

The Effectiveness of Porang (*Amorphophallus oncophyllus*) Powder Solution on Healing Inflammation of The Stomach of Wistar Rats (*Rattus norvegicus*) induced with Acetic Acid

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Abstract: Research was conducted to test the effectiveness of arum *Amorphophallus oncophyllus* flour solution containing glucomannan in curing acetic acid induced gastric wound in wistar rats (*Rattus norvegicus*). Previous studies emphasize the anti-inflammatory characteristics of *A. oncophyllus*. The experiment was conducted in vivo with wistar rats with completely randomized design. Observed parameters were morphology plant, activity of rats, body weight (g), stomach size (cm) and gastric mucosal layer before and after induction and treatment on 1st, 7th, and 14th day. The rats were grouped into control (normal), negative control, positive control and treatments groups. The observed parameters showed that *A. oncophyllus* flour solution has the potential to cure gastric inflammation induced by acetic acid by 25% concentration, with a dose as big as 1.25 mL for 3 days. 3 mL of arum flour solution per day showed great potential and effectiveness in healing diseases and gastric wounds in wistar rats within the 7 days in comparison with the positive control of gastric drugs. This research indicates that *A. oncophyllus* has the potential to be developed as an original Indonesian drug for the treatment of gastric inflammation.

Keywords: Stomach Ulcer, Gastric Inflammation, *Amorphophallus oncophyllus*, Wistar Rats

1. Introduction

Based on data from the Ministry of Health of the Republic of Indonesia, gastric inflammation is in sixth place with a total of 33, 580 cases of inpatients at the hospital 60.86%. Cases of gastric inflammation in outpatients with 201, 083 cases and are in seventh place. The incidence of gastric inflammation in some areas is quite high with a prevalence of 274, 396 cases out of 238, 452, 952 inhabitants or 40.8%. The percentage of cases of gastric inflammation in Indonesian cities, namely, Jakarta 50%, Palembang 35.5%, Bandung 32%, Denpasar 46%, Surabaya 31.2%, Aceh 31.7%, Pontianak 31.2%, while the incidence stomach ulcers in Medan reached 91.6% (Yusfar & Ariyanti, 2019).

The factor that can cause stomach ulcers is vinegar, which is a water-soluble organic acid that is often used as a food flavoring agent. Vinegar acid can cause injury to organs and tissues due to its corrosive and irritant properties. In the stomach, vinegar can increase the damaging agents and cause an imbalance in the stomach and cause gastric

inflammation (Pungus et al., 2020). Also foods that are high in saturated fat such as coconut milk, spicy foods, processed foods or instant foods, gaseous or fizzy foods or drinks (Suwindiri, 2021).

A study published in the International Journal of Biological Macromolecules in 2016, touched on the role of the porang plant as an anti-inflammatory or anti-inflammatory agent. One of the content in the tubers that is very useful is glucomannan (Sari & Suhartati, 2015).

The use of herbs from porang flour solution as a cure for stomach ulcers is not widely known by the public. This is because there is no scientific evidence regarding the efficacy of porang flour from porang tubers (*A. oncophyllus*) as an anti-inflammatory, thus encouraging the implementation of this research.

2. Materials and Methods

The research was conducted at the Biovina Herbal Laboratory located in Jaga 2, Sea Mitra Village, Pineleng District, Minahasa Regency, North Sulawesi Indonesia (Pandiangan *et al.*, 2020). The time for conducting the research was two months.

Tools and Materials

Porang tubers were obtained from farmers in Lolanan Village, Sangklikang District, BolaangMongondow Regency, North Sulawesi Province, Indonesia. Samples of white wistar rats aged 3 months were obtained from the Biovina Laboratory, Minahasa Regency, North Sulawesi Province, Indonesia

Methods

This research is experimental in a completely randomized design (CRD) using 16 wistar rats (Pungus *et al.*, 2020). The test animals have passed the ethical feasibility of the Health Research Ethics Commission of the POLTEKES KEMENKES Manado No. KEPK.01/11/086/2020 (Pandiangan *et al.*, 2020) with the following distribution; (1) Baseline: No induction and drug treatment, (2) Treatment: Administration of acetic acid + porang flour solution, (3) Positive Control: Administration of acetic acid + gastric medicine biovina, (4) Negative Control: Administration of acetic acid + drinking water

Test Animal Preparation

The rats' body weight was first weighed using a simple scale for experimental rats (Nvt 2201 Portable Balance Cup, OHAUS) (Lukman and Christin, 2020). After that, the length and width of the stomach of wistar rats were measured using a Joyko iron ruler (Kololu *et al.*, 2014).

