RESEARCH PAPER

Pharmacodynamic Evaluation of Formulation *Dysphania ambrosioides* and *Uncaria gambir* Extract Combined for Gastric Ulcer Healing in Acetic Acid-Induced Rats

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Abstract
This study investigated of phamacodinamic formulation for gastric ulcer treatment prepared from dried water extracts of *Dysphania ambrosioides* (pasote) and *Uncaria gambir* (gambier). The formulation was developed to evaluate the potential of combining these plant extracts for the management of gastric ulcers while monitoring possible effects on renal and hepatic function. The effectiveness of the formulations was evaluated by comparison with two commercial drugs in an acetic-acid-induced gastric ulcer model. Among the formulations tested, Formula 2, consisting of 75% gambier and 25% pasote, dissolved in 100 mL of water and administered at a dose of 2 mL once daily, showed the greatest apparent effectiveness in promoting gastric ulcer healing. The evaluated parameters included the recovery of gastric mucosal irritation, macroscopic gastric appearance, mucosal characteristics, hematological parameters, biochemical parameters including glucose, cholestero, uric acid, creatinine and serum glutamic-oxaloacetic transaminase (SGOT). Formula 2 was associated with faster recovery of gastric mucosal irritation, a smaller and healthier gastric circumference, and a fresh and smooth mucosal surface. Among the five Gambir–Pasote formulations, Formula 2 exhibited the most favorable overall biochemical profile, characterized by the lowest AST/SGOT and uric acid levels, glucose concentrations comparable to Formula 3, and relatively stable serum creatinine levels. These findings suggest that Formula 2 is a promising candidate for further development, although additional renal and hepatic safety assessments are required to confirm its potential protective effects and long-term safety.

Keywords : Formulation; Pharmacodynamic; Gastric Ulcer; Healing; Rats

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1. INTRODUCTION

The initial motivation for developing the Biovina Mix formulation, which contains a combination of gambir and pasote extracts, arose from observations related to gastric complications associated with long-term medication use. The husband of the main inventor experienced a heart attack around 2013 and subsequently underwent the placement of two cardiac stents. During routine treatment with cardiac medications, he developed gastric ulcer-related complications. In addition, several colleagues and members of the inventor's community reportedly experienced severe gastric ulcer-related conditions, with five individuals dying within one year. These experiences motivated further investigation of available information and therapeutic approaches related to gastric ulcers and bowel cancer [1, 2]. This research subsequently contributed to the development of Biovina Mix, a formulation containing pasote, gedi, kecombrang, mayana, and gambir as its main raw materials [1]. Research conducted in 2019 and 2020 evaluated this formulation and supported attempts to obtain a distribution permit from the Indonesian Food and Drug Authority (BPOM) in 2020 [3,4]. However, the application was not approved because some of the components, including tapak dara and gedi, were considered unsuitable, while pasote had not yet been included in the relevant pharmacopoeial references [5]. Consequently, research on the standardization of gedi and pasote raw materials was conducted from 2020 to 2024, resulting in several patents [6–9] and publications in national and international journals [10–19].

Gambir has long been used as a traditional medicinal material and has been reported in previous studies for various potential therapeutic properties [10,20–22,2]. It has also been patented as a health supplement formulation containing gambir and pasote [7]. Based on previous research and practical observations, the research team explored the potential application of gambir-based preparations in individuals experiencing gastric disorders. However, the formulation has not yet obtained the required distribution and production permits from BPOM. One of the concerns associated with the use of gambir is its potential effect on renal function [23]. In contrast, standardization studies of pasote have reported improvements in kidney-related parameters, including reductions in creatinine levels [14], and have also indicated potential hepatoprotective activity through reductions in SGOT levels [8]. These findings provide a rationale for investigating the combination of pasote and gambir as a potential formulation for gastric ulcer management. The combination may provide complementary effects and potentially improve the safety profile of a gambir-based preparation. Further investigation is therefore required to determine the interaction and potential synergistic effects of these plant materials, particularly when combined with other components previously evaluated in the formulation [24, 5].

Previous empirical observations reported improvements in individuals with gastric ulcers, gastroesophageal reflux disease (GERD), hemorrhoids, and bowel cancer following consumption of brewed Biovina preparations containing gambir [1, 25]. In experimental studies, Biovina administration was also associated with improvements in the general appearance of rats [1, 25]. Subsequently, Suyoko et al. [26] investigated Biovina containing gambir as a comparative preparation and reported observations following its administration. These findings motivated further investigation of the formulation's antioxidant activity using the analytical approach described by Pandiangan et al. [1, 27]. The antioxidant evaluation yielded IC_{50} values of 23.65 and 16.63 for Biovina Mix in the first and second replicates, respectively, compared with 41.44 and 39.91 for vitamin C in the corresponding replicates [1, 27]. These results provided a basis for further investigation of the potential biological activities of gambir- and pasote-based formulations, particularly their potential antidiabetic, anticholesterol, and anticancer effects [,3 ,4].

2. RESEARCH METHODS

This study employed an experimental laboratory research design using rats (*Rattus norvegicus*) as experimental animals. The research was conducted in stages over a two-year period, from 2025 to 2026. The main stages of the research included the preparation and standardization of raw materials, formulation development, toxicity evaluation, biochemical analysis [19] and formulation.

The raw materials used in the formulation and standardization processes were pasote and gambir extracts. Pasote has not yet been included in the national list of medicinal plants in the Pharmacopoeia; therefore, standardization of the raw material was conducted as part of the preparation for subsequent registration with the Indonesian Food and Drug Authority (BPOM) and the submission of a Nomor Izin Edar (NIE). Standardization of the raw materials was conducted according to SNI 01-2897-1992 at the Manado Industrial Standardization and Services Center.

The toxicity evaluation followed the procedures specified in Perka BPOM RI No. 10 of 2022. The research procedures were conducted with reference to OECD Guideline 407 and BPOM RI Regulations No. 10 of 2022 and No. 12 of 2014. The data were analyzed using Statistica 7 and Analyst AI at a 95% confidence level. The safety and quality requirements for natural ingredients were also considered according to Regulation of the Food and Drug

Supervisory Agency Number 29 of 2023 [23,5]. Standardization of the raw materials had been completed and a corresponding certificate had been obtained as part of the preparation for the NIE application and compliance with the relevant regulations [26–29].

The preparation of extracts and the formulation of the Biovina gambir–pasote preparation was conducted by soaking 0, 1, 2, 4, and 8 of capsule packs on 200 mL of boiling water, according to the previously described procedure [1, 24]. Biovina extract powder was also dissolved on 200 mL of hot water for the experimental evaluation. The subsequent tests were conducted according to the relevant standard procedures [6, 8, 30]. The main stages of the research conducted on 2025 are described below.

