nitrite, remained within normal reference ranges, with no meaningful differences between groups. Although elevated ketone levels and low urine specific gravity were observed, these findings occurred in both groups. Overall, no treatment-related alterations in urinalysis parameters were detected under the conditions of this study. These findings suggest a comparable urinary safety profile between groups, indicating that Gedi leaf brewing does not induce detectable urinary abnormalities in this experimental model. Further studies are needed to confirm these findings.

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