

Original Research

Received 07 June 2026

Revised 15 July 2026

Accepted 30 July 2026

Natural Resources for Human Health 2026, Vol. 6 (13s): 839–855

KEYWORDS:

Preclinic; Acetic acid; Biovina; effectiveness; bloating; gastric ulcers.

Preclinical Test of Biovina Health Supplement Extract on the Healing of Gastric Ulcers and the Bloated Rat (*Rattus norvegicus*) That Acetic Acid Induce

Dingse Pandiangan^{1*}, Sarla Bukari², Nelson Nainggolan³, Enih Rosamah⁴, Michael Vallery Tumbol⁵, Redsway Truman Douglas Maramis⁶, Agnes Virginia Simamora⁷, Silvana Tulandi⁸, Ivana Christy Nainggolan⁹.

^{1,2,10} Biology Study Program, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado, North Sulawesi 95115, Indonesia

³ Department of Mathematics, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado, North Sulawesi 95115, Indonesia

⁴ Department of Forestry, Mulawarman University, East Kalimantan, Indonesia

⁵ Department of Medical Laboratory Technology, Health Polytechnic Ministry of Health Manado, North Sulawesi, 95129, Indonesia

⁶ Department of Pests and Diseases, Faculty of Agriculture, Sam Ratulangi University, Manado, North Sulawesi, 95115, Indonesia

⁷ Department of Agrotechnology, Faculty of Agriculture, Universitas Nusa Cendana. Jl. Adisucipto, Kupang 85001, East Nusa Tenggara, Indonesia

⁸ Biology Department, Faculty of Mathematics and Natural Sciences, Tomohon Christian University, North Sulawesi 95444, Indonesia

⁹ Harapan Kita Mother and Child Hospital, Jakarta 11420, Indonesia

* Corresponding author: dingsepan@unsrat.ac.id

ABSTRACT: Gastric ulcers and bloating are gastrointestinal tract disorders related to gastric mucosa damage, inflammation, and oxidative stress. Acetic acid induction models in experimental animals are widely used to represent these conditions experimentally. This study aims to test the effectiveness of Biovina health supplement extract on the healing of peptic ulcers and bloating in male white rats (*Rattus norvegicus*) induced by acetic acid. This study used a laboratory experimental design with the division of baseline (normal), negative control, positive control, and treatment groups given Biovina supplements with formulation variations. Induction of acetic acid is carried out to cause gastric mucosal damage and gastric distension. Effectiveness evaluation was carried out through direct observation of the stomach which included the size of the stomach, mucosal color, and the presence of necrosis and wounds. The results showed that the negative control group experienced significant gastric damage, characterized by a significant enlargement of the size of the stomach (length and width), discoloration of the mucosa from dark red to blackish, and a large amount of necrosis and wounds. This condition reflects the occurrence of acute inflammation and distension in the gastric tissues. In contrast, the positive control group and the group given Biovina 1, 2 and 3 supplements showed significant improvement, characterized by a large or bloated stomach size to shrink or normal back to or equal to baseline and positive control (commercial drugs). The color of the mucosa from blackish red turns to fresh pink. The stretched mucosal layer and

necrosis and wounds turn into rugae elastic, smooth surfaces and wounds heal the same with positive control and baseline. The conclusion is that Biovina supplements have been shown to be effective in curing peptic ulcers and bloating and have the potential to be a gastroprotective agent due to chemical irritation of acetic acid.

1. INTRODUCTION

The health and fitness of the physical body is very important in the body, but in today's development that makes a fast and consistent way of life of individuals needs to be beneficial and leads to unfavourable ones that can make the body susceptible to diseases. Diseases can be caused by anything including the Minahasa tribe's habit of eating "Gohu" (Nainggolan et al., 2022), which is always mixed with vinegar acid (acetic acid) which can cause stomach ulcers and bloating if the concentration is excessive, even small habits that are often done such as delaying mealtimes to irregular diets, which can cause excessive acid production so as to trigger the emergence of stomach acid disease (Sinaga & Fajrin, 2022), (Pandiangan et al., 2022). The results of a survey from the North Sulawesi health office in 2021 recorded 29,260 gastritis patients (Kuna et al., 2023).

Gastric ulcer is one of the disorders of the digestive tract that has a high prevalence. This condition is mainly caused by excessive stomach acid production which is influenced by the increased presence of aggressive factors, including foods containing acetic acid, the use of non-steroidal anti-inflammatory drugs, alcohol consumption, smoking habits, and the presence of *Helicobacter pylori* bacterial infections (Ifada et al., 2022), (Pandiangan et al., 2025).

Biovina supplements are antidegenerative traditional medicinal plants (Pandiangan et al., 2024), that have benefits as antioxidants (Pandiangan et al., 2018), anticancer (Malingkas et al., 2022), anticholesterol (Pandiangan et al., 2023), and antidiabetic (Pandiangan, et al. 2025). Biovina products are a technology of medicinal products made from herbs or traditional medicinal plants that have often been used by the public in general, biovina health products are new products that have never existed because they are based on regional traditional medicinal plants (Pandiangan et al., 2021). According to the patent ID 000009577 that Biovina's health supplement contains raw materials Pasote (*Dysphania ambrosioides*) (Pandiangan et al., 2018) and dry extract of Gambir (*Uncaria gambir*). Related to the production method of health supplements that use raw materials in the form dried extract of Pasote (*Dysphania ambrosioides*) and dried extract of Gambir (*Uncaria gambir*). The process of making this supplement includes several stages, namely: first, preparing dried extracts from the Pasote plant; second, prepare dried extract of Gambir leaves; third, grind Pasote extract until it reaches a particle size of 40 mesh; fourth, puree dried extract of Gambir leaves also up to a size of 40 mesh; and fifth, mixing 25% Pasote extract with 75% gambier leaf extract evenly using a mixing device in the form of a mixer (Patent ID 000009577).

The habit of the people of North Sulawesi with food containing acetic acid called "Gohu" can cause stomach ulcers and bloating if the concentration of vinegar acid is excessive because there is no right level of the taste of the maker (Pandiangan et al., 2018). Therefore, it is necessary to research the use of herbal medicines from Biovina supplements as a cure for gastric ulcers so that the problem of this disease can be overcome (Pandiangan et al., 2026). It is necessary to research how effectively it is used as a peptic ulcer medicine by preclinic test .

The problem in this study is whether the administration of Biovina health supplements is effective in the healing process of gastric ulcers and bloating induced with acetic acid? Therefore, the title of this study is a test of the effectiveness of Biovina health supplement extract on the healing of rat gastric ulcers (*Rattus norvegicus*) induced by acetic acid. The goal is to determine the effectiveness of Biovina's health supplement on the healing of gastric wounds and bloating in rats (*Rattus norvegicus*) induced with acetic acid to be developed into a new Indonesian Natural Ingredient Medicine.

2. MATERIALS AND METHODS

2.1 Research Location

This research was conducted at the Manado Unsrat Pharmacy Laboratory and the Biovina Herbal Laboratory located at Jaga 2, Sea Mitra Village, Pineleng District, Minahasa Regency, North Sulawesi Indonesia.

2.2 Tools and Materials

Sonde, Gloves, Erlenmayer, measuring cup, Beaker glass, iron ruler, jar, scissors, tweezers, Biovina Supplement, chlorosome acetic acid, gastric medicine (Promag), aquaades, alcohol, caliper, binocular microscope,

2.3 Research Design

The research design used was a Complete Random Design (RAL) experimentally in the laboratory using the research object of 18 Wistar rats (Suyoko et al., 2023; Solfaine & Supriyanto, 2022). The experimental group was divided into 6 groups, including:

Baseline (Normal control): normal life (no given acetic acid)

Negative Control: Acetic Acid + Aquaades

Positive Control: Acetic Acid + Gastric Medicine (Promag)

Biovina 1: Acetic Acid + Biovina Supplement 1 mg/mL

Biovina 2: Acetic Acid + Biovina Supplement 2 mg/mL

Biovina 3: Acetic Acid + Biovina Supplement 3 mg/mL

2.4 Research Procedure

2.4.1 Rat Maintenance

This study used a test animal of wistar rats (*Rattus norvegicus*) with a weight of about 150-300 g and $\pm$ 10 months old. The rats were kept individually in a 25 cm x 40 cm plastic cage with a wire cover that had been equipped with a drinking water bottle and a feeding place. Then the rats were stabilized for 3 weeks to reduce the stress gained due to the differences in the new environment in the research site and the environment (Rejeki et al., 2018).