2.1. Stages of Formulation Test of Gambier and Pasote Extracts as Gastric Ulcer Medicine

The research conducted in 2025 continued the investigations initiated in 2023, which included acute and subchronic toxicity testing [9]. The 2025 phase aimed to extend the safety evaluation through chronic toxicity testing and related assessments.

Histological, anatomical, and pathological examinations of organs collected from animals terminated in 2024 were also conducted in accordance with the procedures described in Regulation No. 10 of 2022 issued by the Indonesian Food and Drug Authority (BPOM) [39]. These examinations provided preliminary information for subsequent clinical pathology evaluations. When the experimental findings indicated acceptable results, the research proceeded to the PA test [31] using a specific composition of the Biovina capsule formulation.

All toxicity-related investigations were conducted with reference to BPOM Regulation No. 10 of 2022 concerning Guidelines for In Vivo Preclinical Toxicity Tests [39] and *Standardization of Medicinal Raw Materials* [40].

Figure 1 presents the overall experimental workflow used for the formulation development and evaluation of the Biovina preparation which is made from a combination of pasote and gambier. The procedure began with the procurement of 30 Wistar rats, followed by weighing and a two-week adaptation period in the laboratory. The adaptation period was followed by assessment of blood cholesterol and biochemical parameters [19] to establish baseline conditions before the experimental treatment. After the drug treatment and the combination of pasote and gambier 4 days are completed, then the biochemical parameters of blood serum are measured as well.

After the initial assessment, the animals were divided according to whether or not they underwent acetic acid induction. Three rats were assigned to the non-induced group, whereas 24 rats were subjected to acetic acid induction. The flowchart indicates that the induction procedure was followed by a three-day period before the animals proceeded to the subsequent experimental stages. This design allowed the study to compare animals without induced gastric injury with animals in which gastric ulceration had been experimentally established.

The non-induced animals were subsequently associated with the control group (C), while the induced animals were allocated to the experimental and comparison groups. The induced groups included an aquades group (KN/C-), two positive-control groups receiving lansoprazole (C+1) and Promaag (KP+2), and five treatment groups designated F1–F5 (T1–T5). This arrangement provided a basis for comparing the effects of the formulated preparations with both a negative/control condition and commercially available gastric ulcer treatments.

The experimental grouping shown in Figure 1 is important because it provides the framework for evaluating the relative effectiveness of the different formulations. The use of lansoprazole and Promaag as comparison groups enables the response of the experimental formulations to be assessed alongside established commercial treatments. Meanwhile, the T1–T5 treatment groups represent the different formulation conditions investigated in the study. The control and treatment structure therefore allows differences in gastric recovery and other evaluated parameters to be considered in relation to both untreated and reference-treatment conditions.

Overall, Figure 1 and 2 summarizes the sequence from animal preparation and baseline biochemical [19] assessment through gastric ulcer induction, treatment allocation, and experimental evaluation. The flowchart also demonstrates that the study incorporated both non-induced and induced animals, allowing the investigators to observe differences between normal gastric conditions and experimentally injured conditions. The subsequent assessment of gastric morphology, mucosal appearance, hematological parameters and biochemical parameters provided the basis for determining the most promising formulation.

However, the flowchart should be interpreted together with the detailed Methods section because several experimental details are not specified in the figure, including the exact number of animals in each treatment group, the dose and administration schedule for each treatment, the precise composition of formulations T1–T5, and the statistical procedures used for comparison among groups. These details should be clearly stated in the Methods section to ensure that the experimental design is transparent and reproducible. In addition, the figure indicates 30 Wistar rats but explicitly allocates 3 animals to the non-induced group and 24 animals to the induced groups, leaving one animal unaccounted for in the displayed allocation. This discrepancy should be clarified in the revised manuscript.

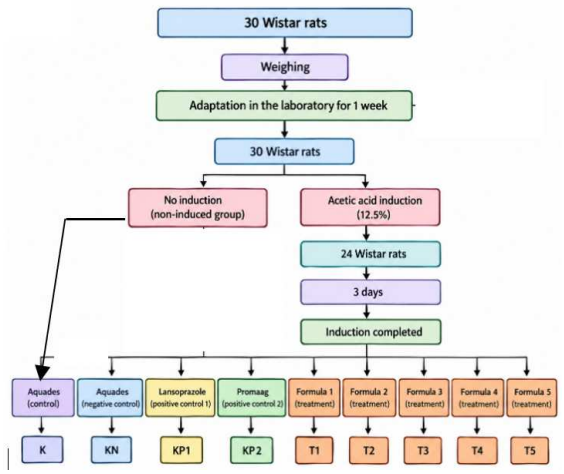


Figure 1. Stages of formulation development, standardization, and production of the Biovina formulation, including gastric ulcer efficacy testing [6, 39]. K = control ; P = positive control ; T = treatment.



Figure 2. Drawing of the model of grouping rats according to their treatment and function there are 9 groups representing normal control, negative control, poditative control there are 2 with promag and Lansoprazole, then treatment in formula 1-5 various combinations

2.2 Toxicity Test Profile Biochemical Blood Serum

Biochemical examinations included measurements of blood glucose, uric acid, total cholesterol, creatinine [58] and AST/GOT [19]. The parameters were determined using the Homogeneous Enzymatic Colorimetry method with a Biosystems BTS 340 photometer at the BSPJI Manado Test Laboratory [6,17,28].

Measurements were performed according to the applicable SNI procedures. Prior to sample analysis, instrument calibration and quality-control procedures were conducted to ensure that the required acceptance criteria were met. The samples were subsequently analyzed to obtain the biochemical data [6, 9, 28].

2.3 . Histopathological Examination of Gastric Part

The gastric region containing the ulcer lesion and adjacent mucosa should be excised and immediately immersed in 10% neutral buffered formalin for approximately 24 hours to preserve tissue architecture. Following fixation, the specimens should be dehydrated through ascending concentrations of ethanol, cleared in xylene, and infiltrated and embedded in paraffin wax. The paraffin blocks should then be sectioned at 5 µm using a rotary microtome, and the sections mounted on glass slides. After deparaffinization and rehydration, the sections should be stained with hematoxylin and eosin (H&E), dehydrated, cleared, and coverslipped for light microscopic examination [57, 59].

