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Histological Structure of Male Wistar Rats' Stomach Fed with Yam Tuber Flour Supplementation

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Abstract

A residential rat might harm human health because it acts as a disease reservoir. It has been many efforts to control this rate using synthetic rodenticide. Nevertheless, synthetic rodenticides broke the environment and made rats resistant. Yam (*Dioscorea hispida*) tube application on rat's bite could solve the problem. This study evaluated the histology of male Wistar rats (*Rattus norvegicus*) fed with bite block supplemented with different yam tuber flour concertation to control residential rat populations. Five different treatments were applied with five replications. The treatments were negative control and brodifacoum 0.005% (positive control), 30%, 50%, and 70% of yam tuber flour. The results show that yam tuber supplementation caused damage to male Wistar rat stomachs, as indicated by mucosal erosion and the presence of inflammatory cells. The statistical test indicated that stomach damage significantly differed among treatments, with the severest damages caused by 50% yam tuber supplementation. It could be concluded that the rat's bite containing yam tuber flour caused stomach damage, and the feed bite containing 70% yam tuber flour was the most effective. This result proved that yam tuber has good potential as a natural rodenticide to control residential rat populations.

Keywords: disocorin, gastric, residential rat, rodenticide, yam

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Introduction

Rats are one of the pests in the agricultural industry, so it is economically very detrimental (Shiels *et al*., 2014; Adeshina & Emikpe, 2020). In addition, rats can act as a reservoir for various disease-causing organisms that can be zoonotic (Younis *et al*., 2021). Several diseases transmitted through rats and mice include rickettsiosis, leishmaniasis, spirochetes, tularemia, leptospirosis, tick-borne encephalitis, and listeriosis, which can cause endemic outbreaks in an area (Boey *et al*., 2019; Prompiram *et al*., 2020). This phenomenon has prompted the Ministry of Health of the Republic of Indonesia to include rats as one of the disease-causing animals that must be controlled, as stated in Minister of Health Regulation No. 50 of 2017, especially rats that live in residential areas, offices, and hospitals. This effort must be made to prevent the spread of various dangerous diseases (Younis *et al*., 2021).

So far, rat control has been carried out using synthetic rodenticides. Synthetic rodenticides contain active compounds with acute ingredients, such as zinc phosphide and chronic compounds, such as bromadiolone and brodifacoum. Acute ingredients are chemicals that take effect suddenly, in a short time and are usually causing severe indications. Chronic compounds are chemicals that do not directly react or kill the target animal. These compounds function as anticoagulants (Nosal *et al*., 2020), which cause primary poisoning if consumed by organisms (Eisa & Yassin, 2016; Khidr *et al*., 2018). This phenomenon becomes

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reason synthetic rodenticides are considered more effective in controlling rat pests (Kalinin *et al*., 2017; Kappes *et al*., 2022). It is because, with only a few active ingredients, these rodenticides can kill rats within hours (Saraf *et al*., 2015; Trakulsrichai *et al*., 2017) or are anticoagulants that can be lethal for 3–5 days (Eisa & Yassin, 2016; Khidr *et al*., 2018). Nevertheless, synthetic rodenticides can harm the surrounding environment (Kalinin *et al*., 2017; Kappes *et al*., 2022). On the other hand, synthetic rodenticides containing acute ingredients deter rats (Trakulsrichai *et al*., 2017), and rats become resistant to chemical compounds, which causes the utilization of chemical compounds to be ineffective (Agboola *et al*., 2022).

One effort to control rat populations without causing detrimental effects is to utilize the active ingredients of plants as natural rodenticides (Cruz *et al*., 2022). Plants that can be used as a source of active ingredients for natural rodenticides are yam tubers (*D. hispida*) because they contain dioscorin, diosgenin, dioscin, and cyanide acid (HCN), which are very toxic (Ojimelukwe *et al*., 2021). Previous research stated that block bait containing grated yam tuber (*D. hispida*) as much as 30% was very effective in killing *R. norvegicus* rats (Posmaningsih *et al*., 2014), while a concentration of yam tuber (*D. hispida*) as much as 70% could cause bleeding and killed all *Mus musculus* mice (Ningtyas & Cahyati, 2017) and *Rattus* sp. (Sari *et al*., 2020). However, there has been no scientific report on the effect of yam tuber flour (*D. hispida*) on the control of residential rats with histological parameters of the stomach of white male rats (*R. norvegicus* Berkenhout, 1769) Wistar strain.