2.4.2 Induction and Treatment of Gastric Ulcers and Bloating

Acetic acid is used as an inducing agent for gastric ulcers and bloating due to its ability to cause obvious local health disorders in the abdominal cavity so that it is easy to observe (Dong et al., 2025), the pain that occurs is caused by a decrease in pH in the abdominal cavity which causes ulcers in the gastric tissue (Lara et al., 2021). The manufacture of Acetic Acid induction solution is made by dissolving 25% Acetic Acid as much as 50 mL, in 50 mL of aqueducts, so that an acetic acid solution with a concentration of 12.5% is obtained. The solution (Inducer) is injected or given to rats according to body weight with the provision of 1 mL/200 g body weight.

The administration of Biovina supplement extracts and gastric drugs to test animals was carried out by the oral administration method (checked). Biovina supplement extract 100 mg is dissolved in an aqueduct of 100 mL and then administered orally using a sonde (check) for 7 days. During the treatment period, the animals were observed to see changes in the behavior and morphology of the Wistar rat. The research object used was 18 male Wistar rats divided into 6 groups (Sundalangi et al., 2016). Group I (3 baseline or normal rat), group II (3 Negative Control rat), group III (3 Positive Control rat), group IV (3 Biovina 1 rat), group V (3 Biovina 2 rat), (3 Biovina 3 rat). The control group was not given an inducing solution and treatment. The negative control group was given 12.5% Acetic Acid for 2 days then given aqueducts of 1 mL/200 g body weight for 7 days and then termination of day 7. The positive control group was given 12.5% Acetic Acid for 2 days then continued with the administration of Promag gastric drug for 7 days, then terminated on the 7th day. The Biovina 1 group was given 12.5% acetic acid for 2 days then continued with the administration of Biovina solution 1 mg/mL for 7 days and terminated on the 7th day. The Biovina 2 group was given 12.5% acetic acid for 2 days then continued with the administration of Biovina solution 2 mg/mL for 7 days and terminated on the 7th day. The Biovina 3 group was given 12.5% acetic acid for 2 days then continued with the administration of Biovina 3 mg/mL solution for 7 days and terminated on the 7th day.

2.5 Observation Parameters

2.5.1 Observation of Rat Growth

Mouse growth was determined through weight measurements and observation of activity and behavior (Suyoko et al., 2023). Weighing the weight of Wistar rats (g) aims to determine the health of test animals and monitor weight changes (Ismiranty et al., 2023). The method of data collection and tabulation is as shown in Table 2.

2.5.2 Morphological or Behavioral Observation Parameters

This parameter is observed by visible appearance and is carried out by paying attention to movements, breathing movements, eyes, muzzle, hair, eating and drinking activities.

2.5.3 Measurement of Stomach Length and Width (Bloating Parameters)

To find out the occurrence of ulcers and gastric bloating, the length and width (cm) of the rat's stomach were measured with a digital caliper, which would be the result of multiplication, which would be the magnitude of bloating (cm²).

2.5.4 Microscopic Observation of Gastric Ulcers

This microscopic observation is carried out to see gastric ulcers through the parameters of looking at the color of the mucosa, the surface of the mucosal layer (there is a wound or its number) per 1 field of view, and necrosis of the inside of the stomach.

2.6 Data Analysis

The data will be analyzed using the Excel program to tabulate by averaging and looking for standard deviations and graphing. Then the difference statistics were tested with variance analysis (ANOVA) using SPSS. If there is an ANOVA with a significant difference in $\alpha = 0.05$, then it is further tested using the Duncan test at a confidence level of 95%.

3. RESULTS AND DISCUSSION

3.1 Growth of Wistar Rats During the Adaptation Period

Observation of the growth of Wistar rats during the adaptation period or before treatment is necessary to ensure that the rat used are healthy and grow well before the treatment is carried out. Observation of the growth of the rats before treatment was carried out through periodic weight weighing every 3 days. Weighing lasted for 18 days before treatment or the adaptation period to the mean and standard deviation can be seen in Table 1. The increase in the growth of these rat over 18 days can be seen in Figure 1 showing that all of the rat used are growing and healthy. Figure 1 shows that the lowest average body weight of Wistar rat before treatment (adaptation period) was the Biovina 2 group with the highest average body weight of 257.00 ± 66.78 g on day 18 of the adaptation period, while the highest body weight over time (days) occurred in the Biovina 3 treatment with an average of 306 ± 66.78 g. It can be ascertained that the Rat used were healthy rat (Figure 1).

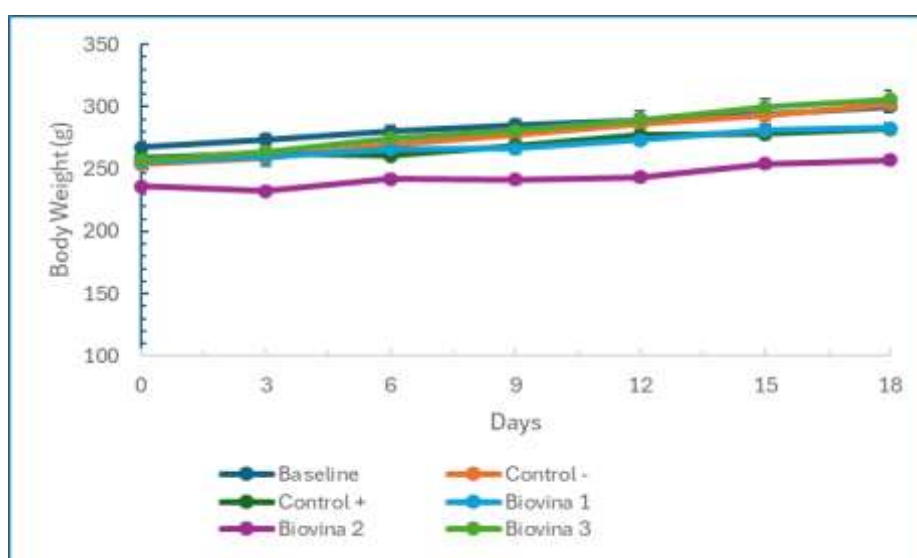


Figure 1. Wistar mouse growth graph of each treatment group baseline (blue line), Negative Control (Orange color), Positive Control (dark green color line), Biovina 1 (light blue line), Biovina 2 (purple line), Biovina 3 (Light green).

Figure 1 shows weight observation over 18 days, it can be seen that most of the groups experienced a gradual increase in weight from the beginning to the end of the adaptation period. The Biovina 2 group showed the lowest weight gain with an average

weight gain of 21.00 ± 4.00 , the Negative Control group showed the highest increase with an average of 48.33 ± 16.65 . It shows that all groups experienced good growth during the adaptation period.

Table 1. Growth and weight gain (g) of Wistar rat Adaptation Period or before treatment for 18 days showing good growth.

Sample code	Mean and standard deviation of baseline weight (g)/Day 0	Mean and standard deviation of final weight (g) day 18	Mean and standard deviation of weight gain (g)
Baseline (Normal)	267.33 ± 7.44	300.33 ± 15.01	33.00 ± 5.58
Negative Control (K ⁻)	253.67 ± 6.71	302.00 ± 12.05	48.33 ± 6.65
Positive Control (K ⁺)	259.33 ± 3.08	282.33 ± 9.44	23.00 ± 6.46
Biovina 1 mg/mL (B1)	256.00 ± 4.91	283.33 ± 11.34	27.33 ± 3.58
Biovina 2 mg/mL (B2)	236.00 ± 9.48	257.00 ± 6.78	21.00 ± 4.00
Biovina 3 mg/mL (B3)	257.33 ± 3.47	306.00 ± 6.78	33.00 ± 10.58

The weight of Wistar rat before treatment experienced a gradual increase in weight in all groups. The weight gain in the Baseline group increased by 33.00 ± 10.58 g, K⁻ increased by 48.33 ± 16.65 g, K⁺ increased by 23.00 ± 16.46 g, Biovina 1 increased by 27.33 ± 13.58 g, Biovina 2 increased by 21.00 ± 4.00 g, increased by 33.00 ± 10.58 g. The highest weight gain was seen in Negative Control and the lowest increase in Biovina 2. That Wistar rat during the adaptation period experienced a good increase, this increase was also seen morphologically in Wistar rat (Table 1).