Test Animal Maintenance

This study used wistar rats (*Rattus norvegicus* L) (Figure 1) with a weight of 85 - 100 g and aged \pm 3 months obtained from the Biovina Sea Mitra Laboratory (Pandiangan *et al.*, 2020). The rats were kept individually in a plastic cage measuring 25 cm x 40 cm with a wire cover that was equipped with a drinking water bottle and a feeding holder (Pandiangan *et al.*, 2019). Wistar rats were adapted to laboratory conditions for 3 days by being fed and drinking ad libitum (Pandiangan *et al.*, 2018) and (Sihombing, 2011).



Figure 1: Wistar Rat Maintenance at the Biovina Sea Mitra Laboratory

Porang Powder Preparation

The manufacture of porang powder follows the working method (Aryanti *et al.*, 2015). Prepare the porang tubers, then clean the porang tubers under running water then drain, after that do the soaking using table salt for 30 minutes and drain, then the tubers are cut into thin slices like chips, after that do the drying by drying in the sun for approximately for 2 - 3 days, after the porang is dry, pound it using a mortar until it becomes a powder, then filter it using a 40 mesh flour sieve, then after it is smooth like flour then store the container in a flour plastic.

Preparation of Porang Powder Solution

Porang powder solution was prepared by following the working method (Aryanti *et al.*, 2015) and (Dewi *et al.*, 2019). Before being made into a solution, 3.0 g of alum solution (aluminumsulfate) was prepared which was dissolved in 100 mL of water. Then 3.0 g of porang powder was dissolved in 100 mL of alum solution. The solution was stirred in an Erlenmeyer flask using a spatula for approximately 3 - 5 minutes until the solution thickened.

Treatment of Test Animals

This study follows (Pungus *et al.*, 2020) which has been modified by the use of vinegar (acetic acid). The research object used was 16 male wistar rats which were divided into 4 groups, namely Group I (4 baseline rats), Group II (4 positive control rats), Group III (4 negative control rats) and Group IV (4 treatment mice).

The Baseline group was not given the induction or treatment solution. The positive control group was given 25% acetic acid for 3 days and continued treatment with 0.3 mg/mL gastric medicine for 7 days and terminated on the 8th day. The negative control group was given 25% acetic acid for 3 days, followed by no treatment for 7 days, then terminated on the 8th day. The treatment group was given 25% acetic acid for 3 days then continued with 0.3 mg/mL porang flour solution for 7 days and terminated on the 8th day.

Data analysis

The data will be analyzed descriptively by using data tabulation and graphing both quantitative and qualitative data.

3. Results and Discussion

Growth of Wistar Rats Before and After Treatment

The results of observations of the growth of rats through periodic weighing of rats. Rat body weight was weighed with a weighing time of week 1 before giving the solution or an adaptation period for 7 days, induction of inflammation for 3 days, then week 3 (day 26) after giving porang flour solution (Lukman & Christin, 2020). The results of the weight measurements are presented in Table 1. Body weight before (initial) adaptation period averaged Baseline 96 g, treatment PTP 89 g, K - 82 g, K+ 90 g. After 21 days of treatment, the average body weight of mice (g) was Baseline 186, Treatment 173, K - 159, K+ 178 g. The results of the final body weight measurement after the treatment was reduced by the initial body weight obtained the weight of each treatment group Baseline 90 g, Treatment 84 g, K - 77 g, K+ 88 g. It is clear that the growth of rats is good and it is

certain that the growth process is going well and healthy during the adaptation and treatment period. Weight gain Baseline 90, Treatment 84, K - 72, K+ 88 g.

Table 1: Growth results of wistar rats (g) before and after being given treatment

| Sample Code | Initial average weight (g) | Final average weight (g) | Weight gain (g) |
|------------------------|----------------------------|--------------------------|-----------------|
| Baseline | 96 | 186 | 90 |
| Treatment PTP | 89 | 173 | 84 |
| Negative Control (K -) | 82 | - | - |
| Positive Control (K+) | 90 | 178 | 88 |

The body weight of the mice during the study can be seen in Figure 1. There was an increase in body weight in the Treatment and K+ groups during the treatment process. The results of measuring the weight of the wistar rats Table 1 and Figure 2 show that not all of them experienced an increase in the weight of the wistar rats. Improvements occurred in baseline controls and K+ or gastric drug Biovina. The highest increase in body weight was seen in the treatment of gastric inflammation drug Biovina K+ with a final body weight of 178 g from an initial body weight of 90 g. This increase was also seen directly in the morphology of the mice.

