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EFFECTS OF GAMAL LEAF EXTRACT (*Gliricidia sepium* Jacq.) ON BODY WEIGHT AND INTERNAL ORGAN PERFORMANCE OF MICE (*Mus musculus* L.)

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Abstract: Gamal leaves (*Gliricidia sepium* Jacq.) contain toxic compounds that have the potential to act as vegetable rodenticides, such as alkaloids, tannins, dicumerol, and hydrogen cyanic acid (HCN). Coumarin compounds can bind vitamin K, disrupt the circulatory system, and damage liver cells. The compounds dicumerol and warfarin are used as rat poison because of their anticoagulant properties, which prevent blood clots in the heart. Alkaloid and tannin compounds can damage liver cells through liver necrosis and bleeding in the digestive system. This study aims to determine the effect of Gamal leaf extract on body weight and internal organ performance in mice (*Mus musculus* L.). The experimental research method is based on a Completely Randomized Design (CRD) pattern. A total of 16 mice weighing 20-22 grams consisting of 4 treatments, namely A0 (water control), A1 (extract 10 mg/g BW), A2 (extract 15 mg/g BW) and A3 (extract 20 mg/g BW). The extract was given orally for 14 days, and body weight was measured every 3 days to obtain data on changes in body weight (PBB). At the end of the treatment, the mice were sacrificed using chloroform and then dissected to observe the performance of the liver, kidney and heart organs in color appearance and organ weight. Quantitative data were analyzed using one-way ANOVA and further DMRT test α=0.05. The research showed that mice's mean change in body weight (PBB) decreased significantly as the dose of Gamal leaf extract increased. The percentage of liver organ weight (BOH), kidney organ weight (BOG), and heart organ weight (BOJ) decreased as the dose of Gamal leaf extract increased. The research results concluded that Gamal leaf extract caused color changes in the internal organs of mice and significantly affected the percentage change in body weight (PBB) and internal organ weight (BOD) of mice along with increasing doses of Gamal leaf extract.

Keywords: *Gliricidia sepium* Jacq., *Performance, Liver, Kidney, Heart, Mus musculus*

INTRODUCTION

Gamal leaves contain toxic compounds such as alkaloids, tannins, dicumerol, hydrogen cyanic acid (HCN), and coumarin compounds, which bind to vitamin K and can disrupt the circulatory system. The alkaloid compounds in Gamal leaves can damage several internal organs in mice because the alkaloid compounds require a long time to be excreted and metabolized by the body, so the contact between the alkaloid and liver cells takes longer and can damage liver cells. Tannin compounds can damage internal organs because these compounds can cause toxic effects in the form of liver necrosis and bleeding in the digestive system [1].

Measuring the body weight of mice is a measurable number that shows the physiological condition of mice. Body weight and internal organ weight of experimental rodents kept in the laboratory differ according to age and sex [2]. A decrease in feed consumption in animals will cause body weight to decrease due to the lack of nutritional intake in the body as an energy provider so that it cannot synthesize the components that make up the body ideally. It is believed that giving Gamal leaf extract containing active compounds will affect the performance of rodents' internal organs.

Toxic compounds can damage the performance of internal organs. One indicator of the performance of internal organs is the appearance of the color and weight of the internal organs. Normal internal organ performance in mice, liver, kidneys, and heart are generally bright red, while abnormal internal organ performance has a dark, pale color and a mottled surface. The normal liver weight of adult mice ranges from 2.05 grams [3]. The relative weight of a mouse's kidney is 0.4-0.9% of body weight, while the normal weight of an adult mouse's heart is around 0.6% of body weight [4]. Abnormalities in the internal organs of mice exposed to toxic substances can be characterized by changes in color from yellowish red to dark red [5].

Giving Gamal leaf extract can affect the performance of the internal organs in mice, namely the liver, kidneys, and heart. This is related to organ function. The kidneys function as an organ for excreting substances that the body does not need, the heart is a blood-pumping organ, and the liver functions as an organ that metabolizes toxins and very easily interacts with toxic compounds. Damage to internal organs in mice due to toxic substances can be influenced by several factors, including the type of substance involved, the size

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of the dose given, and the duration of exposure to the substance [6].