Wistar rat that are healthy and well adapted to the laboratory environment generally show gradual weight gain over time, especially if feed and water intake is administered ad libitum. This condition reflects that the nutritional and energy needs of rats are well met, so that metabolic processes, tissue growth, and physiological functions of the body can take place normally, stable weight gain shows that rats do not experience excessive environmental stress, metabolic disorders, or pathological conditions that can inhibit growth (Fitria *et al.*, 2019).

3.2 Rats Morphology and Behavioral Activity

Observation of morphological behavior with the naked eye was carried out with a focus on movement, breathing frequency, eye and muzzle conditions, hair/hair, and eating and drinking activities of rats (Table 2). The results of morphological observations during the initial adaptation period of the study showed uniform and normal conditions of healthy movements, clear clean eyes, healthy clean muzzles, clean white hair, and normal eating and drinking activities.

In the adaptation period, the observation results resembled the initial condition (day 0), because the rat came from the same laboratory so there were minimal changes. Morphological behavior observations on day 18 showed uniform (normal) healthy movements, rhythmic calm breathing, clear clean eyes, healthy clean muzzle, clean white hair, and normal eating and drinking activities (Table 2).

Observations of the morphology and behavior of rat at the induction stage of gastric inflammation with acetic acid began to show differences between the induced group and the baseline group. The induced group displayed fewer active movements than the baseline, rapid breathing with irregular rhythms and panting, eyes often closed, sweaty snout and nose, hair protruding/standing, and appetite and drinking decreased drastically during induction (Table 2) conditions worsened for 2 days with enlarged stomach, shortness of breath, and near-death.

Morphological appearance and activity of the Baseline group maintained normal morphology and activity (Table 2) agile and active movements, normal and regular breathing, accompanied by frequent scratching of the ears and muzzle (Figure 2b). The eyes are clean and clear, while the white hair plumage appears smooth, dense, and shiny (Figure 2b).



Figure 2. Morphological observations and activity of induced (a), non-induced (Baseline) Wistar rat (b) (Pandiangan et al., 2026c)

The morphological appearance and activity of the Negative Control treatment after acetic acid induction (Figure 2a), were seen still, little movement, shortness of breath, decreased appetite, frequent sleeping, flatulence or swelling, and raised and standing feathers. On day 2 activity decreases with slight movement, irregular and shortness of breath, enlarged or bloated stomach, and sweaty nose (Figure 2a). On the seventh day, the test animals died (Table 2).

Table 2. Morphological observations and behavioral activity of Wistar rat Baseline (normal), Negative control (K-), Positive control (K+), Biovina treatment 1, 2, 3 mg/mL during 27 days of observation (Pandiangan et al., 2026c).

Groups	Observation of the morphology, activity and behavior of Wistar rats		
	Adaptation period 18 days	Induction (acetic acid) 2 days	After treatment (7 days)
Baseline (Normal)	Agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, fine and dense white hair.	Agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, fine and dense white hair.	Agile movements, regular breathing, normal eating activities, clean and clear eyes, frequent scratching of the ears, smooth and thick, clean hair.
K-	Agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, fine and dense white hair.	Slight movement, shortness of breath, decreased appetite, frequent sleeping, flatulence/swelling, feathers raised and standing.	Not much movement, irregular breathing, not much movement, enlarged/bloated, sweaty nose. The second day died.
K+	Agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, fine and dense white hair.	Slight movement, decreased appetite, frequent sleeping, flatulence/swelling, feathers raised and standing.	Agile movements, normal and calm breathing, frequent scratching of the ears, clean and swollen eyes, clean and dense fur.
B 1	Agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, fine and dense white hair.	Slight movement, shortness of breath, decreased appetite, frequent sleeping, flatulence/swelling, feathers raised and standing.	Agile movements, normal and calm breathing, normal eating activities, frequent stroking of his nose, frequent scratching of his ears, clean and clear eyes, fine and dense white fur.

B 2	Agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, fine and dense white hair.	Slight movement, shortness of breath, decreased appetite, frequent sleeping, flatulence/swelling, feathers raised and standing.	Agile movements, normal and calm breathing, normal eating activities, frequent stroking of his nose, frequent scratching of his ears, clean and clear eyes, fine and dense white fur.
B 3	Agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, fine and dense white hair.	Slight movement, shortness of breath, decreased appetite, frequent sleeping, flatulence/swelling, feathers raised and standing.	Agile movements, normal and calm breathing, normal healthy eating activities, frequent stroking of his nose, frequent scratching of his ears, clean and clear eyes, fine and dense white fur.

Observations of the morphology and behavior of rat at the induction stage of gastric inflammation with acetic acid began to show differences between the induced group and the baseline group. The induced group displayed fewer active movements than the baseline, rapid breathing with irregular rhythms and panting, eyes often closed, sweaty snout and nose, hair protruding/standing, and appetite and drinking decreased drastically during induction (Table 2) conditions worsened for 2 days with enlarged stomach, shortness of breath, and near-death.

Manifestation of morphological behavior and activity of the Positive Control treatment (Table 2) prior to induction, showed agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, and smooth and dense white hair. During acetic acid induction, there is a decrease in activity with slight movement, shortness of breath, decreased appetite, frequent sleep, flatulence and swelling, and raised and standing feathers as a result. After administration of the drug solution, the condition recovered significantly with agile return movements, normal and calm breathing, frequent scratching of the ears, clean and clear eyes, and clean and dense fur.

Biovina 1 treatment prior to induction, showed agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, and fine and dense hair. After induction, there is a decrease with slight movement, shortness of breath, decreased appetite, frequent sleep, flatulence and swelling, and raised and standing feathers. At the administration of Biovina 1 mg/mL, (Table 2) the condition was recovered, characterized by agile movements, normal and calm breathing, normal eating activities, frequent stroking of the nose, frequent scratching of the ears, clean and clear eyes, and smooth and dense white hair.

Biovina 2 treatment before induction showed agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, and fine and dense white hair. After induction, (Table 2) the condition worsens with slight movement, shortness of breath, decreased appetite, frequent sleeping, flatulence or swelling, and raised and standing feathers. After administration of Biovina 2 mg/mL solution, the recovered condition is indicated by agile movements, normal and calm breathing, normal eating activities, frequent stroking of the nose, frequent scratching of the ears, clean and clear eyes, and smooth and dense white hair.

Biovina 3 treatment before induction, shows agile movements, normal breathing, frequent scratching of the ears, clean and clear eyes, and fine and dense white hair. (Table 2) After induction, slight movement, shortness of breath, decreased appetite, frequent sleeping, flatulence and swelling, and raised and standing feathers. After administration of Biovina solution 3 mg/mL, the condition is recovered, characterized by agile movements, normal and calm breathing, normal healthy eating activities, frequent stroking of the nose, frequent scratching of the ears, clean and clear eyes, and smooth and dense white hair.

3.3 Stomach Length and Width

Measurements of the length and width of the stomach can be used as parameters to see bloating. The size of the length and width of the stomach is a morphological change to assess changes in the structure of the stomach during the healing process. The results of the measurement of the length and width of the rat's stomach can be seen in Table 3. The table shows that in the baseline group, the average length of the stomach is 2.20 ± 0.26 cm and the width is 1.30 ± 0.15 cm. Negative Control appears to enlarge with an average hull length of 5.30 ± 1.41 cm and width of 2.90 ± 0.51 cm. Positive Control Hull length 2.30 ± 0.36

and width 1.40 ± 0.50 cm. Biovina 1 has a hull length of 2.40 ± 0.78 cm and a width of 1.40 ± 0.50 cm. Biovina 2 has a hull length of 2.30 ± 0.36 and a width of 1.40 ± 0.50 cm. Biovina 3 shows a hull length of 2.80 ± 0.36 cm and a width of 1.40 ± 0.45 cm.

Table 3. Length and width of the stomach of wistar rats (cm) in the treatment group Baseline (normal), Negative control (K), Positive control (K+), Biovina treatment 1, 2, 3 mg/mL for 27 days of observation (Pandiangan et al., 2026c).