The stomach functions to store and process food before it is passed on to the duodenum. Therefore, the stomach is continuously exposed to various factors that can damage stomach tissue. The stomach has a defense system to avoid mucosal damage by producing mucus bicarbonate. Prostaglandins regulate mucosal bicarbonate production, so if prostaglandin production is disturbed, the stomach can experience inflammation or gastritis. The most common pathogenesis of acute gastritis is mucosal irritation. The stomach encounters increased exfoliation of surface epithelial cells due to chemical compounds that have an irritating effect. Gastric epithelial irritation is caused by two damaging factors, namely endogenous destroyers (HCl, pepsin, and bile salts) and exogenous destroyers (chemical compounds and bacteria) (Paulsen & Waschke, 2019; Teng *et al*., 2013).

This study aimed to determine the effect of yam tuber flour (*D. hispida*) mixed in block bait on the gastric histology of male rats (*R. norvegicus* Berkenhout, 1769) Wistar strain. The benefit of this research is to provide information about the potential of yam tuber as a source of plant-based rodenticides for controlling rat populations, primarily residential rats.

Research Methods

Materials and Tools

The research object used during the study were white male rats (*R. norvegicus* Berkenhout, 1769) of the Wistar strain, as many as 25 individuals aged eight weeks old and with an average weight of 180 grams. The ingredients for the production of block feed are yam tubers (*D. hispida*), rice grain, corn flour, granulated sugar, desiccated coconut, paraffin, and food coloring ingredients. The chemicals used are 70% alcohol, 80% alcohol, 90% alcohol, 100% alcohol (absolute alcohol), xylol, 0.9% physiological sodium chloride, distilled water, 10% neutral buffered formalin (NBF) with a pH of 6.5- 7.5, dye hematoxylin-eosin (HE), Ketamine HCl 10%, paraffin and entellan. The additional material used is sawdust.

The tools used in this study were glassware, surgical instruments, sample vials, a blender, cover glass, tissue blocks, hot plates, ovens, paraffin dispensers, rotary microtomes (RE 100-S DLAB), water baths, tissue cabinets, distilled water bottles, stopwatches, base mold, staining rack, light microscope (XSP-13AE 1250X Yazumi), analytical balance (Otsuka type Ja203 p), jerry can, spatula, pH meter (Otsuka type Ja203 p), electric stove, heated aluminum bowl, and feed mold. The additional equipment used was a single cage for test animals (size 30x25x10 cm^3), a wire cage cover, a drink bottle, and a food container.

Experimental design

This research was conducted experimentally in the laboratory using a completely randomized design (CRD). Test animals were selected for each treatment randomly. Mice (*R. norvegicus* Berkenhout, 1769) were grouped into five treatments, namely, negative control, positive control, P1, P2, and P3 (Table 1). Each treatment consisted of 5 test animals as replications. Treatment was given for four consecutive days, and observations were made daily. On day 5, all rats were sacrificed by injecting a 10% ketamine HCl dose of 70 mg/kg BW.

Acclimatization and treatment

The experimental rats were acclimatized for seven days before treatment so that the test animals could adapt. The single cage measured 30 cm x 25 cm x 10 cm. The room temperature of the test animal cages is room temperature with a temperature range of 22 -25 °C. The test animals were fed as much as ± 25 grams/rat/day and distilled water ad libitum (Hardiningsih & Nurhidayat, 2006). Replacement of sawdust as the base of the cage is done every day. The treatment was given for four consecutive days.

Isolation and histological preparations of stomach organs

Mice that died during the experiment (4 days) were immediately dissected. Meanwhile, rats that had not died until the fifth day were then injected with 10% ketamine HCl 70 mg/kg BW and then sacrificed by neck dislocation and dissection. The isolated gastric organs were washed thoroughly using 0.9% physiological NaCl to remove residual blood. Gastric histological preparations were carried out in the following stages. Gastric organs were fixed using a 10% neutral buffered formalin (NBF) solution with a pH of 6.5-7.5. The comparison between the gastric organs and the solutions is 1:10. The gastric organs were put into graded alcohol with a formula of 70%, 80%, 90%, and 100% for 30 minutes each.

