JEJUNUM HISTOLOGICAL PROFILE OF WISTAR MALE RATS AFTER FEEDING *GADUNG (DIOSCOREA HISPIDA*) TUBER BLOCK BAIT

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ABSTRACT

Rodent management practices routinely involve the use of chemical products, mainly anticoagulant rodenticides (ARs). Chemical rodenticide certainly has an efffect to the enviromental such as living residues. Gadung tuber contain secondary metabolites dioscorine and cyanide acid which cause damage to the structure and function of visceral organs. The aim of this study was to determine the effect of gadung tuber block bait on the histological profile of the jejunum of Wistar male rats. The method used is an experimental method with a Completely Randomized Design (CDR). The test animals consisted of 24 male rats with an average weight of ±180 g grouped into 4 treatment groups namely KN (block bait without gadung tuber), P1 (30% gadung tuber block bait), P2 (50% gadung tuber block bait), and P3 (70% gadung tuber block bait). The treatment was given for 4 days and on the 5th day, the rats were sacrificed by neck dislocation. Furthermore, jejunum were isolated and histological preparations were made. Parameters observed were the number of goblet cells and the severity of epithelial necrosis on histological incisions. Data on the number of goblet cells were analyzed using ANOVA and Duncan, while data on the severity of epithelial necrosis were analyzed using the Kruskal Wallis test and the Mann Whitney test. The results showed that gadung tuber significantly decreased the number of goblet cells and increased the severity of jejunal epithelial necrosis. It can be concluded that Gadung tuber has the potential to be developed as a natural rodenticide with an effective concentration of 50%.

Keyword: *Gadung tuber, Histology of jejunum, rodenticide.*

1. INTRODUCTION

Wild animals such as rats can become pests because they can cause disadvantages in human health, agriculture, animal husbandry, and other fields. The many disadvantages caused by rats are the main reason for the importance of rat pest control by humans. Rat pest control that is commonly used is synthetic rodenticides because they are considered more effective than other methods [1]. Synthetic rodenticides have many advantages but are difficult to decompose in nature, so their residues can pollute the environment, kill non-target animals, and cause pest resistance [2]. As a result, more environmentally friendly pest control for rats is required.

An alternative that can be used as a substitute for synthetic rodenticides is plant-based rodenticides, namely natural rodenticides. Plants are considered as a substitute for synthetic rodenticides because they contain secondary metabolites. The effectiveness of vegetable rodenticides is not as effective as synthetic rodenticides, but they are relatively safer and also environmentally friendly. The compounds contained in plants are easily broken down by microorganisms, easily soluble in water, and easily broken down when oxidized and exposed to sunlight, so that the residue is easy to remove and will not kill non-target organisms [2].

Gadung tuber (*Dioscorea hispida* Dennst.) is one of the plants that can be used as a natural rodenticide because it contains secondary metabolites that dissolve easily in water, so it will not leave residue or pollute the environment.

Several studies have proven the effectiveness of Gadung tuber as a vegetable rodenticide. Gadung tuber in the form of block bait with a concentration of 70% caused 100% death of rat on the ninth day of treatment [3]. Gadung tuber with a concentration of 30% mixed with feed also caused 55.56% rat mortality on the fifth day of treatment. Secondary metabolite compounds contained in Gadung tubers include dioscorine and cyanide acid [4].

Dioscorine is a neurotoxic alkaloid compound that acts as an anticholinesterase, which is a chemical compound that inhibits the action of acetylcholinesterase in breaking down the neurotransmitter acetylcholine, which can cause dizziness, nausea, seizures, and fatal paralysis of the nervous system [5]. Dioscorine is also known to damage visceral organs and increase blood pressure in rats [6]. Cyanide acid (HCN) contained in Gadung tuber in the form of cyanogenic glycosides can inhibit the use of oxygen at the cellular level, resulting in cellular dysfunction which can develop into cell death and cause organ failure [7]. The body that is exposed to toxic substances in high concentrations or doses will cause damage to cell death thereby disrupting metabolic functions and ultimately disrupting normal organ function, inhibiting growth, and reducing body weight [1].

Secondary metabolites in Gadung tuber that enter the rat's body orally will pass through the small intestine for absorption. Jejunum as a part of the small intestine, functions as a primary absorption site for nutrients such as carbohydrates, fats, and amino acids [8]. Jejunum is one of the organs that has the most potential to be damaged by secondary metabolites of Gadung tuber because of this function. Several studies state that the secondary metabolites of Gadung tuber can cause damage to the morphology of the small intestine. The small intestine of rats turns black after being given Gadung tubers [6]. In another study, it was also stated that rats fed Gadung tuber blocks had dark red intestines which indicated bleeding [3].