Groups	Hull Size (cm)		Mean and standard deviation Hull Size (cm)	
	Length	Width	Length	Width
Baseline (I)	2,30	1,30	2.20 ± 0.26 ^a	1.30 ± 0.15 ^a
Baseline (II)	2,40	1,50		
Baseline (III)	1,90	1,20		
K- (I)	5,50	2,50	5.30 ± 1.41 ^b	2.90 ± 0.51 ^b
K- (II)	6,60	3,50		
K- (III)	3,80	2,80		
K+ (I)	1,50	3,80	2.10 ± 0.65 ^a	1.80 ± 0.81 ^a
K+ (II)	2,80	1,40		
K+ (III)	2,20	1,40		
Biovina 1 (I)	1,50	2,00	2.40 ± 0.78 ^a	1.40 ± 0.50 ^a
Biovina 1 (II)	2,80	1,00		
Biovina 1 (III)	2,90	1,40		
Biovina 2 (I)	2,40	1,50	2.30 ± 0.36 ^a	1.40 ± 0.40 ^a
Biovina 2 (II)	2,60	1,80		
Biovina 2 (III)	1,90	1,00		
Biovina 3 (I)	3,20	1,90	2.80 ± 0.36 ^a	1.40 ± 0.45 ^a
Biovina 3 (II)	2,70	1,50		
Biovina 3 (III)	2,50	1,00		

Remarks: The letter 'a' indicates no significant difference, the letter 'b' indicates a significant difference.

The size of the rat's stomach in the Negative Control treatment appeared to be enlarged with an average stomach length of 5.30 ± 1.41 cm and a width of 2.90 ± 0.51 cm (Table 3). This increase in size indicates the occurrence of enlargement or gastric bloating due to the administration of acetic acid (Figure 2b). Acetic acid serves as a corrosive agent that irritates the gastric mucosa, impairs the integrity of the epithelium, and triggers acute local inflammation. This response causes tissue edema, mucosal ulceration, and vasodilation accompanied by leukocyte infiltration, which macroscopically appears as swelling or bloating of the stomach (Ateufack *et al.*, 2015).

The size of the stomach in the Positive Control treatment was similar to that of the Baseline group, which was 2.10 ± 0.65 cm long and 1.80 ± 0.81 cm wide (Table 3). that the stomach that had previously experienced enlargement or bloating due to acetic acid induction had improved after commercial drug administration, positive control treatment showed results close to the

baseline group, which was characterized by reduced gastric bloating, thus suggesting that commercial drugs were effective in repairing acetic acid-induced gastric damage.

Biovina 1 showed a normal stomach size, with a length of 2.40 ± 0.78 cm and a width of 1.40 ± 0.50 cm. It shows that the stomach that previously experienced enlargement or bloating due to acetic acid induction has healed after the administration of Biovina 1 mg/mL, which is characterized by reduced bloating in the stomach. The smaller size of the stomach shows that gastric bloating is reduced, this is influenced by flavonoids and tannins in *Dysphania ambrosioides* that help inhibit inflammation, as well as catechins from *Uncaria gambir* which can improve the gastric lining by lowering TNF- α and increasing prostaglandin E2 so that epithelial cells more quickly repair damage to the gastric mucosa (Oswari *et al.*, 2019)

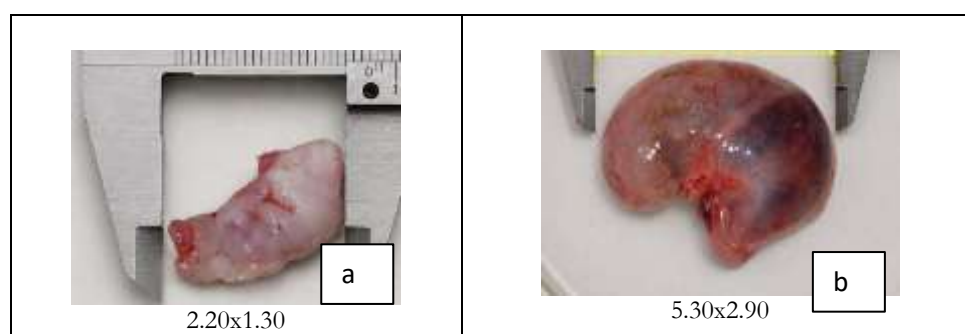
Biovina 2 has a hull length of 2.30 ± 0.36 and a width of 1.40 ± 0.50 cm. Shorter gastric size suggests that administration of Biovina 2 mg/mL may relieve acetic acid-induced gastric bloating. The result is due to the presence of the antioxidant catechins from *Uncaria gambir* that protect the mucosa from oxidative damage, as well as the protective effects of quercetin and kaempferol compounds in *Dysphania ambrosioides* (Riberiro *et al.*, 2023). The quercetin compound is able to inhibit the production of the hormone histamine in the gastric tissues. Histamine is one of the main triggers for gastric cells to produce acid (Ahmed, 2024).

Biovina 3 shows a hull length of 2.80 ± 0.36 cm and a width of 1.40 ± 0.45 cm. A larger gastric size than Biovina 1,2 indicates that administration of Biovina 3 mg/mL may cure gastric bloating due to the administration of acetic acid, even if given in different doses. Tannins from *Uncaria gambir* are thought to form a tannin-protein complex in the damaged mucosa, protecting tissues from further irritation and promoting epithelial regeneration (Jesus *et al.*, 2012). Suyoko *et al.* (2023) reported that Biovina supplements can restore gastric morphology to almost normal within 4–7 days. It shows that Biovina 1, 2, and 3 treatments given 1 mg/mL, 2 mg/mL, and 3 mg/mL have the potential and can cure gastric ulcers and bloating in rats.

Data analysis used One-Way ANOVA to identify the effect of treatment on the parameters of the length and width of the rat stomach in each treatment group Negative Control, Positive Control, Biovina 1, Biovina 2, Biovina 3. Before the ANOVA test, the variance homogeneity test was carried out using the Levene test. The test results showed a significance value of 0.123 for the length of the hull and 0.204 for the width of the hull (with a significance level of > 0.05), so that the data met the assumption of homogeneous variance.

The One-Way ANOVA test showed significant differences between groups in the parameters of hull length, with values $F = 7.943$ and $p = 0.002$ (with significance level < 0.05). Designation of a significant effect of treatment on stomach length. In the stomach width parameters, there were also significant differences with $F = 4.265$ and $p = 0.018$ (with significance level < 0.05), which confirmed the significant effect of the treatment on stomach width.

The Duncan test on the stomach length parameter showed that the Negative Control group had a highest average of 5.3 cm, which differed significantly compared to all the much lower baseline, Positive Control, and Biovina 1, 2, and 3 groups. The Duncan test on the rat stomach width parameters showed that the Negative Control group had the highest average value of significantly 2.9 cm, while the Positive Control and Biovina 1, 2, and 3 groups recorded significantly lower stomach width. The significant decrease in gastric length in Biovina groups 1, 2, and 3 reflects Biovina's effectiveness in inhibiting the formation of gastric ulcers.



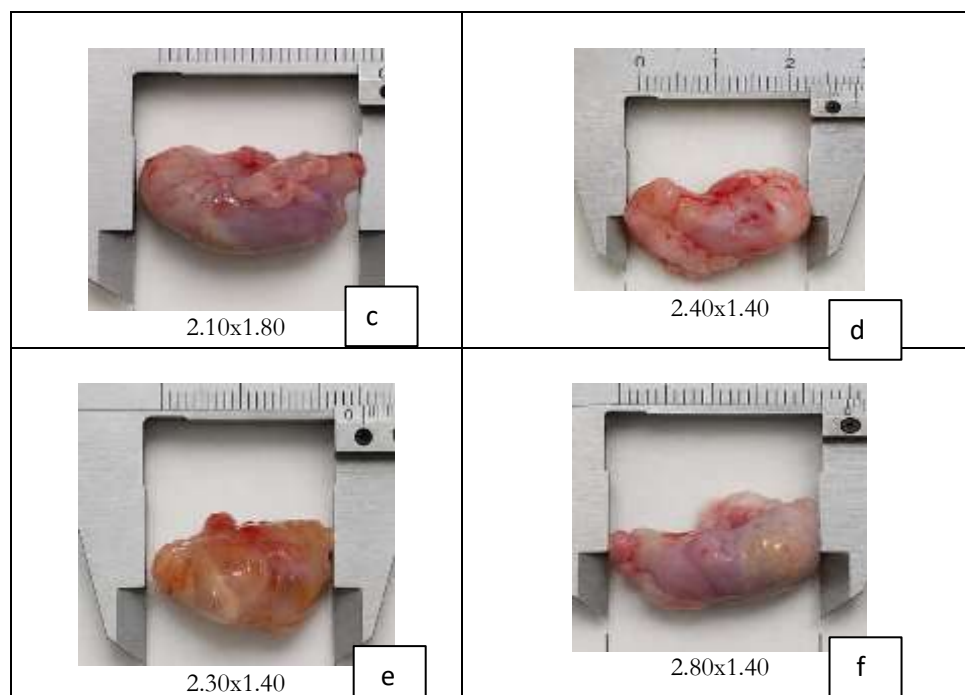


Figure 3. Treated rat stomach, length x width (cm) (a) Baseline, (b) Group K-, (c) Group K+, (d) Biovina 1, (e) Biovina 2. (f) Biovina 3