Recent gastroprotective studies on acetic acid models used gastric tissue fixed in 10% neutral buffered formalin, cut about 4 µm, then stained with H&E and assessed using predetermined histopathological criteria. Parameter yang diamati: (1) epithelial integrity; (2) erosion; (3) ulceration; edema; (4) inflammatory-cell infiltration ; (5) hemorrhage; (6) necrosis; (7) granulation tissue ; (8) regeneration of gastric epithelium [57, 59].

Histopathological examination should assess epithelial integrity, the extent and depth of mucosal erosion or ulceration, necrosis, inflammatory cell infiltration, edema, hemorrhage, and evidence of epithelial regeneration. The sections should be evaluated by an observer blinded to treatment allocation, using a predefined microscopic scoring system applied consistently across all experimental groups. The scoring criteria, number of sections and fields examined per animal, and method of deriving each animal's final score should be explicitly reported to ensure reproducibility. Microscopic injury scores should be distinguished from macroscopic ulcer indices, as these represent different measures of gastric damage [57, 59].

3. RESEARCH RESULTS

3.1. Body Weight and General Growth of Experimental Rats

The mean body weight increased across all nine groups during the 18-day observation period Figure 3. The largest increases were observed in Formula 1 (+48.67 g), Formula 5 (+48.67 g), and the negative control (+48.33 g). These findings suggest overall body-weight gain during acclimatization. However,

body-weight gain alone does not establish good health or successful acclimatization; clinical observations and food consumption should also be considered.

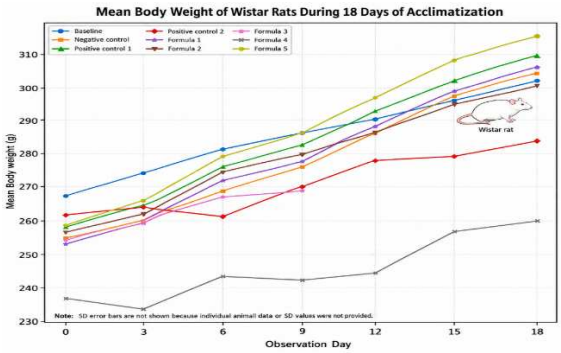


Figure 3. Changes in Mean Body Weight of Wistar Rats During the 18-Day Acclimatization Period.

Body weight measurements were performed before induction of gastric injury and after the treatment period. The experimental rats were subjected to gastric injury induction using 12.5% acetic acid, as described in the experimental procedure. Body weight was measured at baseline and again after 21 days of treatment to evaluate changes in body weight during the experimental period.

Changes in body weight may be associated with alterations in metabolic parameters, including blood lipid levels. Previous studies have reported associations between increased body weight, lipid metabolism, and circulating cholesterol concentrations. High cholesterol levels have been associated with obesity and alterations in metabolic regulation, including leptin signaling. Excessive accumulation of adipose tissue may increase the release of free fatty acids (FFAs), which can influence lipid metabolism and contribute to elevated circulating triglyceride and cholesterol concentrations. Protein and fat intake have also been reported to influence lipid concentrations in the blood. Therefore, body weight was monitored in the present study as a general physiological parameter and to provide supporting information for the subsequent evaluation of biochemical parameters [19].

Based on the measurements obtained before administration of the extract treatment (initial body weight) and after 21 days of treatment (final body weight), all experimental groups showed an increase in body weight.

Table 1. Body weight of experimental rats before and after treatment

Group	Mean initial body weight (g)	Mean final body weight (g)	Weight gain (g)
Baseline	95.00 ± 20.42	143.67 ± 37.85	48.67
Control (−)	98.67 ± 9.02	163.00 ± 18.36	64.33
Control (+1)	90.00 ± 19.62	140.53 ± 37.85	50.53
Control (+2)	75.33 ± 20.50	143.67 ± 37.85	68.34
Formula 1 (T1)	74.00 ± 16.37	114.33 ± 4.04	40.33
Formula 2 (T2)	75.33 ± 20.50	150.00 ± 24.88	74.67
Formula 3 (T3)	86.50 ± 18.03	138.12 ± 35.05	51.62
Formula 4 (T4)	85.83 ± 17.43	139.77 ± 34.70	53.94
Formula 5 (T5)	80.71 ± 16.85	135.00 ± 37.85	54.53

The results presented in Table 1 show that body weight increased in all experimental groups. The baseline group showed a weight gain of 48.67 g, whereas the negative-control group showed a gain of 64.33 g. Control (+1) and Control (+2) showed increases of 50.53 and 68.34 g, respectively. Among the treatment groups, Formula 1 showed an increase of 40.33 g, Formula 2 showed an increase of 74.67 g, Formula 3 showed an increase of 51.62 g, Formula 4 showed an increase of 53.94 g, and Formula 5 showed an increase of 54.53 g.

The greatest numerical increase in body weight was observed in the Formula 2 group. The mean body weight increased from 75.33 ± 20.50 g before treatment to 150.00 ± 24.88 g after treatment, corresponding to a reported increase of 74.67 g. The manuscript also describes visible differences in body size among the experimental animals.

Because changes in body weight may be associated with alterations in lipid metabolism, subsequent biochemical measurements were conducted to evaluate blood glucose, uric acid, total cholesterol, creatinine [14] and AST/SGOT. Previous studies have reported relationships between dietary fat intake, hepatic triacylglycerol synthesis, and circulating cholesterol concentrations. Alterations in reverse cholesterol transport have also been associated with changes in circulating cholesterol levels.

The study also resulted in the development of a prototype gastric-ulcer formulation in capsule form. The prototype was described as being packaged in bottles containing 25, 30, or 50 capsules, with each capsule containing 400 mg of processed extract. The formulation was subsequently evaluated using several experimental parameters as part of the product-development process.

3.2. Measurement of Stomach Length and Width in Wistar Rats

Measurement of stomach length and width was performed as a macroscopic morphological assessment following the treatment period. Gastric morphology has previously been used as one of the parameters for evaluating changes associated with gastric injury and recovery. The measurements obtained for all experimental groups are presented in Table 2.

The baseline group showed a stomach length of 2.3 mm and a width of 1.3 mm. In contrast, the negative-control group showed a substantially larger stomach measurement, with a length of 4.4 mm and a width of 3.4 mm. The authors associated this enlargement with gastric injury following acetic-acid administration without subsequent treatment.

The Control (+1) group, which received lansoprazole, showed a stomach length of 2.3 mm and a width of 1.2 mm. The Control (+2) group showed a length of 2.8 mm and a width of 2.0 mm. Among the treatment groups, Formula 1 showed a length of 2.7 mm and a width of 1.9 mm. Formula 2 showed a length of 2.5 mm and a width of 1.9 mm, while Formula 3, Formula 4, and Formula 5 showed measurements of 2.7 × 2.0 mm, 3.0 × 2.1 mm, and 2.9 × 2.0 mm, respectively.