One alternative for controlling mice is using Gamal leaves as a vegetable rodenticide. The results of research by Tangendjaja (1991) showed that mice that were given 2500 ppm of coumarin compounds in their feed or only 25 ppm over one year caused severe liver damage [7]. Several reports state that gamal plant extract has biological activity as an anti-fungal and botanical insecticide. The results of research by Tariq et al. (2016) show that the dicumerol compound in gamal leaves is known to be toxic, causing poisoning effects in Bandicota bengalensis mice, so it can be concluded that the gamal plant can have an influence on several organs in rodents [8].

Botanical rodenticides are made from plants prepared using a mixture of other natural ingredients that function as an alternative to suppress rat pest populations. The gamal plant can be used as a vegetable rodenticide that suppresses rat pests by mixing it with wheat flour and seasonings [8]. People in Central America use the leaves and bark of Gamal mixed with boiled corn kernels as rodent poison. It is hoped that the results of this research will allow the Gamal plant to be used as an alternative plant-based rodenticide to reduce the explosive population of rat pests biologically in nature.

RESEARCH METHODS

Preparation of Test Animals

A total of 16 adult mice weighing around 20 grams were divided into 4 treatments, namely water control (A0), Gamal leaf extract at a dose of 10 mg/g BW (A1), a dose of 15 mg/g BW (A2) and a dose of 20 mg/g BW (A3). Mice were divided by giving a mark for each replication. Mice were placed in cages made of plastic basins with a wire cover on top. Mice were acclimated for 7 days and given pelleted food and water ad libitum. Mice were given 10 grams of food per animal daily every morning at 07.00 [10]. Lighting is provided for 12 hours of light and 12 hours of darkness, and the cage's cleanliness is maintained by changing the husks every 3 days. Before being given treatment, the mice were fasted to obtain the same physiological conditions. Then, the mice were weighed before treatment.

Extraction

Fresh Gamal leaves weighing 2 kg are washed and air-dried, then baked in the oven at 70℃ until dry. The dried gamal leaves are then ground using a blender until they become powder. Gamal leaf powder for a dose of 10 mg/g BW was weighed as 500 grams, a dose of 15 mg/g BW was weighed as 750 grams, and a dose of 20 mg/g BW was weighed as 1000 grams [11]. Gamal leaf powder is soaked in 70% ethanol and left for 48 hours, stirring occasionally. The soaking is filtered to separate the residue from the ethanol. The filter results are placed in a Rotary Evaporator at a temperature of 70℃ until the solvent evaporates and the ethanol is separated from the extract until no ethanol drips and a concentrated extract is obtained. Soaking is carried out for 48 hours to maximize the solvent's ability to attract active compounds [12].

Treatment

A total of 16 adult mice weighing around 20 grams were divided into 4 treatments, namely A0 (water control), A1 (Gamal leaf extract dose 10 mg/g BW), A2 (dose 15 mg/g BW) and A3 (dose 20 mg/g BB) Gamal leaf extract is given orally using a syringe every morning at 07.00 for 14 days. The observation variables are as follows:

a. Weight change

Data on changes in body weight were obtained by weighing the mice before administering Gamal leaf extract to obtain initial body weight. Every 3 days, the body weight is weighed to obtain the final body weight. The body weight data obtained was calculated using the Body Weight Change formula [10].

$$\text{Body Weight Change} = \frac{\text{Final BW ((g/head))} - \text{Initial BW (g/head)}}{\text{Length Of Rearing (days)}}$$

Keterangan:

Final BW = Body Weight During Maintenance

Initial BW = Initial Body Weight Before Treatment

b. performance of internal organs

The internal organ performance variables in the study were the external appearance/change in color and weight of the liver, kidney, and heart after surgery at the end of treatment. The surgical method was carried out according to the working procedure of [13], namely that the mice were anesthetized using chloroform, and the surgery started from the abdomen using surgical scissors. The chest was opened by cutting the ribs at the sternum, then each organ was taken and separated then the color appearance was observed and weighed. The organ weight data obtained was calculated using the internal organ weight change (PBOD) formula. The surgical method is according to the [13] procedure: Mice were placed in a container containing chloroformed cotton, covered tightly, and left for several minutes until the mice died. Mice were positioned on a surgical board using a pin, and surgery was carried out starting from the abdomen using surgical scissors. The chest space is opened by cutting the ribs at the sternum. Then, the organs are removed [14]. Percentage Change in Internal Organ Weight is

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calculated using the following formula [10].

$$\text{Percentage Change in Internal Organ Weight} = \frac{\text{Internal Organ Weight (g)}}{\text{Live BW (g)}} \text{x } 100\%$$

Information:
Live BW = Body weight of live mice

Data analysis

Data were analyzed using one-way ANOVA and the Duncan Multiple Range Test (DMRT) at a confidence level of 95% [15].

RESULTS AND DISCUSSION

Change in Body Weight

The results of observations of body weight per animal for 14 days are presented in Table 1.

Table 1. Mean Change in Body Weight (g/head) After Giving Gamal Leaf Extract

Treatment	Body Weight Average (g/head)
A0	22.53 ± 0.60^{c}
A1	18.09 ± 0.41^{b}
A2	17.69 ± 0.97^{ab}
A3	16.66 ± 1.19^{a}

Note: Numbers followed by different letters indicate there is a significant difference in the BNT test with a 95% confidence level

Table 1 shows that the change in body weight of mice given Gamal leaf extract in each treatment showed a decrease. It can be seen that the higher the dose of gamal leaf extract, the more the mice's body weight decreases. At a dose of 10 mg/g BW (A1), the result was a change in body weight of 18.09 g/head, a dose of 15 mg/g BW (A2) of 17.69 g/head and a dose of 20 mg/g BW (A3) of 16.66 g/head. In the water control (A0), the body weight was 22.53 g/head. Based on these data, it is known that Gamal leaf extract causes weight loss in mice. This is by research by Nurpalinri *et al*. (2023) that the active compounds contained in Gamal leaf extract have quite an effect on reducing the weight of mice as indicated by the excretion of quite a lot of feces and urine due to food cannot be digested completely [16].

The reduced body weight of mice was caused by the active compounds such as alkaloids, tannins, and HCN contained in Gamal leaf extract disrupting the mice's digestive process. This is to research put forward by Muliani (2011), that the cause of the decrease in weight of mice is due to disruption of the digestive process due to toxic substances entering the body, which causes mice to need much energy to neutralize these toxic substances so that growth does not occur which causes weight gain. The body of mice decreases [17]. Highly toxic substances will cause cell damage and even death. The large number of dead cells will disrupt metabolic processes in the body, which can disrupt the normal function of organs, ultimately reducing growth and weight loss [17].

The change in mice's body weight may also be due to the mice experiencing stress due to the distinctive smell of Gamal leaf extract, thereby reducing feed consumption. When the extract was given, the mice experienced changes in behavior. Namely, they immediately became limp after being force-fed, and their breathing was irregular, like convulsions. However, this incident was only momentary because the mice could move normally again. This is likely the sharp smell of Gamal leaf extract, which mice do not like. Symptoms of poisoning can be seen in the behavior of mice when they are force-fed, drinking lots of water rather than eating pellets. At a treatment dose of 20 mg/g BW (A3), 1 mouse died on the seventh day after treatment. This death was probably due to stress, which resulted in physiological disorders in the mice's bodies.

Internal Organ Performance

The effect of Gamal leaf extract on the internal organs of mice in this study can be seen from the color appearance and percentage change in weight of the mice's liver, kidneys, and heart.

a. Liver organ weight

The results of observations of the average percentage change in liver organ weight (%PBOH) in all gamal leaf extract treatments are presented in Table 2.