Figure 3 shows the difference in the morphology of the stomach, in the Baseline group (Figure 3a) the stomach has a normal size of 2.20 cm long and 1.30 cm wide, the outside of the stomach is fresh pink. Negative Control Treatment (Figure 3b) the stomach swelled to a length of 5.3 cm and a width of 2.9 cm, it appears that the outside of the stomach is reddish-black showing inflammation in the stomach. The Positive Control Treatment (Figure 3c) shows that the outside of the stomach is pink, showing that the normal return size is 2.10 cm long and 1.80 cm wide. Biovina 1 treatment (Figure 3d) shows the outside of the stomach being fresh red with a shrinking stomach size with a length of 2.40 cm and a width of 1.40 cm. Biovina 2 treatment (Figure 3e) shows the outside of the stomach is fresh pink with a smaller stomach size with a length of 2.30 cm and a width of 1.40 cm. Biovina 3 treatment (Figure 3f) fresh red gastric outer splint with a shrinking gastric size of 2.80 cm long and 1.40 cm wide. Based on the external shape of the stomach, it shows that Biovina 1, 2, 3 treatment is effective in healing swollen stomachs.

3.4 Healing of Gastric Ulcers and Bloating

Macroscopic observations of the stomach of Wistar rats were carried out on several observations, namely the color of the stomach, the size of the stomach, the surface of the gastric mucosa, and the necrosis of the mucosa in each treatment group during 27 days of observation. Based on macroscopic observations on the stomachs of Wistar rats, there were variations in size, mucosal surface conditions, mucosal necrosis, and gastric color between treatment groups.

Table. 4 Macroscopic observation of gastric bloating in Wistar rats after 7 days of Baseline (normal), Negative control (K-), Positive control (K+), Biovina treatment 1, 2, 3 mg/mL (for 27 days of observation).

Parameters	Groups			Treatment		
	Baseline	K- Brownish	K+	B1	B2	B3
Color	Pink	red / black	Pink	Pink	Pink	Pink
Hull size (cm ²)	2,86	15,37	3,78	3,36	3,22	3,92

Surface Gastric mucosa						
	Thickness/shiny/Slippery	Thinning/dull	Thickness/shiny/Slippery	Thickness/shiny/Slippery	Thickness/shiny/Slippery	Thickness/Glossy/Smooth
Necrosis mucosa						
	None/healthy	Abundant and hardened	None/healthy	None/healthy	None/healthy	None/healthy

In the Baseline group, normal gastric conditions were characterized by pink, gastric size of 2.86 cm², thick, shiny, and slippery mucosal surfaces, and no necrosis was found on the mucosal surface. This condition shows a healthy stomach condition without any damage disorders.

The Negative Control treatment showed the most significant changes. The color of the stomach changes to brownish-black, red, with a stomach size of 15.37 cm², a thin and dull mucosal surface, and the discovery of abundant and hardened mucosal necrosis. These changes are the impact of acetic acid induction which causes inflammation and damage to the gastric mucosa due to acetic acid without any treatment given.

Positive Control treatment showed healing of the stomach, with the color of the stomach returning to pink, the size of the stomach decreased to 3.78 cm², the mucosal surface was thick, shiny, and slippery, and no mucosal necrosis was found. These results show that commercial drugs are able to effectively repair gastric damage induced by acetic acid.

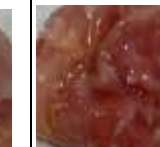
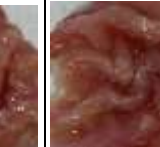
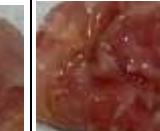
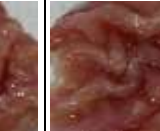
Biovina treatment of 1 mg/mL showed results close to Positive Control, with pink color, 3.36 cm² gastric size, thick, shiny, and slippery mucosal surface, and no mucosal necrosis found. This shows that the administration of Biovina 1 is able to cure bloating and ulcers due to the administration of acetic acid.

Biovina 2 mg/mL certification also showed improved gastric condition, with pink color, 3.22 cm² gastric size, thick and shiny mucosal surface, and no mucosal necrosis found. The size of the stomach in this treatment was smaller than Biovina 1, which showed better healing as the dose increased.

Biovina treatment of 3 mg/mL showed a pink color and a thick, shiny mucosal surface, and no mucosal necrosis was found. The size of the hull is 3.92 cm², slightly larger than that of Biovina 1 and Biovina 2. These results show that too high a dose increase can cure ulcers and gastric bloating due to the administration of acetic acid.

Microscopic observations of the stomach of Wistar rats were carried out with two observations, namely the surface of the gastric mucosa and mucosal necrosis in each treatment group during the 27 days of observation. In the Baseline group, the surface of the gastric mucosa showed a normal condition of rugae that was clearly visible, shiny, and slippery, and no necrosis was found on the mucosal surface. Negative Control Treatment showed that the mucosal surface was thinned and appeared dull, and that mucosal necrosis was abundant and hardened. Positive Control Treatment showed a noticeable recovery. The surface of the mucosa again shows a clear, shiny, and slippery rugae structure, and no necrosis is found on the mucosal surface. Biovina treatment of 1 mg/mL showed an improved gastric mucosal surface, with a clear, shiny, and slippery surface of the rugae, and no necrosis was found on the mucosal surface. Biovina 2 mg/mL treatment showed improved gastric mucosal surface, clear, shiny, and slippery rugae, and no necrosis on the mucosal surface. Biovina treatment of 3 mg/mL also showed improved gastric mucosal surface, rugae were clear, shiny, and slippery, and no mucosal necrosis was found.

Table. 5 Microscopic Observations of Gastric Ulcers and Gastric Bloating in Wistar Rats after 7 days of Baseline (normal), Negative Control (K-), Positive Control (K+), Biovina Treatment 1, 2, 3 mg/mL for 27 days of observation through the parameters of the gastric mucosal wall and necrosis or wound.

Parameters	Groups Treatment					
	Baseline	K-	K+	B1	B2	B3
Surface Gastric mucosa	 Rugae/shiny/ Slippery	 flat/dull/stiff	 Rugae/shiny/ Slippery	 Rugae/shiny/ smooth	 Rugae/shiny/ smooth	 Rugae/shiny/ Slippery
Necrosis mucosa	 None/healthy	 Lot/hardened	 None/healthy	 None/healthy	 None/healthy	 None/healthy

Macroscopic observation of the outer stomach in the Baseline group, the stomach appeared normal and healthy, with a pink color, a supple and smooth outer surface texture (Table 4), and an inner structure that showed wavy, thick, pink mucosal folds (plica) (Table 5). This condition is consistent with the image of a healthy stomach that has not been damaged, where the gastric mucosa remains intact and elastic, retaining a protective mucous layer that covers the wavy epithelial surface (Nakao *et al.*, 2014).

In the acetic acid-induced Negative Control treatment for 2 days, changes were seen macroscopically, the rat's stomach appeared to be enlarged in size or bloating, mucosal color change to dark red to blackish (Table 4), and a lot of necrosis and ulcers (Table 5) (Suyoko *et al.*, 2023). The stomach appears to be enlarged with a blackish-red color, indicating bleeding accompanied by thinning of the mucosa, necrosis is also seen in the form of pale and hard hollow areas, indicating severe tissue damage due to exposure to acetic acid (Usman, 2016; Pandiangan *et al.*, 2026c).

In the Positive Control treatment with the administration of Acetic Acid for 2 days, followed by the administration of commercial drugs with 1 time a day for 7 days, the outer surface of the stomach is pink again (Table 4), with a wavy and thick mucosal structure without necrosis (Table 5), indicating the recovery of the gastric mucosa after the occurrence of ulcers (Pandiangan *et al.*, 2026c). Consistent administration of the drug suppresses acid secretion, reduces inflammation, and accelerates the regeneration of gastric epithelium until it reaches a macroscopic condition close to normal (Sigit *et al.*, 2012).

In Biovina 1 treatment with acetic acid for 2 days, Biovina was continued with 1 mg/mL for 7 days, the outside of the stomach was pink, small without any swelling (Table 4), smooth surface texture (Table 4), and wavy and thick inner mucosal folds without necrosis (Table 5) (Pandiangan *et al.*, 2026c).