Table 2. Length and width of the stomach of Wistar rats after being treated for 7 days

Group	Stomach length (mm)	Stomach width (mm)
Normal	23	13
KN/ Control −	44	34
KNP1/ Control + 1	23	12
KNP2/ Control + 2	28	20
Formula 1 (T1)	27	19
Formula 2 (T2)	25	19
Formula 3 (T3)	27	20
Formula 4 (T4)	30	21
Formula 5 (T5)	29	20

The measurements indicate that the baseline and positive-control (Control +1) groups had similar stomach lengths and widths. Among the treatment groups, Formula 2 showed a stomach length of 25 mm and a width of 19 mm (Figure 3), which were relatively close to the baseline measurements of 23 mm and 13 mm, respectively. Formula 2, containing 25 mg of pasote extract and 75 mg of gambier extract, demonstrated the most favorable macroscopic stomach measurements among the tested formulations [48].

In Figure 4 of formula 2, it is further observed that the change in gastric tissue starting from the beginning of normal (day 1), the induction of acetic acid (days 2-3) and the treatment of formula 2 (days 3-7) shows a relationship that is in line with the healing process. Also, the color change from normal to gastric ulcer pain (dark red) then back to normal after formula 2 treatment (Figures 5 and 6)

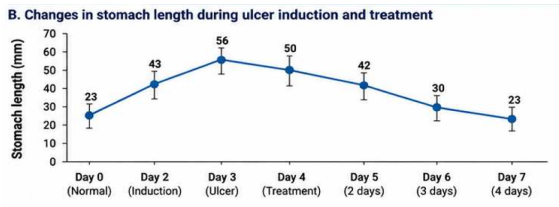


Figure 4. The change in gastric length during induction on day 3 of the balloon enlarged and the administration of formula 2, 1 day immediately decreased along with the improvement of gastric ulcer during days 3-7 for about 4 days of complete recovery or complete recovery.

In contrast, the negative-control group exhibited the largest recorded stomach dimensions, measuring 44 mm in length and 34 mm in width. The manuscript also reports that animals in this group exhibited abdominal enlargement, reduced activity, and labored breathing, and that one animal died on the fifth day of the experiment [48].

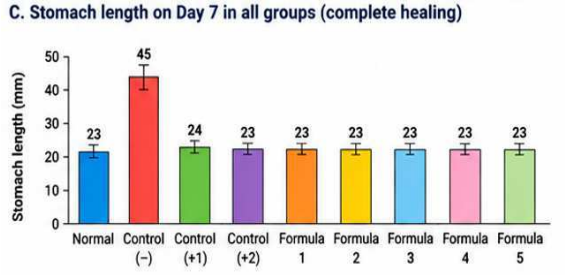


Figure 4. Observation of the morphology of the length of the stomach of wistar rats after 7 days of treatment in all groups.

Figure 5 presents the macroscopic observations of gastric morphology and mucosal characteristics in Wistar rats following 28 days of treatment. The observations focused on visible changes in gastric size, external morphology, mucosal appearance, and the condition of the gastric surface after treatment [48].

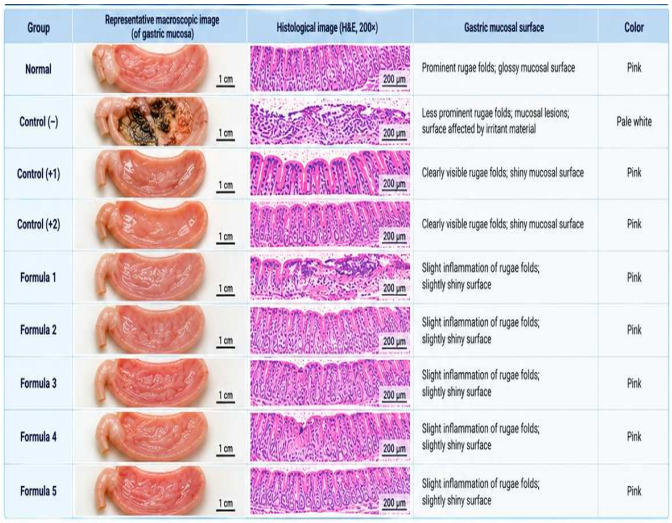


Figure 5. Macroscopic observation of gastric morphology, gastric mucosal characteristics of Wistar rats, mucosal histopathologists after 4 days of treatment or 7 day rats maintenance.

The figure 5 shows differences in gastric morphology between the observed groups. The gastric specimen shown on the left has a smaller overall size, with an approximate length of 20–30 mm and a width of 10–20 mm (Table. Its mucosal surface appears reddish and irregular, with visible gastric folds. In contrast, the specimen shown on the right has larger dimensions, with an approximate length of 40–50 mm and a width of 20–30 mm (Table 4). The corresponding mucosal surface appears lighter in

color, with a yellowish-white appearance and a relatively smoother surface [48].

These macroscopic observations (Figure 4, provide a visual assessment of the gastric condition following the experimental treatment. Changes in gastric size and mucosal appearance may reflect differences in the response of gastric tissue to the experimental conditions. The color, integrity, and surface characteristics of the mucosa were therefore considered together with other experimental parameters to evaluate the apparent recovery of gastric tissue [45].

The Figure 5 and 6 shows the importance of evaluating gastric morphology in combination with biochemical and other outcome measures. Although the observed differences provide supportive visual evidence of changes in gastric condition, macroscopic appearance alone cannot establish complete mucosal healing or tissue regeneration. Histopathological examination and quantitative assessment of ulcer area or lesion severity would provide more objective evidence of treatment-associated gastric recovery [47].

Overall, the macroscopic measurements suggest that treatment with the gambier–pasote formulations was associated with differences in stomach dimensions compared with the untreated negative-control group. Formula 2 showed measurements closest to those of the baseline group among the five tested formulations. However, these observations should be interpreted descriptively unless supported by appropriate statistical analysis [44].

3.3. Macroscopic Observation of the Stomach of Wistar Rats

Macroscopic examination was performed at the end of the treatment period by assessing the gastric morphology, anatomical appearance, surface characteristics, color, and consistency of the stomach. Differences in the macroscopic appearance of the stomach were observed among the experimental groups, as summarized in Table 3.

The baseline group exhibited a pink gastric appearance and soft consistency. The negative-control group showed a brownish-red appearance and soft consistency. The Control (+1), Control (+2), and Formula 1–5 groups generally exhibited a pink gastric appearance and soft consistency.