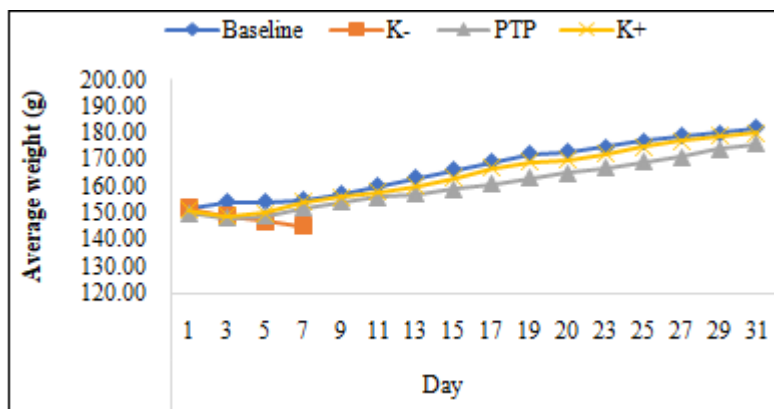


Figure 2: Growth of wistar rats during 31 days of observation

Based on Figure 2, it shows that in the Treatment and K+ groups there was an increase in almost reaching the growth rate in the baseline group, in this case the treatment given triggered an increase in appetite in wistar rats. Meanwhile, in the K - group day by day there was a decrease so that on the 7th day the rats died because the prevention was only given water. Wistar rats with high weight gain have larger bodies, and if they are directly held, their bodies will feel soft because they have accumulated a lot of fat (Gurnida and Rosifah, 2011).

Morphological Observations of Rats and Their Activities

Morphology or visual appearance is carried out when weighing body weight by paying attention to movement, respiratory movements, eyes, snout, hair, eating and drinking activities (Table 2). The results of morphological observations showed that at the beginning of the study during the adaptation period, all morphological observations showed a healthy (normal) uniformity of movement, his eyes were clean and clear, his muzzle was clean and healthy, his hair was clean and white, his eating and drinking were normal.

During the adaptation period, it also showed the same thing as the initial observation period (day 0) because the rats were obtained from the same laboratory, so the conditions did not change much. Morphological observations during the adaptation period (7th day) showed healthy (normal) uniformity of movement, calm breathing movements and regular rhythm, clean and clear eyes, clean and healthy muzzle, clean and white hair, normal eating and drinking.

Observations of morphology, activity or behavior of rats at the induction stage of gastric inflammation with acetic acid began to show different appearances from those induced and not induced. Induction with acetic acid showed more movement activity than not induced (control), more breathing movements than control and irregular rhythm, eyes often closed, nose sweating, hair protruding or standing up than control, eating and drinking less reduced and not wanting to eat (from day 0 to 3 during induction), the same condition continued for 3 days and growing stomach and gasping for breath and really like dying eventually leading to death. Uninduced mice showed the same morphology and activity as the adaptation period (Table 2).

Table 2: Morphological observations and activities of Wistar rats

| Treatment Group | Observation of morphology, activity and behavior of Wistar rats | | |
|-----------------|---|---|---|
| | Adaptation period | Induction of gastric inflammation with acetic acid | Treatment after inflammation induction with acetic acid |
| Baseline | Agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, fine and thick white hair | Agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, fine and thick white hair | Normal eating activity, normal movement, normal calm breathing, frequent scratching of the ears, clean and clear eyes, fine and thick white fur |
| K - | Agile movements, normal breathing, frequent scratching of the ears, clean and clear | Little movement, decreased appetite, frequent sleep, flatulence, hair rises and stands up | Head bent 30 degrees, nose sweating, irregular breathing, hair began to fall out, not moving much, stomach enlarged finally death occurred on day 5 |

| | | | |
|-----|---|---|---|
| | eyes, fine and thick white hair | | |
| PTP | Agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, fine and thick white hair | Little movement, decreased appetite, frequent sleep, flatulence, gasping for breath, raised hair and standing | Movements began to be agile, normal breathing, but weight loss, normal appetite, more or less movement and normal, normal breathing, clean and clear eyes, fine and thick white fur |
| K+ | Agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, fine and thick white hair | Little movement, decreased appetite, frequent sleep, flatulence, hair rises and stands up | Agile movements, normal breathing, normal healthy eating activities, lots of movement, normal breathing, often stroking his nose, often scratching his ears, clean and clear eyes, fine and thick white fur |