In the Biovina 2 treatment with the administration of acetic acid for 2 days, followed by the administration of Biovina as much as 2 mg/mL for 7 days the outside appears pink, small without swelling (Table 4), on the inside there are wavy and thick pink folds of the gastric mucosa without necrosis (Table 5). This shows that the administration of Biovina 2 mg/mL can accelerate the healing of gastric ulcers due to the administration of acetic acid (Pandiangan *et al.*, 2026c).

In Biovina 3 treatment with acetic acid for 2 days, followed by Biovina 3 mg/mL for 7 days, the outer stomach appears pink, the outer surface texture is smooth (Table 4), and the wavy and thick inner mucosal folds appear to return to normal without necrosis (Table 5) (Pandiangan *et al.*, 2026c). There is no significant difference between the Positive Control treatment and the Biovina 1, 2 and 3 treatments, showing that all Biovina treatments are effective in the healing process of gastric ulcers (Pandiangan *et al.*, 2026c).

3.5 Discussion

The healing mechanism is strongly suspected to involve antioxidant and anti-inflammatory activity from the bioactive compounds Pasote (Pandiangan et al., 2026a) and Gambir. Pasote (*Dysphania ambrosioides*) is known to contain flavonoids, phenolics, terpenoids, and essential oils that have the ability to capture free radicals (ROS) thereby reducing oxidative stress on the gastric mucosa (Pandiangan et al., 2026a). Decreased ROS plays a role in preventing lipid peroxidation of gastric epithelial cell membranes which is one of the main causes of tissue necrosis (Kangwan et al., 2014; Pandiangan et al., 2026c).

Gambier (*Uncaria gambir*) is rich in catechins and epicatechins, polyphenol compounds with strong antioxidant activity. Catechins play a role in stabilizing cell membranes, accelerating epithelial regeneration, and promoting the formation of new blood vessels, thereby accelerating the wound healing process in the gastric mucosa (Faadhilah et al., 2025). The antioxidant, anti-inflammatory, and increased protective prostaglandins, explain why Biovina groups 1, 2 and 3 show a gastric with a near-normal size, fresh mucosal color, and the absence of necrosis or ulcers (Table 5) (Pandiangan et al., 2026a and 2026c).

In the three Biovina treatments tested, it was seen that all groups showed smaller gastric size than the Positive Control group (Pandiangan et al., 2026c). The pink and fresh color of the mucosa and reduced necrosis are the main indicators of histological recovery of the stomach influenced by anti-inflammatory (Pandiangan et al., 2026a) and antioxidant mechanisms (Dong et al., 2025).

The administration of commercial drugs in the Positive Control and Biovina 1,2,3 treatment showed a healing effect of gastric ulcers and bloating which can be seen in (Table 4) showing the size of the stomach and bloating to be reduced (Pandiangan et al., 2026c), which can be seen from the return of the size of the stomach to close to normal (Table 4), the color of the mucosa which is fresh pink, and the reduction and even the loss of necrosis and wounds (Table 5). Biovina treatment 1, 2 and 3 showed that the size of the stomach looked smaller than that of the Positive Control group, which showed the effectiveness of Biovina 1, 2 and 3 in improving bloating and peptic ulcers (Pandiangan et al., 2026c). The effectiveness of healing stomach and bloating by Biovina health supplements is a potential for it to be developed into Indonesian Natural Medicine (Yuslianti et al., 2016).

The healing effect is thought to be strongly related to the antioxidant and anti-inflammatory activity of the bioactive compounds contained in Pasote (*Dysphania ambrosioides*) (Pandiangan et al., 2026a) and Gambir (*Uncaria gambir*). Phenolic compounds, flavonoids, catechins, and tannins play a role in lowering oxidative stress, inhibiting inflammatory mediators, and increasing protective prostaglandins of the gastric mucosa (Pandiangan et al., 2026c). The combination of these mechanisms contributes to the restoration of mucosal structure (Pandiangan et al., 2026c), increased epithelial protection (Pandiangan et al., 2026a), and prevention of further damage to the stomach (Oswari et al., 2019). In addition, the side effects of chronic gambier use so far conveyed by BPOM are that kidney disorders can be overcome by administering pasote water extracts with indications of a decrease in serum creatinine content in rats (Pandiangan et al., 2023, Taghulihi et al., 2022). This shows that the combination of these two natural ingredients is synergistic with each other for the cure of increased creatinine disorders and bloating or gastric ulcers ((Pandiangan et al., 2026c, Taghulihi et al., 2022).

The use of the results of this test combination of natural medicinal plants (Biovina), especially local plants in Indonesia, hopes to develop government steps to downstream natural medicinal products in the future (Diah et al., 2024). In the future, if clinical trials have been carried out on humans empirically, the results of this research can be commercialized both nationally and globally in this 4.0 era (Diah et al., 2024, Pandiangan et al., 2025).

4. CONCLUSION

Based on the results and discussion above, it is concluded that the administration of Biovina health supplements based on Pasote and Gambir has proven to be effective in treating gastric ulcers and bloating in Wistar rats or potentially as a gastroprotective and therapeutic agent in the treatment of bloating and gastric damage in rats due to acetic acid chemical irritation.

ACKNOWLEDGEMENTS

The funding or budget for this research comes entirely from the 2025 Prototype Application-Output Research with decree no.0070/C3/AL.04/2025 and agreement/contract no. 320/UN12.13/LT/2025 with the title Formulation, Standardization and Chronic Toxicity Test of Gambier (*Uncaria gambier*) Extract and Pasote (*Dysphania ambrosioides*) Drugs for Gastric Ulcers and Immunomodulators chaired by Prof. Dr. Dra. Dingse Pandiangan, M.Si.

AUTHOR CONTRIBUTIONS

Dingse Pandiangan: Conceptualization, Methodology, Investigation, Project Administration, Funding Acquisition, Writing—Original Draft.

Sarla Bukari: Investigation, Data Collection, Laboratory Analysis, and Validation.

Nelson Nainggolan: Methodology, Formal Analysis, Statistical Analysis, and Data Curation.

Enih Rosamah: Supervision, Validation, and Writing—Review and Editing.

Michael Vallery Tumbol: Investigation, Laboratory Analysis, Visualization, and Data Interpretation.

Redsway Truman Douglas Maramis: Investigation, Validation, Resources, and Writing

Agnes Virginia Simamora: Review and Editing manuscript

Silvana Tulandi: Supervision, Validation, and Writing—Review and Editing.

Ivana Christy Nainggolan: Investigation, Data Collection, Laboratory Analysis, and Validation

All authors reviewed and approved the final version of the manuscript

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

The animal study protocol was reviewed and approved by the Health Research Ethics Committee of Poltekkes Kemenkes Manado Indonesia (Manado Health Polytechnic, Ministry of Health, Indonesia) under Approval No. DP.04.03/FXXX.28/568/2025.

REFERENCES

- [1] Ahmed, Z.A., 2024. Gastroprotective effect of quercetin and misoprostol in ethanol-induced gastric ulcer in rats. *The Turkish Journal of Gastroenterology* 35(11), 822–830. <https://doi.org/10.5152/tjg.2024.24209>.
- [2] Aisyah, S., Gumelar, A.S., Maulana, M.S., Amalia, R.A.H.T., 2023. Identification of vertebrate animal characteristics of white rat mammals (*Rattus norvegicus*) based on their anatomical morphology. *Proceedings of Semnas Bio*.
- [3] Annaz, H., Abdelaal, S., Mandour, D.A., Mahdi, I., Mahmoud, M.F., Sobeh, M., 2023. Mexican tea (*Dysphania ambrosioides* (L.) Mosyakin & Clemants) seeds attenuate tourniquet-induced hind-limb ischemia–reperfusion injury by modulating the ROS and NLRP3 inflammasome pathways. *Journal of Functional Foods*.
- [4] Ateufack, G., Mokam, E.C.D., Mbiantcha, M., Feudjio, R.B.D., Nana, D., Kamanyi, A., 2015. Gastroprotective and ulcer-healing effects of *Piptadeniastrum africanum* on experimentally induced gastric ulcers in rats. *BMC Complementary and Alternative Medicine* 15, 214. <https://doi.org/10.1186/s12906-015-0713-5>.
- [5] Beserra, A.M.S., Calegari, P.I., Souza, M.C., dos Santos, R.A.N., Lima, J.C. da S., Silva, R.M., Balogun, S.O., Martins, D.T.O., 2011. Gastroprotective and ulcer-healing mechanisms of ellagic acid in experimental rats. *Journal of Agricultural and Food Chemistry* 59(16), 9117–9124. <https://doi.org/10.1021/jf2003267>.
- [6] Buana, A.T., Jasaputra, D.K., Tiono, H., 2020. Comparison of carminative effects of ethanol extracts of turmeric (*Curcuma longa* L.) and kencur (*Kaempferia galanga* L.) on intestinal motility of Swiss Webster mice. *Journal of Medicine and Health* 2(6), 188–196.
- [7] Deswati, Afriani, T., Salsabila, N.P., 2022. Antioxidant benefits of the gambier plant (*Uncaria gambir* Roxb.) for health, cosmetics, and food: A literature review. *Afyyah* 6(2).
- [8] Dong, S., Wang, X., Liu, Y., Qiao, L., Xue, Q., Zhou, Y., Xu, Z., 2025. A novel gastric ulcer model in rats using filter paper with acetic acid. *PLOS ONE* 20, e0319096. <https://doi.org/10.1371/journal.pone.0319096>.