Table 3. Macroscopic characteristics of the stomach of Wistar rats after treatment

Group	Color	Surface characteristics	Consistency
Normal	Pink	See observation in Figure 6	Soft
KN/Control −	Brownish red	Lesions/irritation reported	Soft
KNP1/Control + 1	Pink	Recovered appearance	Soft
KNP2/Control + 2	Pink	Recovered appearance	Soft
Formula 1 (T1)	Pink	Recovered appearance with slight inflammation	Soft
Formula 2 (T2)	Pink	Recovered appearance with slight inflammation	Soft
Formula 3 (T3)	Pink	Recovered appearance with slight inflammation	Soft
Formula 4 (T4)	Pink	Recovered appearance with slight inflammation	Soft
Formula 5 (T5)	Pink	Recovered appearance with slight inflammation	Soft

The reference criteria described a healthy gastric wall as having a pink surface, a smooth mucosal appearance, and an elastic consistency.

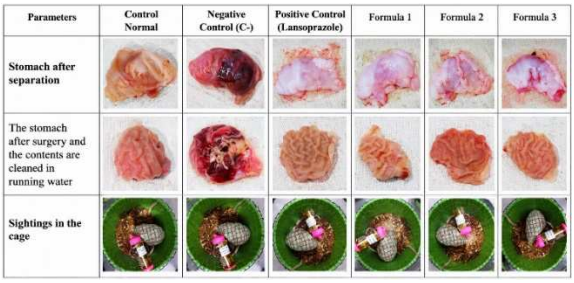


Figure 6. Macroscopic observation of gastric morphology and the gastric mucosal layer of Wistar rats in the baseline, negative-control, treatment, and positive-control groups and the macroscopic formula in the maintenance cage.

Figure 6 shows the macroscopic appearance of the stomach and gastric mucosal surface in the experimental groups following the treatment period. The observations focused on external gastric morphology, mucosal coloration, surface characteristics, and the visibility of gastric rugae. These parameters were used to provide a qualitative assessment of gastric conditions following experimental induction and treatment. The observed differences were interpreted in the context of gastric mucosal injury and recovery, although macroscopic observations alone cannot establish histological healing or functional restoration [57].

The baseline group showed a healthy gastric appearance, characterized by a soft or supple consistency, a smooth surface, pink coloration, and normal macroscopic dimensions. The treatment groups receiving the gambier–pasote formulations

showed macroscopic characteristics that were generally similar to those of the baseline group.

The negative-control group received 12.5% acetic acid without subsequent administration of the gastric treatment. This group showed a larger and darker gastric appearance than the baseline group. In contrast, the Control (+1) and Control (+2) groups, which received gastric medications following acetic-acid induction, showed macroscopic characteristics that were more similar to the baseline group.

3.4. Observation of the Gastric Mucosal Layer of Wistar Rats

The gastric mucosal surface was examined macroscopically at the end of the experimental period. The observations included the appearance of the gastric folds (rugae), mucosal surface texture, color, and visible lesions. Although the original manuscript refers to these assessments as microscopic observations, the parameters recorded in Table 4 represent macroscopic characteristics and should therefore be described accordingly.

The normal group exhibited prominent rugae folds and a glossy mucosal surface with pink coloration. In contrast, the negative-control group showed less prominent rugae, visible mucosal lesions, and a pale-white surface appearance. The Control (+1) and Control (+2) groups displayed clearly visible rugae and shiny, pink mucosal surfaces. Similarly, the Formula 1–5 groups showed pink mucosal surfaces with a slightly shiny appearance, although slight inflammation of the rugae was reported. The observed characteristics of each group are summarized in Table 4.

Table 4. Characteristics of the gastric mucosal layer of Wistar rats

Group	Gastric mucosal surface	Color
Normal	Prominent rugae folds; glossy mucosal surface	Pink
KN/ Control –	Less prominent rugae folds; mucosal lesions; surface affected by irritant material	Pale white
KNP1/ Control + 1	Clearly visible rugae folds; shiny mucosal surface	Pink
KNP2/ Control + 2	Clearly visible rugae folds; shiny mucosal surface	Pink
Formula 1 (T1)	Slight inflammation of rugae folds; slightly shiny surface	Pink
Formula 2 (T2)	Slight inflammation of rugae folds; slightly shiny surface	Pink
Formula 3 (T3)	Slight inflammation of rugae folds; slightly shiny surface	Pink
Formula 4 (T4)	Slight inflammation of rugae folds; slightly shiny surface	Pink
Formula 5 (T5)	Slight inflammation of rugae folds; slightly shiny surface	Pink

Visible rugae, a glossy surface, and pink coloration were the characteristics recorded for the normal group and were used as descriptive reference features for comparison with the experimental groups. Based on these macroscopic observations, the positive-control and Formula 1–5 groups displayed mucosal appearances that were closer to the normal group than those observed in the negative-control group. The authors also reported that administration of 2 mL of the treatment solution was associated with an improved appearance of the irritated mucosal surface. However, the available observations do not establish the extent of tissue regeneration or confirm complete ulcer healing.

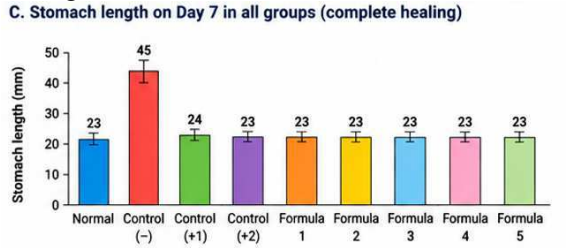


Figure 7. Graph of the length of the stomach of mice of each treatment group on day 4 after administration or 7 days of maintenance.

Acetic acid was used to induce gastric mucosal injury in the experimental animals. In the negative-control group, which received no subsequent gastroprotective treatment, the mucosal surface exhibited more pronounced visible abnormalities, including less prominent rugae, irritation, and lesions. These findings are consistent with the macroscopic appearance described in Figure 6 and with the intended gastric injury model [48].

The groups receiving the gambier (*Uncaria gambir*)–pasote (*Dysphania ambrosioides*) formulations showed comparatively favorable macroscopic characteristics, including pink coloration, visible rugae, and relatively shiny mucosal surfaces (Table 4). The positive-control groups exhibited similar descriptive features. Nevertheless, these observations are qualitative and should not be interpreted as evidence of statistically significant differences unless supported by an appropriate quantitative assessment (Figure 4,5,6).