Morphological appearance and activity after treatment of porang solution (Treatment) and drug solution (K+), distilled water only (K -), and baseline (normal control) showed the difference between negative control (K -) after 5 days of induction experienced death or day 2 aquadest treatment after induction, while the baseline was healthy and grew normally (Table 2). However, the PTP treatment showed similar morphology and activity to the positive control (K+) although the Biovina drug treatment was healthier and more agile than the porang flour solution (PTP) treatment as shown in Table (2). Another difference that appears in the PTP is that the body weight is lower than the aquadest treatment (baseline), even K - die when not given treatment. Gastric inflammation induced with 25% acetic acid if not handled properly and properly treated can cause the death of Wistar rats (Kololu et al., 2014). These results indicate that it is also possible for humans to do this, but further evidence is needed.

Wistar Rat Stomach Size

The results of observations of the length and width of the stomach can be used as gastric morphological parameters as an indicator of wound healing (Kololu et al., 2014). Table 3 shows the results of each measurement per treatment group. Where the measurement of the length and width of the stomach of wistar rats showed that in the baseline treatment the stomach size was normal (Kololu et al., 2014). reaches a length of 2.3 cm and a width of 1.3 cm. In the K - group, the size of the stomach reached 4.4 cm long and 3.4 cm wide. This is due to an infection that occurs in the stomach due to the administration of acetic acid that is not treated with medication so that the stomach enlarges and its lining thins. In the PTP treatment group, the stomach size was 2.5 cm long and 1.9 cm wide, so it was still at the normal standard of stomach size. In the K+ group, the stomach size was 2.3 cm long and 1.2 cm wide (Pungus et al., 2020).

Table 3: Wistar rats stomach length and width (cm) in four treatment groups

| Group Treatment | Stomach Size (cm) | | Average Stomach Size (cm) | |
|-----------------|-------------------|------|---------------------------|------|
| | Long | Wide | Long | Wide |
| Baseline (I) | 2, 1 | 1, 0 | 2, 3 | 1, 3 |
| Baseline (II) | 2, 3 | 1, 3 | | |
| Baseline (III) | 2, 5 | 1, 5 | | |
| Baseline (IV) | 2, 4 | 1, 3 | | |
| PTP (I) | 2, 7 | 1, 9 | 2, 5 | 1, 9 |
| PTP (II) | 3, 0 | 2, 7 | | |
| PTP (III) | 2, 6 | 1, 6 | | |
| PTP (IV) | 2, 5 | 1, 4 | | |
| K - (I) | 4, 3 | 3, 6 | 4, 4 | 3, 4 |
| K - (II) | 5, 0 | 4, 2 | | |
| K - (III) | 4, 0 | 2, 9 | | |
| K - (IV) | 4, 3 | 3, 0 | | |

| | | | | |
|----------|------|------|------|------|
| K+ (I) | 2 | 1, 1 | 2, 3 | 1, 2 |
| K+ (II) | 2, 3 | 1, 3 | | |
| K+ (III) | 2, 3 | 1, 3 | | |
| K+ (IV) | 2, 1 | 1, 2 | | |

Measurement of the length and width of the hull (morphological) that the baseline and K+ are almost the same in length and width. This indicates that there is a healing process in the enlarged and long stomach caused by acetic acid (wound induction) (Pungus et al., 2020).

The treatment of porang flour solution showed that the length and width of the stomach were much shorter (small) with an average of 2.5 cm and a width of 1.9 cm, approaching the length and width of the baseline (control/normal) with an average length of 2.3 cm and a width of 1, 3 cm (Table 3). This shows that the administration of porang solution in gastric inflammation caused by acetic acid is very effective or the same as the gastric inflammation drug Biovina. Meanwhile, K - who had gastric ulcers and eventually died, the results of the measurement of stomach length were on average 4.4 cm and width 3.4 cm (Table 3). The large size of the K - stomach was in line with the observation of the morphology of the stomach being enlarged (bloated) and its activity was slow and gasping for breath (Table 2) and finally on day 5 it died. This shows that the treatment of porang flour solution has the potential or can cure stomach inflammation and stomach swelling (gastritis). This needs further evidence with a focus on the process of reducing gastric gas.