Previous studies have shown the effect of gadung tuber on the morphology of the small intestine, but there has been no research discussing its histological effect on the small intestine, especially the jejunum as the primary site of absorption of nutrients. Histological changes in the small intestine can be observed through the number of goblet cells and the severity of epithelial necrosis [9. 10]. Therefore, this study was conducted with the aim to determine the effect of gadung tuber block bait on the histology of the rat jejunum by using parameters of goblet cell count and severity of epithelial necrosis. Gadung tuber is made in the form of block bait because block bait is a form of rodenticide that is commonly circulating in the market which aims to protect toxic substances from various external environmental factors [11].

2. Materials and Methods

2.1 Block bait production

There are four steps in making block bait per 1000 grams. The first step is the preparation of block bait materials. The ingredients for making block bait are rice, corn flour, granulated sugar, grated coconut, gadung tubers, and paraffin. The gadung tubers used in this study were obtained from Kampung Simpang, Desa Tambak Mekar, Cagak District, Subang. The next step is the production of gadung tuber simplicia. The gadung tuber is washed, then cut into small pieces and crushed using a blender. The next step is the manufacture of block bait. The block bait is being made based on research by Murjani with modifications [12]. The composition is shown in Table 1. The final step is block bait molding. 5 grams of the mixture were put into a 2 x 2 x 2 cm mold and allowed to stand for 15 minutes. The block bait was removed from the mold and dried for 60 minutes.

Table 1. Block bait composition

Treatment	Composition					
	Gadung tuber (gram)	Rice (gram)	Corn flour (gram)	Paraffin (gram)	Granulated sugar (gram)	Grated coconut (gram)
KN	0	300	200	300	50	150
P1 (30%)	300	210	140	210	35	105
P2 (50%)	500	150	100	150	25	75
P3 (70%)	700	90	60	90	15	45

2.2 Aclimatization of test animals

The test animals were acclimatized for seven days before being treated so they could adapt to the laboratory environment. Rats were acclimatized in 30 x 25 x 10 cm cages at a temperature of 22-30 ^{o}C. Rat were given ±20 grams of standard feed pellets (CP 551) and drinking water (aquadest) ad libitum. The bedding of the cage is also routinely replaced three times a week.

2.3 Treatment

The research was carried out in the Pharmacology and Therapeutic Laboratory, Eijkman Building, Unpad Faculty of Medicine Hospital, Bandung and the Structure and Function Laboratory Biology Department of the Faculty of Mathematics and Natural Sciences Unpad, Jatinangor in October – December 2022. Treatment was given for four consecutive days. Each treatment was given block bait according to the respective concentration of 10% of the weight of the rats, which is ±20 grams [13]. The remained block bait is weighed every day, so that the weight of the block bait consumed can be known [3]. Body weight and wet weight of rat feces were also weighed every day.

2.4 Preparation of jejunum histological slides

Rats were injected with ketamine at a dose of 70 mg/kg BW and killed on day 5 by neck dislocation, and then the jejunum and spleen of the rats were isolated. Organs were washed with NaCl solution 0.9% to remove residual blood and dried. The organs were then weighed and histological slides were made. Preparation of jejunum histological slides were carried out using the paraffin method. The tissue was sliced using a rotary microtome with a thickness of 5 µm, then stained using the hematoxylin eosin (HE) technique.

2.5 Histological observation of jejunum

Histological observation of the jejunum was carried out by observing two parameters, namely the number of goblet cells and the severity of epithelial necrosis. Parameters of the number of goblet cells were counted on 20 villi with a magnification of 400x [9]. The parameters of the severity of epithelial necrosis were observed in 5 different fields of view with a magnification of 400x and were assessed based on the assessment criteria in the study of Mordue et al. (2001) with modification [14]

Table 2. Parameters of epithelial necrosis severity

Score	Criteria
0	In one field of view, no necrosis was found in the observed part
1	In one field of view found 1-20% necrosis in the observed section
2	In one field of view found 21-50% necrosis in the observed section
3	In one field of view found 51-75% necrosis in the observed section
4	In one field of view found more than 75% necrosis in the observed section

2.6 Histological observation of jejunum

The results of histological observations of the jejunum were then statistically analyzed using the IBM SPSS Statistics 25 program. Data on the number of goblet cells were tested by ANOVA test and if there was a significant difference it was continued with Duncan test. Meanwhile, the data on the severity of jejunal epithelial necrosis was tested with the Kruskal Wallis test and if there was a significant difference it was continued with the Mann Whitney test.