Furthermore, the stomach organs undergo a clearing process using xylol. The infiltration process was carried out by immersing the pieces of stomach organs in a solution of xylol: paraffin with a ratio of 3:1, 1:1, 1:3, and pure liquid paraffin for 15 minutes each in an oven with a temperature of ± 60ºC. The paraffin melting point is 54.17 °C. Therefore, the used temperature (60ºC) was reliable for making liquid paraffin. The organs were placed in a paper box filled with liquid paraffin and left for 24 hours to harden. Afterward, the frozen paraffin block was removed from the paper box, and the excess paraffin was thrown away. The paraffin block containing the organs was sliced using a microtome. The sample was sliced with 5 µm thickness.

The stomach slices were put in a water bath with a temperature of 37-40˚C and leave until the paraffin tape expanded. Paraffin tape was attached to the object glass dripped with Meyer's albumin. Then, the object was placed on a hot plate at 40˚C. The object glass is given a little distilled water to help stretch the incision results. The incision tape was extended on an object glass and dry. The stomach incision was stored for 24 hours (Susatyo *et al*., 2022). The next step was deparaffinization by immersing the slide containing the coupes in xylol solution for 5 minutes. The process was repeated two times. The hydration stage was carried out using an alcohol solution of the formula by immersing a glass slide containing a gastric incision in absolute alcohol, 90%, 80%, and 70% for 2 minutes each.

Hematoxylin-Eosin (HE) staining was carried out in a staining jar by immersing the preparation in 1% hematoxylin dye solution for 10-30 minutes, then washing it with running water. It was stained with eosin for 5-10 seconds. The gradual dehydration process of 70%, 80%, 90%, and absolute alcohol was repeated two times for 3 minutes each (Susatyo *et al*., 2022).

Histological observation

The histological structure of the male Wistar *R. norvegicus*'s stomach was observed using a light microscope with 40 x 10 magnification and five different view fields. The parameters observed included mucosal erosion (epithelial degradation), inflammation of the mucous lining (inflammatory cells), and dilation of blood vessels (capillary vasodilatation) (Shafira *et al*., 2016). These parameters are then given a score. The criteria for assessing gastric organ damage were

determined based on the score presented in Table 1 (Kuswandi, 2017).

Analisis Data

Data on the results of scoring measurements (non-parametric) were analyzed using the Kruskall-Walis test. If they are significantly different, proceed with the Mann-Whitney test with p-value = 0.05.

Table 1. Feed composition

Treatment	Yam	Brodifacoum	Rice groats	Cornstarch	White sugar	Dry coconut	Paraffin
KN	0	0	30	20	5	15	30
	0 g	0 g	300 g	200 g	50 g	150 g	300 g
KP	0	0,005	30	20	5	15	30
	0 g	0.05g	300 g	200 g	50 g	150 g	300 g
P1	30	0	20	5	5	10	30
	300 g	0 g	200 g	50 g	50 g	100 g	300 g
P2	50	0	20	0	5	5	20
	500 g	0 g	200 g	0 g	50 g	50 g	200 g
P3	70	0	10	0	0	0	20
	700 g	0 g	100 g	0 g	0 g	0 g	200 g