Macroscopic Observation of the Stomach of Wistar Rats

Direct observation with the naked eye and macroscopically begins at termination through observations of morphology, gastric anatomy, surface, color, and consistency (Kololu et al., 2014). Based on observations of the wistar rats' stomachs, macroscopically the size, surface, and color of the wistar rats' stomachs varied from one group to another, for gastric consistency, all of them had a soft consistency (Table 4).

Table 4: Macroscopic observation of the stomach of Wistar rats after treatment with porang solution (PTP), drug (K+), negative control (K -) and base line (control)

| Parameter Observation | Treatment Group | | | | Information |
|-----------------------|-----------------|---------|--------------|---------|--|
| | Baseline | PTP | K - | K+ | |
| Colour | Pink | Pink | Brownish red | Pink | Permukaan dinding lambung berwarna merah muda (Pasaribu, Loho and Lintong, 2013) |
| Surface | Fine | Fine | Fine | Fine | Permukaan lapisan lambung tampak halus (Pasaribu, Loho and Lintong, 2013) |
| Consistency | Springy | Springy | Springy | Springy | Konsistensi lambung kenyal (Pasaribu, Loho and Lintong, 2013) |

Information;

Baseline: Control P: Porang Powder treatment

K -: negative control K+: positive control

The morphological picture of the stomach in the baseline control group (normal) showed a healthy stomach condition, with a visible gastric consistency (Kololu et al., 2014) chewy, smooth and pink in color and of normal size. The gastric morphological picture of the PFP group with 1.25 mL of 25% acetic acid for 3 days followed by the administration of porang flour solution at a dose of 3 mL once per day for 7 days showed that the stomach morphology recovered as in the baseline group.

Morphological picture of the stomach in the K - group with 1.25 mL of 25% acetic acid for 3 days followed by only 3 mL of water, the morphological appearance of the stomach (Pungus et al., 2020) is large and black in color. The morphological picture of the stomach in the K+ group with 1.25 mL of 25% acetic acid for 3 days followed by gastric drug solution at a dose of 1 time per day as much as 3 mL for 7 days showed that the stomach morphologically recovered as in the baseline group.

The results of morphological observations of the stomach of wistar rats in Figures 2b and d show that the stomach condition recovered and returned to normal as in the baseline group. While in Figure c, the stomach looks worse in terms of size when compared to other groups it is very large, this happens because (Pasaribu et al., 2013) acetic acid inhibits prostaglandin synthesis so that blood flow to the gastric mucosa is reduced and causes loss of the mucous layer. which protects the gastric mucosa.

Stomach Mucous Layer of Wistar Rats

Microscopic observations began at termination through observations of anatomy, surface, color, and consistency (Kololu et al., 2014). Based on observations of the stomach of wistar rats, microscopically the structure of the mucosal layer and color (Table 5).



Figure 2: Macroscopic stomach of wistar rat (R. norvegicus). (a) normal baseline group, (b) PTP group, (c) K - group, (d) K+ group

Table 5: Gastric mucosal layer of wistar rats appearances

| Parameter | Kelompok | | | | References |
|-----------------------|---|--|--|--|---|
| | Baseline | PTP | K - | K+ | |
| Gastric Mucosal Layer | Prominent rugae folds Surface coating that looks shiny | The rugae folds seem to still have a little inflammation The surface area of the coating looks a little shiny | The rugae folds look less clear. Injuries to the mucosal lining The coating surface is filled with irritation | The rugae folds are clearly visible The surface of the mucous layer looks shiny | The surface of the stomach wall appears to have rugae folds, the reflection of a shiny mucus layer (Yuliasuti et al., 2016) . |
| Colour | Pink | Yellowish Pink | Yellowish white | Whitish Pink | The surface of the stomach wall is pink (Pasaribu, Loho and Lintong, 2013) |

Information;

Baseline: Control PFP: Porang flour treatment

K -: Negative control K+: Positive control

Table 5 above shows that the PTP and K+ groups managed to recover as in the baseline group by giving 3 mL of a solution, this is because the administration of a thick porang flour solution can regenerate irritated mucosal layers in 4 - 7 days and continues for several weeks depending on the level

of inflammation that occurs. Meanwhile, in the K - group there was worsening inflammation which was indicated by irritation covering all mucosal layers and there were also wounds in the mucosal layer (Figure3c).