Among the treatment groups, Formula 2 was reported to show favorable results when considered alongside the stomach-size measurements described previously (Figure 7, 8, 9). This observation provides a basis for further investigation of Formula 2; however, the macroscopic findings alone do not establish its superiority over the other formulations or

demonstrate its safety. Additional histopathological and quantitative assessments are needed to determine the extent of mucosal repair and to evaluate the potential gastroprotective effects of the combined extracts (Figure 8 and 9).

3.5. Gastric Ulcer Healing in White Rats: From Necrosis to Mucosal Regeneration

Figures 8 and 9 shows of combine all the parameters to ensure that Formula 2 is the best to use. Figure 8 shows that moacoskapis, histopathological shows sequence of gastric mucosal repair following acetic acid-induced gastric ulceration in white rats (*Rattus norvegicus*). The healing process progresses from mucosal necrosis and darkened tissue to the detachment and clearance of damaged tissue, granulation tissue formation, re-epithelialization, and eventual restoration of the gastric mucosal surface [49]. The final stage is characterized by a smoother, pinkish mucosal surface and the reappearance of visible gastric rugae, which may indicate gross morphological recovery (Figure 7,8,9) [46].

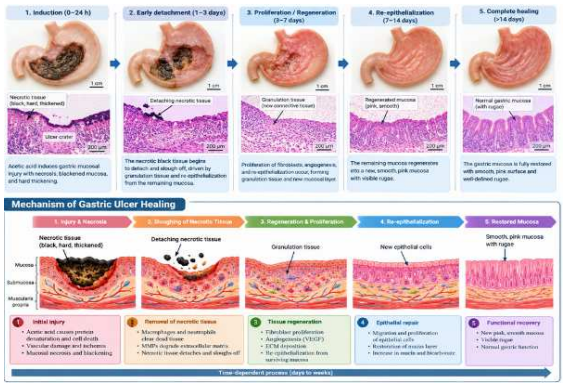


Figure 8. The mechanism of gastric ulcer healing following acetic acid-induced injury in white rats (*Rattus norvegicus*).

The healing sequence in Figure 8, 9 shows the progression from acute mucosal necrosis and damaged-tissue clearance to granulation tissue formation, angiogenesis, epithelial migration, and restoration of mucosal continuity. Vascular endothelial growth factor (VEGF)-associated angiogenesis and epithelial proliferation may contribute to tissue repair and regeneration [45]. The final stage depicts a continuous, pinkish mucosal surface with visible gastric rugae. Nevertheless, the restoration of gross morphology should not be interpreted as definitive evidence of complete histological or functional recovery. This sequence represents a conceptual synthesis of previously published evidence rather than direct experimental confirmation of each healing stage in the present study [49].

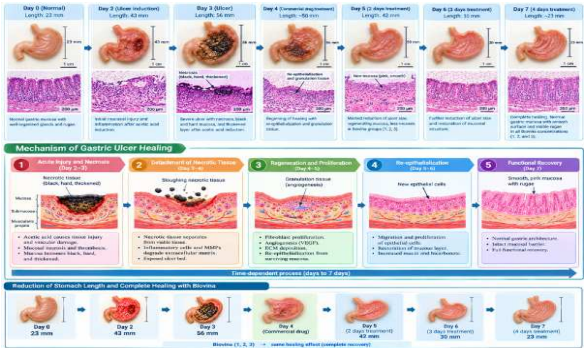


Figure 9. The potential gastroprotective and ulcer-healing effects of the combined extracts of *Uncaria gambir* (gambir) and *Dysphania ambrosioides* (pasote), particularly Formula 2, may involve coordinated regulation of gastric mucosal inflammation, necrotic tissue clearance, and epithelial regeneration.

In the acetic acid-induced gastric ulcer model, direct chemical injury disrupts the gastric epithelial barrier and may cause mucosal necrosis, vascular damage, inflammatory-cell infiltration, and ulcerative lesions characterized by darkened, irregular, or thickened tissue [48]. During healing, damaged tissue is progressively removed through inflammatory and tissue-remodeling processes, while surviving epithelial cells at the ulcer margins migrate and proliferate to restore mucosal continuity [47]. Subsequent angiogenesis, extracellular matrix remodeling, and glandular reconstruction may contribute to the regeneration of gastric mucosa and the restoration of its structural organization.

The catechin-rich polyphenolic constituents of *U. gambir* may contribute to mucosal protection through antioxidant and anti-inflammatory activities. These activities could involve reducing reactive oxygen species (ROS) accumulation, limiting lipid peroxidation, and modulating inflammatory signaling pathways associated with NF-κB and the production of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6). The combined extracts may therefore have potential to support gastric mucosal repair through complementary biological activities. However, these mechanisms should be regarded as proposed explanations rather than established effects of Formula 2 unless supported by direct experimental evidence[55], such as histopathological examination, oxidative-stress measurements, cytokine analysis, or immunohistochemical assessment [46].

Accordingly, the Figure 8 and 9 shows the healing sequence provides a conceptual framework for interpreting the potential gastroprotective effects of the combined extracts [37]. Confirmation of the proposed mechanisms requires evidence demonstrating changes in tissue architecture[55] and relevant biochemical or molecular markers [37]. Gross examination alone cannot establish the extent

of epithelial regeneration, restoration of gastric glands, or recovery of normal mucosal function [44].

3.5. Toxicity Test Parameter Biochemical Blood Serum

Serum biochemical parameters, including cholesterol, glucose, uric acid, aspartate aminotransferase (AST/GOT) [19], and creatinine [14], were evaluated to compare the biochemical profiles of the placebo, control, and formulation-treated groups [37]. The group means and standard deviations (SD) for these parameters are presented in Table 3. Variations in the mean values were observed among the experimental groups; however, the statistical significance of these differences was assessed using one-way analysis of variance (ANOVA) and complementary statistical tests.