Table 2. Mean Percentage Change in Liver Organ Weight After Giving Gamal Leaf Extract

Treatment	Liver Organ Weight Mean ± SD
A0	8.47 ± 0.13^{a}
A1	10.14 ± 0.21^{b}
A2	10.00 ± 0.49^{b}
A3	10.98 ± 0.13^{c}

Note: Numbers followed by different letters indicate there is a significant difference in the BNT test with a 95% confidence level

Table 2 shows that the average percentage change in liver organ weight was the highest in treatment A3, 10.98%. This is due to the high dose so that the average weight of the liver organ is lower, which results in a greater percentage change in the weight of the liver organ. This is to Hasana's (2019) statement that liver damage due to toxic substances can be influenced by several factors, including the type of substance involved, the size of the dose given, and the duration of exposure to the substance [6]. At high doses, Gamal leaf extract can reduce the production of enzymes in

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synthesizing protein so that liver muscle tissue shrinks, resulting in a high percentage change in the weight of the liver organ. The liver is the main target organ for the toxic effects of chemical substances because it detoxifies the chemical substances that enter the body through the digestive tract.

A healthy liver organ is bright red, while an unhealthy liver organ is brown to dark red. This is by Hasana et al., (2019) that mouse livers are generally bright red, while abnormal livers have a dark, pale color and a spotted surface [6]. Liver abnormalities exposed to toxic substances can be characterized by changes in liver color from yellowish red to dark brown [5]. The appearance of the liver organ after administration of Gamal leaf extract between treatments can be seen in Figure 1.

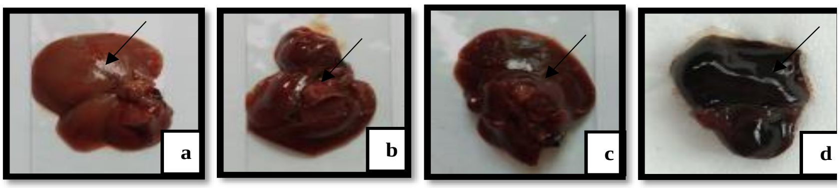

Figure 1. The appearance of the liver of mice after administration of Gamal leaf extract a. Liver A0; looks bright red, b. Liver A1; red-brown, c. Liver A2; red-brown, d. Liver A3: dark red color. (Personal Doc, 2020).

b. Kidney organ weight

The results of observations of the average percentage change in kidney organ weight in all Gamal leaf extract treatments are presented in Table 3 below.

Table 3. Mean Percentage Change in Kidney Organ Weight After Administration of Gamal Leaf Extract

Treatment	Kidney Organ Weight Mean ± SD
A0	0.87±0.02^{bc}
A1	0.89±0.01^{c}
A2	0.85±0.02^{b}
A3	0.78±0.02^{a}

Note: Numbers followed by different letters indicate there is a significant difference in the BNT test with a 95% confidence level

Table 3 shows that the lowest mean percentage change in kidney organ weight (PBOG) was in the treatment dose of 20 mg/g BW (A3), 0.78 g/head. Nugroho (2018) stated that macroscopic changes in the kidneys tend to increase according to the increase in dose given. The higher the concentration, the greater the response, which ultimately reduces the weight of the kidneys due to damage to the tissues that make up them. The decrease in kidney weight was caused by the active compounds in Gamal leaf extract in the form of alkaloids, tannins, HCN, and dicumerol, which were able to interfere with physiological processes in the mice's bodies, resulting in a decrease in kidney weight. Tannin is a compound that can form complex compounds by binding to proteins. Tannins have a spasmolytic effect, which can shrink cell walls or cell membranes, thereby disrupting the permeability of cell membranes, resulting in cells being unable to carry out living activities so that growth is hampered or even dies. Alkaloids are metabolically toxic because they take a long time to be metabolized and excreted, so contact between the alkaloid and liver cells takes longer and damages the liver cells. The alkaloid and tannin compounds of Gamal leaves are antifeedant, so they reduce mice's appetite and cause weight loss. [16].