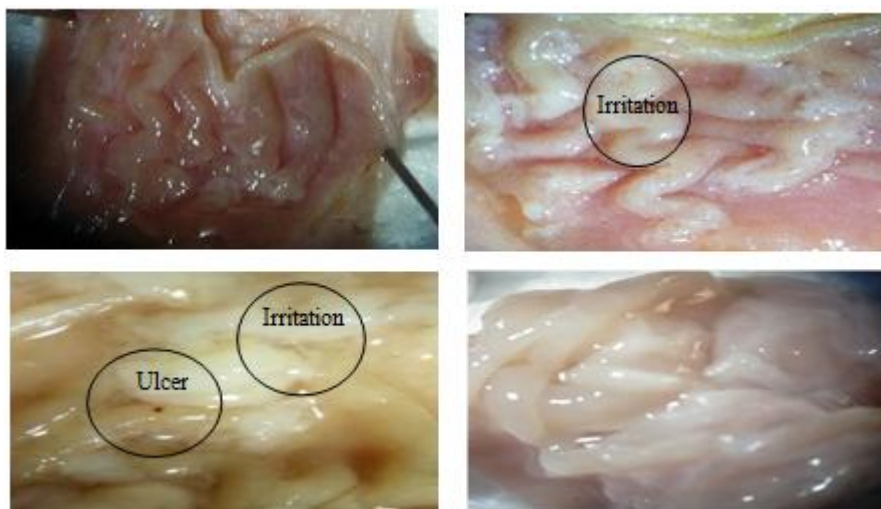


Figure 3: The structure of the rugae folds in the mucosal layer of the wistar rat (*R. norvegicus*). (a) normal appearance in the baseline group, (b) irritation in the PPT group, (c) unclear rugae folds in the group, (d) normal appearance in K+.

Based on the results of the study, it was found that experimental animals induced with acetic acid for 3 days could cause inflammation in the stomach. Acetic acid in this study was used as an aggressive (damaging) factor in the stomach (Yuliastuti et al., 2016). Acetic acid, also known as vinegar, is a water - soluble organic acid that is often used as a food flavoring agent. Acetic acid can cause injury to organs and tissues due to its corrosive and irritant properties. In the stomach, acetic acid can increase damaging agents and cause an imbalance in the stomach and cause gastritis or peptic ulcers (Pungus et al., 2020).

In group K - with acetic acid administration in the absence of gastric protection factor, the condition was very severe, the rugae folds did not stand out and the mucous membrane was lost because the surface of the mucosal layer was filled with irritation due to acid, and there were also wounds on the surface area of the mucosal layer. as in Figure 3c which is marked with a circle.

In the PPT group, the mucosal layer recovered due to the provision of gastric protection factors by administering a solution of porang flour with a solution of 3 mL, the thick porang flour solution can regenerate the mucous layer irritated by acetic acid for 4 - 7 days, the healing process can take place. seen in Figure 3b where little by little the irritation began to disappear and the surface of the other mucosal layers seemed to be reduced as indicated in terms of the color it looked pink, and the mucous membranes also looked back to look like in the baseline group (Figure 3a), the surface of which was still visible a little. The irritation is shown in Figure 3b which is marked with a circle. In the K+ group, the surface condition of the mucosal layer was completely healed and looked like the baseline group with 3 mL of gastric medication with a healing process of 4 - 7 days, which can be seen in Figure 3a. The appearance or results of the PPT tube analysis and drug treatment and the

baseline are close to their appearance or observation. This shows that the treatment of porang flour solution for wistar rats is effective for wound healing starting from day 7 after treatment with a 3 mL sonde per day per rat. This research shows novelty that in the future it can be developed or continued to be used as functional food for stomach ulcers and can also be used as a weight loss diet food.

4. Conclusion

Porang flour solution containing glucomannan is effective for healing gastric inflammation induced by 25% acetic acid. Provision of porang flour solution in the stomach with inflammation has the potential to be used as herbal medicine. And giving porang flour solution can regenerate gastric wounds and restore the surface texture of the layer that is irritated in the gastric mucosal layer within 7 days.

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