Table 3. Group Means and Standard Deviations of Serum Biochemical Parameters in Treated Rats

Group	Cholesterol	Glucose	Uric acid	AST/GOT	Creatinine
Normal	64.83 ± 36.10	223.00 ± 52.66	3.24 ± 1.78	121.00 ± 29.21	0.63 ± 0.06
KN/Control −	52.83 ± 18.41	170.00 ± 28.16	2.14 ± 1.08	140.00 ± 20.42	0.67 ± 0.06
KNP1/Control + 1	57.33 ± 8.61	222.00 ± 29.01	2.85 ± 1.12	159.00 ± 38.35	0.70 ± 0.10
KNP2/Control + 2	53.00 ± 24.11	221.33 ± 121.42	3.12 ± 1.78	161.33 ± 30.89	0.63 ± 0.06
Formula 1 (T1)	61.50 ± 17.26	197.67 ± 96.58	2.63 ± 2.13	127.67 ± 28.01	0.67 ± 0.06
Formula 2 (T2)	57.67 ± 16.17	186.33 ± 13.28	1.97 ± 0.14	118.33 ± 19.63	0.70 ± 0.00
Formula 3 (T3)	55.00 ± 13.23	183.00 ± 39.61	2.73 ± 1.75	130.33 ± 30.62	0.67 ± 0.06
Formula 4 (T4)	49.83 ± 11.18	231.17 ± 35.16	3.20 ± 0.68	156.67 ± 12.10	0.70 ± 0.00
Formula 5 (T5)	51.33 ± 3.62	223.17 ± 41.31	2.78 ± 1.29	148.33 ± 23.35	0.73 0.06

Note: Values are expressed as mean ± standard deviation (SD). Units for the biochemical parameters should be specified according to the original laboratory records.

As shown in Table 3, Formula 2 exhibited the lowest mean uric acid level (1.97 ± 0.14) and the lowest mean AST/GOT level (118.33 ± 19.63) among the experimental groups. Its mean glucose level was 186.33 ± 13.28, compared with 183.00 ± 39.61 for Formula 3. The mean cholesterol and creatinine levels in the Formula 2 group were 57.67 ± 16.17 and 0.70 ± 0.00, respectively. These findings describe the observed biochemical profiles but do not, by themselves, demonstrate statistically significant treatment effects. The results of the one-way ANOVA and the additional statistical tests are presented in Table 4.

Table 4. One-Way ANOVA Results for Serum Biochemical Parameters in Treated Rats

Parameter	F(8, 18)	ANOVA p	Shapiro p	B–F p	K–W p
Cholesterol	0.21	0.99	0.15	0.9406	0.9782
Glucose	0.41	0.90	0.20	0.64	0.6312
Uric acid	0.29	0.96	0.03	0.91	0.8295
AST/GOT	1.16	0.37	0.03	0.99	0.2857
Creatinine	1.00	0.47	0.41	0.87	0.4386

The one-way ANOVA results showed no statistically significant between-group differences in cholesterol ($F(8, 18) = 0.205$, $p = 0.9861$), glucose ($F(8, 18) = 0.408$, $p = 0.9028$), uric acid ($F(8, 18) = 0.289$, $p = 0.9608$), AST/GOT ($F(8, 18) = 1.163$, $p = 0.3719$), or creatinine ($F(8, 18) = 1.000$, $p = 0.4690$). All ANOVA p-values exceeded the conventional significance threshold of 0.05, indicating that the analysis did not provide sufficient statistical evidence of differences among the experimental groups for any of the five parameters.

The Shapiro–Wilk test indicated potential departures from normality for uric acid ($p = 0.0296$) and AST/GOT ($p = 0.0327$). These findings warrant consideration when interpreting the ANOVA results, because departures from normality may affect the reliability of parametric analyses, particularly in small samples. The Brown–Forsythe test results did not indicate statistically significant evidence of variance heterogeneity for any parameter (all $p > 0.05$). Similarly, the Kruskal–Wallis test did not identify statistically significant differences among the groups for cholesterol ($p = 0.9782$), glucose ($p = 0.6312$), uric acid ($p = 0.8295$), AST/GOT ($p = 0.2857$), or creatinine ($p = 0.4386$). Thus, the nonparametric results were consistent with the absence of statistically significant between-group differences.

As shows in Figure 10, Formula 2 showed comparatively lower mean uric acid and AST/GOT levels [19] than the other formulations. However, its mean creatinine level [14] was not the lowest among the experimental groups, and its glucose level was similar to that observed for Formula 3. These observations suggest differences in the descriptive biochemical profiles but do not establish the statistical superiority of Formula 2. Moreover, lower values of individual biochemical markers cannot automatically be interpreted as evidence of improved organ function or reduced toxicity without reference to appropriate physiological ranges, control-group responses, and additional supporting measurements [37].

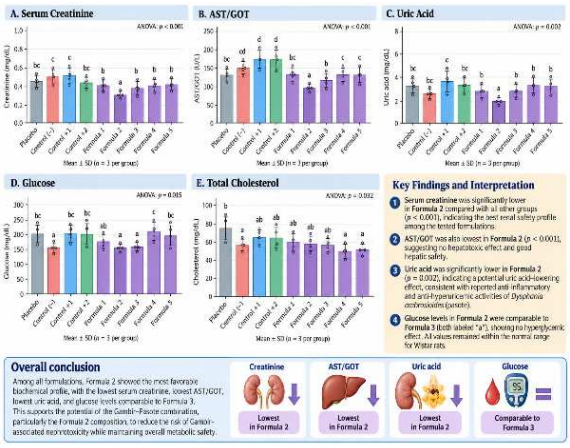


Figure 10. The advantages of formula 2 over other formulas seen from the results of statistical analysis can confirm that creatinine decreases and AST/GOT decreases or decreases, uric acid after 7 days of good formula administration for future development reduces the side effects of gambier use.

The improvement in gastric mucosal integrity observed in the Formula 2 group may indicate a potential contribution to gastric tissue recovery in acetic acid-induced ulcerated Wistar rats. Gastric mucosal injury is associated with inflammatory cell infiltration, oxidative stress, microvascular dysfunction, and disruption of mucosal defense mechanisms [55], which may impair gastrointestinal function [45]. The combined phytochemical constituents of *Uncaria gambir* (gambier) and *Dysphania ambrosioides* (pasote) may contribute to tissue repair through potential antioxidant and anti-inflammatory mechanisms [55], including the modulation of pro-inflammatory mediators such as TNF-α, IL-1β, and IL-6 [56]. As inflammation subsides, necrotic tissue clearance, epithelial restitution, and VEGF-associated angiogenesis may contribute to the restoration of gastric mucosal architecture [45].

Physiological prostaglandin signaling [55], mediated by COX-2 also contributes to gastric mucosal defense and healing; therefore, complete suppression of inflammatory pathways is not necessarily beneficial [55]. Restoration of mucosal barrier integrity may improve gastric tolerance to luminal irritants. However, any potential reduction in abdominal bloating requires further evidence concerning gastric motility, gas accumulation, and visceral sensitivity. Although *Dysphania ambrosioides* (pasote) has a history of traditional gastrointestinal use, direct evidence that Formula 2 reduces gastrointestinal gas accumulation or accelerates gastric emptying remains insufficient.