- [9] Faadhilah, A., Widjastuti, T., Adriani, L., 2025. Identification of antioxidant levels, catechin, tannin, crude protein, and crude fibre from the fermentation of gambier leaves (*Uncaria gambir* R.) using *Rhizopus oligosporus* and *Lactobacillus plantarum*. *Indonesian Journal of Pharmaceutical Science and Technology* 12(1), 112–118. <https://doi.org/10.24198/ijpst.v12i1.54763>.
- [10] Fernandez-Guarino, M., Naharro-Rodriguez, J., Bacci, S., 2024. Aberrances of the wound-healing process: A review. *Cosmetics* 11(6), 209. <https://doi.org/10.3390/cosmetics11060209>.
- [11] Fitria, L., Lukitowati, F., Kristiawati, D., 2019. Reference value for the evaluation of liver and kidney function in Wistar rats (*Rattus norvegicus* Berkenhout, 1769). *Jurnal Pendidikan Matematika dan IPA* 10(2), 243–258. <https://doi.org/10.26418/jpmipa.v10i2.34144>.
- [12] Hera, N., Aprelia, R., Aminuddin, A.T., 2020. Exploration and morphological characteristics of wild gambier plants (*Uncaria gambir* Roxb.) in lowland peatlands in Pekanbaru City. *MENARA Ilmu* 14(2).
- [13] Hewis, L.G., Daeli, G.B.C., Tanoto, K., Carlos, Sahamastuti, A.A.T., 2020. A review of the botany, phytochemistry, and pharmacological effects of *Dysphania ambrosioides*. *Indonesian Journal of Life Sciences* 2(2), 70.
- [14] Ifada, A.S., Pahami, K., Insani, A.K., Karunia, 2022. Modification of the method for testing the effectiveness of gastric protection in alcohol-induced rats (*Rattus norvegicus*). *JIKF* 10(1).
- [15] Irramah, M., Julizar, Irawarti, L., 2017. Effect of *Uncaria gambir* Roxb. on gastric ulcers and ethanol-induced malondialdehyde levels. *Majalah Kedokteran Andalas* 40(1), 1–10. <https://doi.org/10.22338/mka.v40.i1.p1-10.2017>.
- [16] Ismiranty, Sombala, H., Simpan, J.P., 2023. In vivo test of wild onion extract (*Eleutherine americana* Merr.). *Journal of Biotechnology and Conservation in Wallacea* 3(1).
- [17] Jesus, N.Z.T.d., Falcão, H.d.S., Gomes, I.F., Leite, T.J.d.A., Lima, G.R.d.M., Barbosa-Filho, J.M., Tavares, J.F., Silva, M.S.d., Athayde-Filho, P.F.d., Batista, L.M., 2012. Tannins, peptic ulcers, and related mechanisms. *International Journal of Molecular Sciences* 13(3), 3203–3228. <https://doi.org/10.3390/ijms13033203>.
- [18] Jiménez-Ferrer, E., Abarca-Salgado, T., Vargas-Radilla, A.A., Flores-Melgar, J., Abarca-Vargas, R., 2025. *Dysphania ambrosioides* as a source of antioxidant candidates for benign prostatic hyperplasia and prostatitis: A critical review and in silico prioritisation. *Scientia Pharmaceutica* 93, 57. <https://doi.org/10.3390/scipharm93040057>.
- [19] Kangwan, N., Park, J.-M., Kim, E.-H., Hahm, K.B., 2014. Quality of healing of gastric ulcers: Natural products beyond acid suppression. *World Journal of Gastrointestinal Pathophysiology* 5(1), 40–47. <https://doi.org/10.4291/wjgp.v5.i1.40>.
- [20] Kuna, M.R., 2023. Determination of the level of acetic acid (CH₃COOH) in food products circulating in the market. *Dalton: Journal of Chemistry and Chemical Education* 6(2).
- [21] Kuna, M.R., Mokodompit, H.K.N., 2023. Control and increase of public knowledge about hypertension and gastritis. *Journal of Community Service MAPALUS* 1(2), 64–69.
- [22] Lahamendu, B., Bodhi, W., Siampa, J.P., 2019. Ethanol extract test of white ginger rhizome (*Zingiber officinale* Rosc. var. *amarum*) in male Wistar rats (*Rattus norvegicus*). *PHARMACON* 8(4), 928.
- [23] Lara, A.D., Elisma, Sani, F., 2021. Test of analgesic activity of orange leaf infusion (*Acanthus ilicifolius* L.) in male white mice (*Mus musculus*). *Indonesian Journal of Pharma Science* 3(2), 71–80.
- [24] Muhamasari, C., 2020. Web-based expert system for the early diagnosis of gastric disease using the Dempster–Shafer method. *SANTI Journal*.
- [25] Munggari, I.P., Kurnia, D., Deawati, Y., Juleha, E., 2022. Current research on the phytochemical, medicinal, and non-medicinal uses of *Uncaria gambir* Roxb.: A review. *Molecules* 27(19), 6551.
- [26] Nakao, K., Ro, A., Kibayashi, K., 2014. Evaluation of the morphological changes of gastric mucosa induced by a low concentration of acetic acid using a rat model. *Journal of Forensic and Legal Medicine* 22, 99–106. <https://doi.org/10.1016/j.jflm.2013.12.016>.
- [27] Oktrinorma, W.V., Indriyanti, N., Masyitah, Z.N., 2020. Effect of aspirin-induced gastric ulcers on gastric mucosal tissue damage in mice. *Proceedings of the Mulawarman Pharmaceutical Conference*.
- [28] Oswari, L., Hidayat, R., Fatmawati, F., Hayati, L., Alisa, B.S., 2019. Gambir extract (*Uncaria gambir*) decreases inflammatory response and increases gastric mucosal integrity in a Wistar rat model of gastritis. *Open Access Macedonian Journal of Medical Sciences* 7(19), 3149–3152.
- [29] Pandiangan, D., 2023. *Plant Metabolism*. Patra Media Grafindo, Bandung, pp. 46–172.
- [30] Pandiangan, D., Nainggolan, N., 2021. Women’s empowerment in Biovina Herbal MSMEs to improve the drying of BPOM-standard herbal raw materials. *Journal of Indonesian Women and Children* 3(1), 22–29.