3. Results and Discussion

3.1 Histological picture of jejunum Wistar male rats after feeding gadung tuber block bait

In the histological picture of the jejunum treated with KN (Fig. 1.a), jejunal villi with clearly visible goblet cells, normal epithelial cells, and a few necrotic cells were found. This is compatible with the normal histological structure of the jejunum which consists of villi, which are finger-shaped permanent extensions of the lamina propria and the tunica mucosal epithelium of the jejunum. The function of villi is to increase the surface area of the small intestine, thereby expanding the area of absorption [15]. Epithelial cells in the tunica mucosa of the jejunum consist of 7 types of cells, namely absorptive enterocyte cells, enteroendocrine cells, Paneth cells, tuft cells, cup cells, M cells, and goblet cells which play a role in producing mucin as a protective layer and lubricant for the jejunum [16]. In the P1, P2, and P3 treatments (Fig. 1.b, c, d) jejunal villi with fewer goblet cells were observed compared to the KN treatment. In Fig. 1.b, c, d, it can also be observed that necrosis cells are more numerous than Fig. 1.a. This shows that there is an effect of feeding gadung tuber blocks on the histological profile of the rat's jejunum. Damage that occurs in the histological profile of the jejunum can be observed through the number of goblet cells and the severity of epithelial necrosis [9].

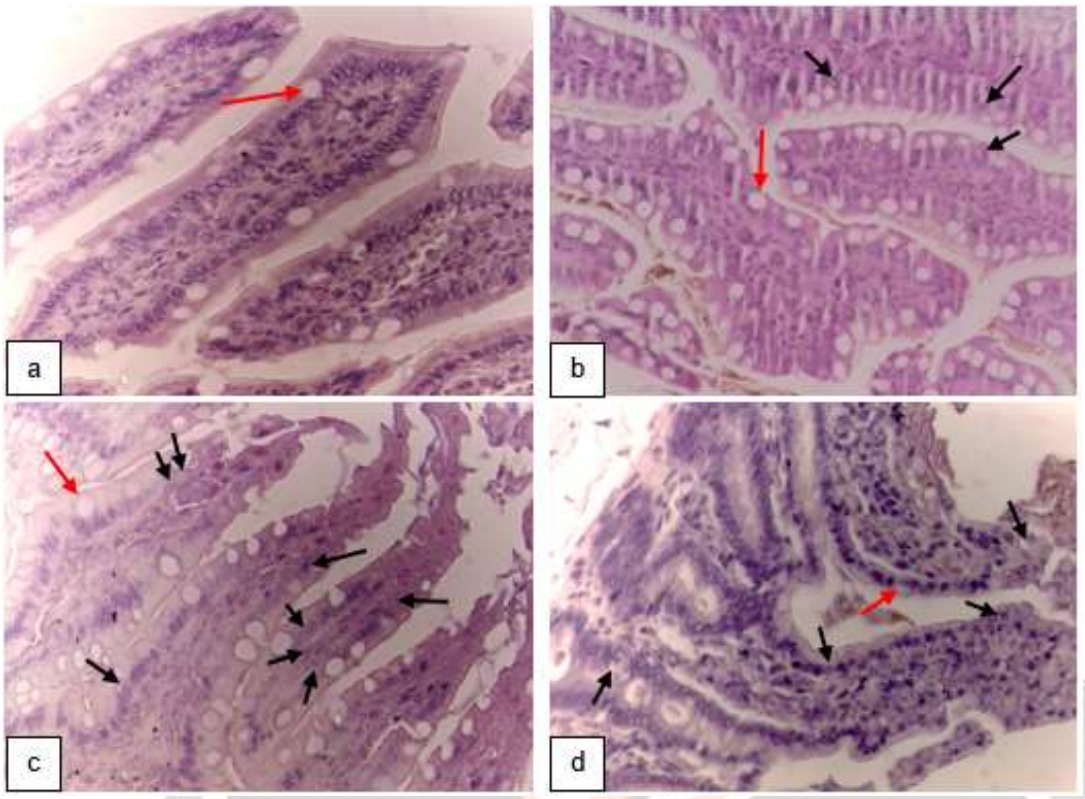

Fig 1. Jejunum histology of male Wistar rats from each treatment with HE staining and 400x magnification.
Notes: a) KN (block bait without gadung tubers); b) P1 (30% gadung tuber block bait); c) P2 (50% gadung tuber block bait); d) P3 (70% gadung tuber block bait). (⟶) Necrotic cells; (⟶) Goblet cells.