Table 1. Scoring of gastric microscopic damage

Score	Damages Percentages (%)
0	<25
1	25 – 50
2	50 – 75
3	>75

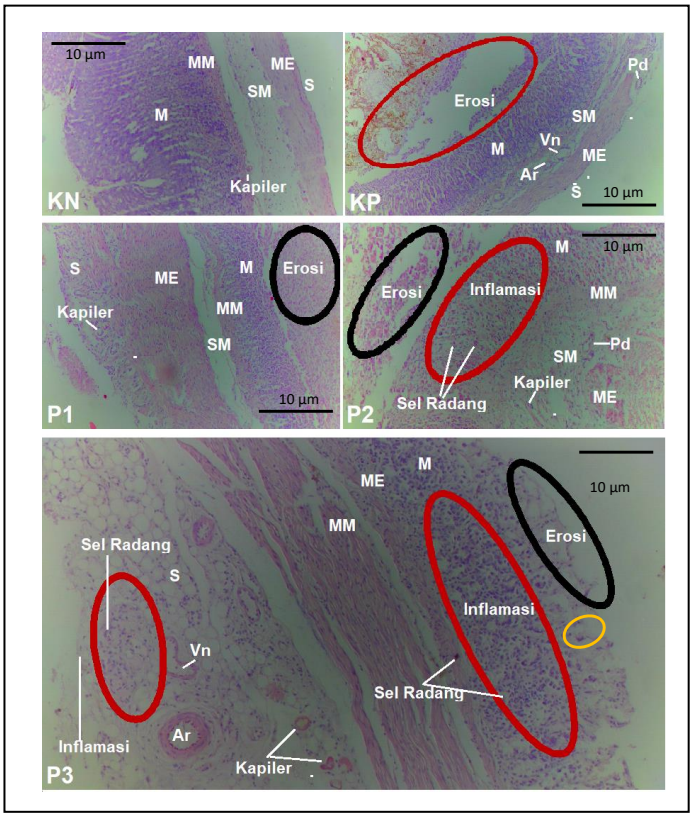

Figure 1. Stomach histology of white male rats (*R. norvegicus*) Description: M: Mucosa, MM: Muscularis mucosa, SM: Submucosa, ME: Muscularis external, S: Serosa, PD: Blood vessels (arterioles [Ar] and venules [Vn]), Red circles indicated areas of inflammation. The black triangles showed the polymorphonuclear cells.

Table 2. Damage level of Wistar rat after bait administration with different levels of yam tuber flour

Treatment	Damage type	Damages Percentages (%)
KN	Mucosa erosion	<25
	Inflammatory cells	0
KP	Mucosa erosion	50 - 75
	Inflammatory cells	0
P1	Mucosa erosion	25 - 50
	Inflammatory cells	0
P2	Mucosa erosion	50 - 75
	Inflammatory cells	25 - 50
P3	Mucosa erosion	25 - 50
	Inflammatory cells	25 - 50

Note:
KN: negative control; KP: positive control; P1: 1st treatment; P2: 2nd treatment; P3: 3rd treatment

Results and Discussion

The histological structure of the stomach of male Wistar rats (*R. norvegicus* Berkenhout, 1769) after four days of block feeding with different concentrations of yam tuber flour supplementation is presented in Figure 1.

In Figure 1, a histological examination of the stomach on a microscopic view showed that the stomach organs in the control treatment (KN) had typical mucosal, submucosal, and capillary cell shapes. This normality is characterized by the absence of inflamed tissue areas, indicated by the lack of inflammatory cells such as neutrophils and other polymorphonuclear cells. In contrast, brodifacoum (positive control/KP) and yam tuber flour (P1, P2, and P3) supplementation in rat's bite has caused stomach damage. The presence of inflammatory cells and mucosal erosion prove these damages. This study did not observe capillary vasodilatation. The results of this study were similar to the observed phenomena in the same rat species but fed with coffee (Kuswandi et al. 2017). The level of stomach damage among treatments is presented in Table 2.

These results indicate an effect of yam tuber flour treatment on the stomach of male Wistar rats. After yam tuber flour supplementation, stomach damage was suggested because of the dioscorin content in yam tuber flour. According to Santi (2010), microscopic damage in the P2 and P3 treatments was caused because the block bait formula eaten contained toxic compounds that entered the stomach, which could cause damage. The gut functions to store and process food before it is passed on to the duodenum. Therefore, the stomach is constantly exposed to various factors that can damage stomach tissue. The stomach increased epithelial cell surface exfoliation due to chemical compounds that have an irritating effect. Gastric epithelial irritation is caused by two damaging factors, namely endogenous destroyers (HCl, pepsin, and bile salts) and exogenous destroyers, such as chemical compounds (Paulsen & Waschke, 2019; Teng *et al*., 2013).