The coumarin compound can cause liver damage in mice with its killing power expressed in the LD50 of 680 mg/kg body weight in mice. Several coumarin derivative compounds, such as dicumerol and warfarin, are widely used as rat poison or to prevent blood clots in the human heart because these compounds have anticoagulant properties [10]. The change in kidney color to dark is probably due to the presence of alkaloid compounds, tannins, and HCN in Gamal leaves, which causes changes in the color and weight of the kidney organ, resulting in disruption in the metabolic process of toxic substances in the proximal tubule, this is due to active absorption that occurs in the proximal tubule which has an impact in the occurrence of changes in the color and weight of the kidney organs [20].

The kidneys are shaped like brownish-red beans and change color to dark when exposed to toxic substances. This is by research by Septiva (2019) that macroscopic observations of the kidneys show that the color of the internal organs becomes dark and the weight of the organs decreases when toxic test materials are administered [20]. The appearance of the kidney organs between treatments after administration of Gamal leaf extract is shown in Figure 2.

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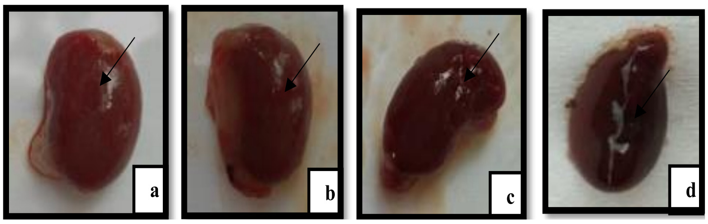

Figure 2. The appearance of the kidneys of mice after administration of Gamal leaf extract. a. Kidney A0 is bright red, b. kidney A1 is red-brown, c. kidney A2 is red-brown, d. A3 kidneys are dark red (Personal Doc, 2020).

The appearance of the kidney organ in Figure 2 shows that the color changes in the kidney organ in this study showed a striking color difference between the control and the Gamal leaf extract treatment. The change in color of the kidneys to dark is due to the presence of alkaloid compounds, tannins, and HCN in Gamal leaves, which cause changes in the color and weight of the kidney organs, resulting in disturbances in the metabolic process of toxic substances in the proximal tubule, this is due to active absorption in the proximal tubule which has an impact on changes—color and weight of kidney organs [19]. Alkaloids in the form of salts (crystals) can degrade cell membranes to enter and damage cells and the nervous system by inhibiting the action of the acetylcholinesterase enzyme. Coumarin compounds can bind vitamin K, disrupt the circulatory system, and damage liver cells. The compounds dicumerol and warfarin are used as rat poison because of their anticoagulant properties, which prevent blood clots in the heart. Alkaloid and tannin compounds can damage liver cells through liver necrosis and bleeding in the digestive system. The entry of alkaloid compounds into the kidney through the process of absorption by the glomerulus, toxic substances trapped in the kidney glomerulus will trigger an inflammatory response so that the glomerulus to become blocked when inflammation occurs and the unblocked glomerulus becomes more permeable, allowing protein and red blood cells to leak from the glomerular capillaries. and enter the glomerular filtrate. The occurrence of abnormalities in glomerular filtration function can cause damage to the components that make up the kidney, such as narrowing of Bowman's space in the glomerulus, which has an impact on changes outside the kidney [20].

Active compounds enter the kidney through the process of absorption by the glomerulus. Toxic substances trapped in the kidney glomerulus will trigger an inflammatory response so that the glomerulus becomes blocked when inflammation occurs, and the glomerulus, which is not blocked, becomes more permeable, allowing protein and red blood cells to leak from the glomerular capillaries. and enter the glomerular filtrate. The occurrence of abnormalities in glomerular filtration function can cause damage to the components that make up the kidney, such as narrowing of Bowman's space in the glomerulus, which has an impact on changes outside the kidney [20].

c. Heart organ weight

The results of observations of the average percentage change in heart organ weight in all Gamal leaf extract treatments are presented in Table 4 below.