3.2 Average number of goblet cells and severity of epithelial necrosis in histological profiles of jejunum wistar male rats after feeding gadung tuber block bait

Based on the image of the histology of jejunum, the number of goblet cells and the severity of epithelial necrosis were counted. The average number of goblet cells and the severity of epithelial necrosis is presented in Table 3.

Table 3. Average Number of Goblet Cells and Severity of Jejunum Epithelial Necrosis in Rats After Treatment

Treatment	Parameter	
	Number of goblet cells	Severity of epithelial necrosis
KN (block bait without gadung tuber)	$204,33 \pm 70,33^{a}$	$0,64 \pm 0,29^{a}$
P1 (30% gadung tuber block bait)	$149,17 \pm 89,37^{ab}$	$2,8 \pm 1,47^{b}$
P2 (50% gadung tuber block bait)	$78 \pm 50,18^{bc}$	$3,16 \pm 1,17^{b}$
P3 (70% gadung tuber block bait)	$55,17 \pm 35,38^{c}$	$3,4 \pm 1,20^{b}$

Note: Data is presented as mean ± SD. Data analysis on the number of goblet cells used the ANOVA test with a 95% confidence level and Duncan test. Data analysis on the severity of epithelial necrosis used the Kruskal Wallis test with a 95% confidence level and the Mann Whitney test. Different letters in one column indicate a significant difference ($p<0.05$).

The results of the ANOVA test with a confidence level of 95% show that F_{count} (6.666) > F_{table} (3.10). This means that the treatment has a significant effect on the number of goblet cells. Duncan's test results in table 3 show that the number of goblet cells in treatment P2 (78 ± 50.18) and P3 (55.17 ± 35.38) was significantly different from KN (204.33 ± 70.33), but P1 (149 .17 ± 89.374) was not significantly different from KN. Treatment P2 had a number of goblet cells that were not significantly different from P3.

The results of the Kruskal Wallis test with a confidence level of 95% indicate that X^2 count (13.447) > X^2 table (7.814). This means that the treatment has a significant effect on the severity of epithelial necrosis. The results of the Mann Whitney test in table 4.1 show that the severity of epithelial necrosis in treatment P1 (2.8 ± 1.47), P2 (3.16 ± 1.17), and P3 (3.4 ± 1.20) was significantly different with the KN treatment (0.64 ± 0.29), while there was no significant difference between the P1, P2, and P3 treatments.

3.3 Discussion

Duncan test results showed that the number of goblet cells in P2 and P3 was significantly different from KN, but P1 not significantly different from KN. This means that the feeding of gadung tuber blocks with a concentration of 50% and 70% had a significant effect on the number of goblet cells in the rat's jejunum. Concentrations of 50% and 70% can cause a decrease in the number of goblet cells which are significantly different from KN because the higher the concentration of toxic compounds, the higher the content [17]. The histological profile of damage becomes higher at high concentrations of toxic compounds. concentration of 30% did not have a significant difference compared to KN presumably due to the low concentration of toxic compounds. P2 had a number of goblet cells that were not significantly different from P3. This shows that gadung tuber block with a concentration of 50% and 70% has a comparable ability to affect the number of jejunal goblet cells in rats.

The decrease in the number of goblet cells occurred due to damage to the mucous layer of the jejunum due to toxic secondary metabolites of gadung tuber, namely dioscorine and cyanide acid. Goblet cells are one of the main cells of the intestinal mucosal epithelium that synthesize and secrete mucin to create the mucus layer. The intestinal mucus lining secreted by goblet cells serves as the first line of defense against physical and chemical injury caused by invading pathogens, toxins, and other environmental irritants, maintains intestinal mucosal homeostasis, and provides lubricant to aid digestion of food. The interaction between the mucous layer, intestinal epithelial cells, intestinal microbiota, and the immune system is very important to maintain intestinal mucosal homeostasis. Disturbances in intestinal homeostasis can be caused by invading pathogens, toxic compounds, and other environmental irritants [18]. Cyanide acid, which is toxic, is known to cause death of intestinal epithelial cells due to inhibition of oxygen use at the cellular level [7]. Dioscorine can affect goblet cell secretion by acting as an anticholinesterase, which is a compound that inhibits the action of acetylcholinesterase in breaking down acetylcholine in the nervous system [5]. Dioscorine can bind to acetylcholinesterase, so that acetylcholine cannot be broken down and accumulates between synapses. This causes continuous stimulation of the muscarinic acetylcholine receptors which can cause hypersecretion and increase peristaltic activity of the gastrointestinal tract [19]. Disturbances in intestinal homeostasis due to toxic compounds can cause damage to the mucous layer and is followed by increased permeability which results in inflammation and injury to the intestinal mucosal layer [18]. Inflammation can cause damage to the intestinal mucosal layer and loss of goblet cells due to epithelial erosion [20].