- [31] Pandiangan, D., Nainggolan, N., Malingkas, H.P., 2021. Community partnership programme to improve the drying process of traditional medicine raw materials for COVID-19 prevention and Biovina MSME products. *Vivabio Journal of Multidisciplinary Service* 3(3).
- [32] Pandiangan, D., Nainggolan, N., Yamlean, P.Y., Maramis, R.T.D., Tumbol, M.V.L., 2025. Immunomodulatory response in *Rattus norvegicus* fed Biovina mixed nanotechnology supplements made from gambier and pasote raw materials. *International Journal for Multidisciplinary Research* 7(5). <https://doi.org/10.36948/ijfmr.2025.v07i05.57163>.
- [33] Pandiangan, D., Taghulihi, Y., Nainggolan, N., Yordan, G., Nainggolan, I.C., Nainggolan, E.A., Tampubolon, P.Y., Van Gobel, A.R., Nainggolan, V.P.G., Maliangkay, P.H., Tumbol, M.V.L., 2023. Pasote plant tea bags (*Dysphania ambrosioides* L.) have kidney creatinine-level reduction activity. *AIP Conference Proceedings* 2694, 050006. <https://doi.org/10.1063/5.0118967>.
- [34] Pandiangan, D., Wahyudi, L., Queljoe, E.D., 2017. Effect of pasote extract on blood sugar levels of male white rats induced with sucrose. *Proceedings of the National Seminar*.
- [35] Park, J.U., Kang, J.H., Abdur Rahman, M.A., Hussain, A., Cho, J.S., Lee, Y.I., 2019. Gastroprotective effects of plant extracts on gastric mucosal injury in experimental Sprague–Dawley rats. *BioMed Research International* 2019, 8759708. <https://doi.org/10.1155/2019/8759708>.
- [36] Pandiangan, D., 2023. Composition of tea bags from pasote plants (*Dysphania ambrosioides* L.) with activity to reduce serum SGOT levels and potential hepatoprotective effects. Indonesian Patent IDS000005565.
- [37] Pandiangan, D., 2023. Tea bags made from pasote plants (*Dysphania ambrosioides*) as an antihypercholesterolaemic product. Indonesian Patent IDS000006325.
- [38] Pandiangan, D., 2023. Process for making antioxidant-rich simplicia from pasote plants (*Dysphania ambrosioides* L.). Indonesian Patent IDS000006454.
- [39] Pungus, M.C., Lintong, M.P., Sambuga, 2020. Effect of ginger extract (*Zingiber officinale*) on the histopathological appearance of the stomach of Wistar rats induced by acetic acid. *Medical Scope Journal* 1(2).
- [40] RaviKumar, V.R., Rathi, S., Patel, B., Singh, S., Chaturvedi, K., Sharma, B., 2023. A comprehensive review of ulcers and their treatment. *Chinese Journal of Applied Physiology*, e20230006.
- [41] Rejeki, P.S., Putri, E.A.C., Prasetya, R.E., 2018. *Oophorectomy in Rats and Mice*.
- [42] Ribeiro, R.M., Brito Junior, E.R.d., Carvalho, M.B.B.d., Lima, B.d.S.F.d., d'Albuquerque, P.M., 2023. *Dysphania ambrosioides*: Phytochemical composition and pharmacological potential of the leaves. *Brazilian Journal of Health Review* 6(4), 18067–18086. <https://doi.org/10.34119/bjhrv6n4-315>.
- [43] Sari, T.F., Usviany, V., 2023. Overview of lansoprazole use in gastritis patients at Assyifa Farma 2 Pharmacy in 2022. *Health Information: Journal of Research* 15(3).
- [44] Sigit, J.I., Rebekah, H., Soemardji, A.A., 2012. Preventive effectiveness of omeprazole against the gastric side effects of non-steroidal anti-inflammatory drugs (acetosal) in female Wistar rats. *Acta Pharmaceutica Indonesia* 37(2), 67–74.
- [45] Sinaga, M.D., Fajrin, A.A., 2022. A web-based expert system for diagnosing acid reflux disease in adults using the forward-chaining method. *Journal of Comasie* 7(7).
- [46] Soares, C.N., Pandiangan, D., Mambu, S.M., 2022. Response of pasote plants (*Dysphania ambrosioides*) treated with plant growth-promoting rhizobacteria. *Journal of Bios Logos* 14(1).
- [47] Solfaïne, R., Supriyanto, M.P.E., 2022. Histopathological appearance of cisplatin-induced renal damage and the effects of torbangun leaf extract (*Coleus amboinicus* L.) in Wistar rats (*Rattus norvegicus*). *Veterinary and Biomedical Clinical Journal* 4(1), 10–15.
- [48] Suminah, S., Ciputri, L., 2023. Analysis of the relationship between stress and triggers of increased stomach acid. *Usada Nusantara* 1(2), 224–228.
- [49] Sundalangi, C.F., Loho, L., Kairupan, C.F., 2016. Histopathological description of the stomach of Wistar rats given soursop leaf extract (*Annona muricata* L.) following aspirin induction. *Jurnal e-Biomedik* 4(1), 1–8.
- [50] Suyoko, E., Pandiangan, D., Tumbol, M.V., Nainggolan, N., Nainggolan, E.A., Nainggolan, V.P.G., 2023. Effectiveness of porang (*Amorphophallus oncophyllus*) powder solution in healing gastric inflammation in Wistar rats (*Rattus norvegicus*) induced with acetic acid. *International Journal of Science and Research* 11(8), 465–472. <https://doi.org/10.21275/SR22804235551>.

- [51] Taghulihi, Y., Pandiangan, D., Antasionasti, I., Nainggolan, N., Nainggolan, E.A., 2022. Effect of pasote steeping water (*Dysphania ambrosioides* L.) on serum creatinine levels in Wistar rats (*Rattus norvegicus*). *International Journal of Science and Research*. <https://doi.org/10.21275/SR22715233930>.
- [52] Uchida, M., Kawano, O., Misaki, N., Saitoh, K., Irino, O., 1989. Relapse of acetic acid-induced gastric ulcer and gastric mucosal prostaglandin I₂ levels in rats. *Chemical and Pharmaceutical Bulletin* 37(3).
- [53] Usiono, Putri, D.A., 2024. Systematic literature review of increased stomach acid. *Detector: Journal of Health Sciences Research Innovation* 2(1), 7–14.
- [54] Usman, S., 2016. Extent of gastric mucosal damage in ethanol-induced model rats. *Medical Pearl* 16(1), 33–40.
- [55] Wahywardani, S., Noor, S.M., Bakrie, B., 2020. Animal-welfare ethics in research and testing: Implementation and constraints. *Wartazoa* 30(4).
- [56] Widianingrum, F.F., Hadoso, S., Aulia, S., 2021. Application of Android-based augmented reality for learning about the human gastric organ. *National Journal of Electrical Engineering and Information Technology* 10(4), 329–334.
- [57] Windiasari, S., Hervani, R., 2023. Effect of soy milk (*Glycine max*) on alloxan-induced blood sugar. *Journal of Amicron Adpertisi* 2(2).
- [58] Woodhall, H., Barrow, K., Brown, S., Roe, T., Bergen, C., 2024. Successful surgical treatment of gastric dilatation and volvulus in a pet domestic rat (*Rattus norvegicus*). *Journal of Exotic Pet Medicine* 51, 10–13.
- [59] Yuslianti, E.R., Bachtiar, B.M., Suniarti, D.F., Sutjiatmo, A.B., 2016. Pharmaceutical standardisation of natural ingredients towards phytopharmaceuticals for the development of Indonesian traditional medicines. *Dental Journal* 19(2), 179–185.
- [60] Yuziani, Hareva, A.A., Khairunnisa, 2023. Nephroprotective effect of curry leaf ethanol extract (*Murraya koenigii* (L.) Spreng.) on blood urea nitrogen and creatinine levels in male Wistar rats (*Rattus norvegicus*) induced by doxorubicin. *Journal of Health Sciences Research (JURRIKES)* 2(2).
- [61] Pandiangan, D., Nainggolan N., Rosanah E., Pongoh J., Tumbol MVL., Nainggolan IC., 2026a. Pasote (*Dysphania ambrosioides* L.): Efficacy, "Anti" Formulation, Standardization, and Safety. Editor Vainggolan VPG. Penerbit Buku Indonesia. Jakarta
- [62] Pandiangan, D., Nainggolan N., Nainggolan IC., Nainggolan EA. 2025. Empirical Ethnomedicine and Medicinal Plant Biodiversity: A Case Study of the Medicine of the Mongondow Tribe of North Sulawesi. Editor Vainggolan VPG. Penerbit Buku Indonesia. Jakarta
- [63] Pandiangan, D., Kaeng M., Nainggolan N., Rosanah E., Maramis RTD., Tumbol MVL., 2026b. GEDI (*Abelmoschus manihot* L.): Utilization, Standardization, Innovation of Herbal Products and Their Safety. Penerbit Buku Indonesia. Jakarta
- [64] Pandiangan, D., Bukari S., Mambu SM., Nainggolan N., Rosamah R., MaramisRDT. 2026c. Efficacy Test of Biovina Health Supplement Extract on The Healing of Gastric Ulcers and The Bloated Rat (*Rattus norvegicus*). *World Journal of Pharmacy and Pharmaceutical Sciences* vol 15 no 6 p.1378-1399.
- [65] Dyah, R.S., Yupono, B., Windhu, P., Irmawati, Soetji, A., Elly K., Pandiangan D., Sushardi. 2024. Linking of digital supply chains and digital transformation on the competitiveness of government companies in the supply chain 4.0 era. *Uncertain Supply Chain Management* vol 12 no 4 p. 2881 - 2890