Table 4. Mean Percentage Change in Heart Organ Weight After Giving Gamal Leaf Extract

Treatment	Heart Organ Weight Mean ± SD
A0	0.69 ± 0.01^{b}
A1	0.77 ± 0.02^{c}
A2	0.69 ± 0.04^{b}
A3	0.62 ± 0.06^{a}

Note: Numbers followed by different letters indicate there is a significant difference in the BNT test with a 95% confidence level

Based on Table 4, it is known that Gamal leaf extract significantly influences changes in the weight of the heart organ. The lowest average percentage change in weight of the heart organ was at a dose of 20 mg/g BW (A3) of 0.62%. Puspitasari et al. (2019) stated that the heart is easily damaged due to the influence of chemical compounds. The heart can be damaged by various substances acting directly on the organs, the central nervous system, or blood vessels [19]. A decrease in the heart organ's weight can indicate damage to the heart organ.

The decrease in heart weight is thought to be due to the active compounds in Gamal leaf extract in the form of alkaloids, tannins, HCN, and dicumarol, which can disrupt physiological processes in the body, resulting in a decrease in the weight of the heart organ. The color appearance of the heart organ after administration of Gamal leaf extract is presented in Figure 3 .

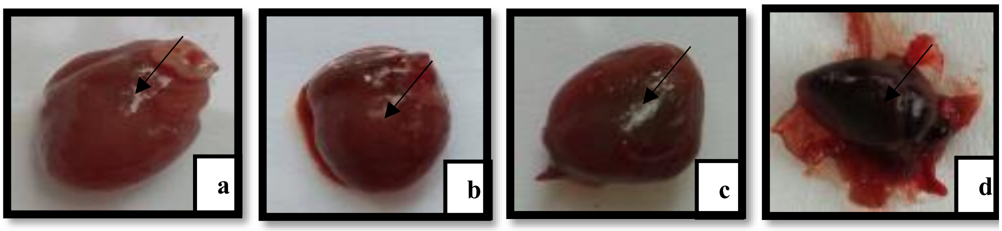

Figure 3. The appearance of the mouse heart after administration of Gamal leaf extract. a Heart A0 is bright red, b. Heart A1 brownish red, c. Heart A2 brownish red, d. Dark red A3 heart. (Personal Doc, 2020)

The heart is an organ that can be a target for toxic substances because the heart can accumulate xenobiotic substances [6]. Giving Gamal leaf extract to mice affects the weight of the heart organ. This is because the alkaloids, tannins, and HCN content in Gamal leaves causes a reduction in heart weight, disrupting the blood pumping process because blood contains toxic substances. Alkaloids and tannins are compounds that, when they enter the body, will undergo absorption, distribution, metabolism, and excretion processes. The intestines will absorb the alkaloid and tannin compounds in Gamal leaf extract, then metabolize them in the liver. The metabolic results of Gamal leaf extract will be distributed throughout the body, including the heart, resulting in color changes [18]. Tannin compounds also cause the death of mice. Tannin can cause disconnection (uncoupling) of the respiratory chain in mitochondria. This causes ATP production to decrease, resulting in cell injury because cell survival depends on oxidative metabolism in the mitochondria. The HCN compound will prevent cell energy production by deactivating the cytochrome oxidase enzyme. Inactivation of cytochrome oxidase will prevent cells from using oxygen so that cells will die quickly. When tissues are unable to absorb oxygen, the condition is called histotoxic hypoxia, one of which is caused by cyanide poisoning. The extract containing the active compound saponin given to mice (Mus musculus L.) orally at high doses resulted in black spots on the heart. It affects the weight of the heart organ[17].

CONCLUSION

Based on the research results, it was concluded that Gamal leaf extract (*Gliricidia sepium* Jacq.) had a significant effect on weight loss and the performance of internal organs in mice (*Mus musculus* L.), namely color changes and a decrease in the weight percentage of the liver, kidneys, and heart.

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