The results of the Kruskal Wallis test with a confidence level of 95% indicate that X^2 count (13.447) > X^2 table (7.814). This means that the treatment has a significant effect on the severity of epithelial necrosis. The results of the Mann Whitney test in table 4.1 show that the severity of epithelial necrosis in treatment P1, P2, and P3 was significantly different with the KN treatment. This means that the feeding of gadung tuber blocks with concentrations of 30%, 50%, and 70% had an effect on the severity of rat jejunal epithelial necrosis. The severity of epithelial necrosis between the P1, P2, and P3 treatments did not have a significant difference. This indicates that gadung tuber block baits with concentrations of 30%, 50%, and 70% have comparable abilities to affect the severity of rat jejunal epithelial necrosis.

Necrosis that occurs in the intestinal epithelium is also caused by dioscorine and cyanide acid in gadung tubers. Necrosis is irreversible cell or tissue death due to cell degeneration that cannot be repaired. Necrosis can be caused by toxic compounds, metabolic disorders, and viral infections. Toxic compounds in general can cause reduced ATP, loss of calcium ions (Ca^{2+}), damaged cytochrome enzymes, decreased NAD and NADP, and disrupt the balance of sodium (Na^{+}) and potassium (K^{+}) ions, resulting in necrosis [21]. Dioscorine is known to inhibit the action of enzymes that play a role in the digestive process, causing metabolic disorders in the body which can lead to necrosis [22]. Cyanide acid is known to inhibit the use of oxygen at the cellular level, resulting in necrosis. This compound has a molecular structure similar to oxygen, so it has the ability to bind to iron ions (Fe^{3+}) from cytochrome oxidase a3 in mitochondria. The bond that is formed between cyanide and iron ions causes inhibition of oxygen utilization by cytochrome oxidase a3 and results in a decrease in the amount of ATP produced. The cell then performs anaerobic metabolism to produce ATP due to the inability to utilize oxygen. Anaerobic metabolism produces little ATP and can cause lactic acidosis. This results in cellular dysfunction which can develop into cell death [7]. The research results obtained are in accordance with the research of Saddamiah [23] who proved that cyanide in the form

of cyanogenic glycosides contained in the ethanol extract of cassava leaves at a dose of 2000 mg/kg BW causes hydropic degeneration, parenchymatous degeneration, and necrosis in rat livers.

The decrease in the number of goblet cells and increase in the severity of epithelial necrosis indicated damage to the histological profile of the rat's jejunum. The number of goblet cells decreased with increasing concentration of gadung tuber block bait while the severity of epithelial necrosis increased. This indicates an increase in damage to the histological profile of the jejunum along with an increase in the concentration of gadung tuber block bait. Gadung tuber block bait concentrations of 50% and 70% had comparable ability to cause a decrease in the number of goblet cells and an increase in the severity of epithelial necrosis. The ideal rodenticide is one that is highly toxic to rats in small quantities [24]. Therefore, 50% concentration of gadung tuber block bait was the most effective concentration in influencing the histological profile of the jejunum compared to other concentrations.

The results showed that the decrease in the number of goblet cells and the increase in the severity of epithelial necrosis in the jejunum was caused by toxic compounds contained in gadung tubers, so that the histological profile of the jejunum was damaged. Based on the results of the study, it can be concluded that gadung tuber block bait with a concentration of 50% was the most effective concentration in influencing the histological profile of the jejunum of male Wistar rats.

4. CONCLUSIONS

Gadung tuber significantly reduced the number of goblet cells and increased the severity of jejunal epithelial necrosis. It can be concluded that gadung tuber has an effect on the histological profile of the jejunum of male Wistar rats. The most effective concentration of gadung tuber block bait in influencing the histology of the jejunum of male Wistar rats is 50%.

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