The stomach or ventriculus is a sac under the diaphragm. The stomach wall comprises four main layers: the mucosa, submucosa, muscularis externa, and serosa. Goblet cells in the mucosa layer produce bicarbonate of mucus, which coats the gastric mucosa from the pepsin and stomach acid (HCl). Mucus bicarbonate is the primary defense against mucosal damage. Prostaglandins influence bicarbonate mucus expression. Prostaglandins are vasodilators that have the effect of dilating blood vessel walls to increase blood flow to tissues. The inhibition of prostaglandins can reduce the flow of blood circulation, one of which is to the stomach. If this process is disturbed, gastritis can occur in the stomach. In our case, administering yam tuber formula can inhibit prostaglandin synthesis so that blood flow to the stomach mucosa is reduced and causes loss of the mucous layer that protects the stomach mucosa, resulting in cell death. Gastric tissue can experience ischemia, which can cause the mucosa to experience erosion (Paulsen & Waschke, 2019).

The Kruskal-Wallis test proved that the treatments significantly affected male Wistar rats' stomach damage, as indicated by quite different damage scores among treatments (p= 0.05; Table 3. Data analysis was continued with the Mann-Whitney test to compare the means of each treatment to the KN treatment. The results of the Mann-Whitney test showed that microscopic damage to the stomach organs occurred in the P2 and P3 treatments. Microscopic damage in the P2 and P3 treatments was caused because the block bait formula eaten contained toxic compounds that entered the stomach, which could cause damage (Santi, 2010).

The appearance of inflammatory areas on gastric histological microscopy is characterized by the abundance of inflammatory cells at P2 and P3, especially neutrophils with abnormal conditions indicating active inflammation. In the early stages of inflammation, arteriolar vasodilatation and increased blood flow increase intravascular hydrostatic pressure and fluid movement from the capillaries (Price *et al*., 2006). The HCl secretion into the mucosa results in mucosal tissue damage. Dioscorin plays a role in inflammatory mediators and is produced by mast cells, basophils, platelets, and supporting tissues in the stroma, especially those around blood vessels. Dioscorin stimulates further acid and pepsin secretion and increases capillary permeability to protein (Wahid *et al*., 2016). Gastric histological damage is caused by increased exfoliation of surface epithelial cells by dioscorin compounds irritating the stomach's outer surface (Paulsen & Waschke, 2019). Damage changes occur because the administration of yam tuber can inhibit the synthesis of prostaglandins so that blood flow to the gastric mucosa is reduced and causes loss of the mucous layer that protects the gastric mucosa, resulting in cell death.

Tissue damage in normal male Wistar rat's stomachs occurred because of the bioactive contents in yam tubers (*D. hispida*) flour, such as dioscorin. Previous studies reported that dioscorin is among the bioactive compounds of various yam tubers (Princewill-Ogbonna *et al*., 2015; Dey *et al*., 2017; Ojuederie *et al*., 2020), including *D. hispida* (Lokman *et al*., 2017; Yalcin *et al*., 2019; Rasid *et al*., 2020; Muhammad *et al*., 2022). Other studies proved that dioscorin prevents ATP production (Rasid *et al*., 2020) and promotes the production of reactive oxygen species (ROS), such as H_2O_2, which is highly poisonous to the cell components and causes cell death (Princewill-Ogbonna *et al*., 2015; Dey *et al*., 2017; Ojuederie *et al*., 2020). It has been reported that dioscorin has caused tissue degeneration in various internal organs of rats (Lokman et al. 2017; Yalcin et al. 2019; Rasid et al. 2020; Muhammad et al. 2022). Therefore, it was reasonable that this study observed gastritis in normal male Wistar rats (*R. norvegicus* Berkenhout, 1769) after yam tuber administration.

50% yam tuber flour was the most effective bite to control the rat population.

This study only observed a single internal organ of a male Wistar rat (*R. norvegicus* Berkenhout, 1769). It is suggested to do similar research in other internal organs of the same rat strain and other rat species to obtain a more effective natural bite to control all residential rat species.

Conclusion and Suggestion

Based on the results, this study concluded that a rat's bite containing yam tuber (*D. hispida*) flour caused damage to the stomach of a male Wistar rat (*R. norvegicus* Berkenhout, 1769) and a rat's bite containing

Table 3. The average damage score of rat stomach after treatment

Treatment	Damage score	p-value
KN	0.00 ± 0.00^{ns}	
KP	0.40 ± 0.89^{ns}	
P1	0.40 ± 0.54^{ns}	0.05
P2	$1.80 \pm 1.09^{*}$	
P3	$2.40 \pm 0.34^{*}$	

Note: ns= non-significant, *= significant

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