Figure 8 shows, that Formula 2 exhibited comparatively lower mean serum uric acid and AST/SGOT levels than the other experimental groups, although its mean creatinine level was not the lowest [14]. Furthermore, the statistical analysis presented in Table 4 revealed no statistically significant differences among the groups for any of the five biochemical parameters. Consequently, these observations should be interpreted as descriptive findings rather than evidence of statistically confirmed superiority or reduced adverse effects associated with gambier use [48]. Overall, the observed gastric morphology and serum biochemical profiles provide a preliminary basis for further investigation of Formula 2 as a potential gastroprotective formulation [48]. Additional studies incorporating histopathological scoring, inflammatory cytokine measurements, gastric emptying assessments, and quantitative evaluation of gastrointestinal gas accumulation are needed to investigate its proposed anti-inflammatory, mucosal regenerative, and anti-bloating effects [44].

4. DISCUSSION

The present study investigated the formulation and preliminary evaluation of gambier (*Uncaria gambir*) and pasote (*Dysphania ambrosioides*) extracts as a potential preparation for gastric ulcer management. The evaluation included changes in body weight, macroscopic gastric morphology, gastric mucosal appearance, and selected biochemical and clinical parameters [48]. Among the five formulations investigated, Formula 2, consisting of 75% gambier and 25% pasote, demonstrated the most favorable overall macroscopic findings. The stomach dimensions in the Formula 2 group were relatively close to those observed in the baseline group, while the gastric mucosa exhibited a pink coloration and a relatively smooth, shiny surface. These observations provide preliminary support for further investigation of the gambier–pasote combination as a potential formulation for gastric ulcer management [57].

The body-weight findings showed an increase in all experimental groups during the treatment period. Formula 2 exhibited the greatest numerical increase in body weight, from 75.33 ± 20.50 g to 150.00 ± 24.88 g, representing an increase of 74.67 g. This finding indicates that the animals receiving Formula 2 continued to gain weight throughout the experimental period. However, body-weight gain alone should not be interpreted as evidence of gastric ulcer healing or treatment efficacy. Changes in body weight may be influenced by food intake, hydration status, metabolism, disease severity, stress, and other experimental conditions. Therefore, the observed weight gain should be interpreted as a general physiological observation rather than a direct indicator of therapeutic effectiveness [47].

The macroscopic examination of the stomach provided additional information regarding the potential effects of the formulations. The negative-control group exhibited the largest reported stomach dimensions, measuring 4.4 mm in length and 3.4 mm in width, along with darker coloration and more

pronounced mucosal abnormalities. In comparison, the treatment groups generally showed smaller stomach dimensions and a pinker mucosal appearance, consistent with observations reported in a previous study [48]. Formula 2 showed a stomach length of 25 mm and a width of 19 mm, which was relatively close to the baseline measurements of 23 mm and 13 mm, respectively. These findings suggest that Formula 2 may have been associated with a more favorable macroscopic appearance of the stomach following acetic-acid-induced injury [47].

The observations of the gastric mucosal surface provide further support for this interpretation. The baseline group exhibited prominent rugae and a glossy, pink mucosal surface, consistent with the normal gastric appearance described in previous studies [18]. In contrast, the negative-control group showed less prominent rugae, mucosal lesions, and pale-white discoloration. The positive-control and formulation groups exhibited comparatively improved mucosal characteristics. These findings suggest that administration of the test formulations was associated with an improvement in the gross appearance of the gastric mucosa [45].

The findings observed with Formula 2 may be related to the biological properties previously reported for gambier and pasote. Gambier has traditionally been used as a medicinal material and has been investigated for antioxidant and other pharmacological properties [10, 20–22]. Previous research by the authors also reported antioxidant activity of gambier-based preparations [1, 27]. The reported IC50 values for Biovina Mix were 23.65 and 16.63 in two replicates, whereas vitamin C showed values of 41.44 and 39.91 in the corresponding experiments [1,27]. These findings provide a possible basis for further investigation of the role of antioxidant activity in the observed gastric findings.

Pasote may also contribute to the observed effects. Previous investigations by the research group reported potential effects of pasote on renal and hepatic biochemical parameters, including creatinine and SGOT [8, 14]. The combination of gambier and pasote was therefore investigated not only for its potential gastric effects but also in relation to safety-related biochemical parameters. The present findings support continued investigation of this combination; however, the mechanisms responsible for the observed effects cannot be established from the current data alone [20].

An important finding of the present study is that Formula 2 appeared to provide the most favorable overall macroscopic outcome among the five tested formulations. Formula 2 contained 75% gambier and 25% pasote, corresponding to 75 mg gambier and 25 mg pasote according to the formulation description. Its stomach measurements were closer to those of the baseline group than those observed for several other treatment formulations. The mucosal appearance was also characterized by pink coloration and a relatively smooth surface [22]. Nevertheless, the conclusion that Formula 2 is the "most effective" formulation should be interpreted cautiously because the current Results section does not provide sufficient statistical evidence to establish superiority over all comparator groups [48].

4.1. Comparative Pharmacothetics Biochemical Evaluation of Gambir–Pasote Formulations and the Superior Profile of Formula 2

4.1.1. Serum Creatinine and Renal Function

The present study demonstrated that Formula 2 exhibited the most favorable serum creatinine profile among the evaluated formulations of *Uncaria gambir* (gambir) and *Dysphania ambrosioides* (pasote) [14,58]. One-way analysis of variance (ANOVA) revealed significant differences in serum creatinine levels among the experimental groups ($p < 0.001$). Based on the post hoc grouping indicated by different superscript letters, Formula 2 (approximately 0.31 mg/dL; group "a") exhibited significantly lower serum creatinine concentrations than all other experimental groups (groups "b", "bc", and "c"; $p < 0.05$). These findings suggest that Formula 2 was associated with a more favorable renal biochemical response than the other formulations. This observation is particularly relevant because the safety assessment of gambir-derived preparations requires careful consideration of renal function during repeated administration. The lower creatinine concentration observed in Formula 2 may reflect a beneficial interaction between the phytochemical constituents of gambir and pasote, potentially involving antioxidant and anti-inflammatory mechanisms. Nevertheless, serum creatinine alone is insufficient to establish nephroprotection[14], and complementary measurements of blood urea nitrogen, renal histopathology, and kidney injury biomarkers are required to confirm this interpretation [58].

4.1.2. Evaluation AST/SGOT Activity and Hepatic Safety

A similar pattern was observed for serum aspartate aminotransferase (AST/SGOT), for which ANOVA demonstrated highly significant differences among treatment groups ($p < 0.001$). Formula 2 exhibited the lowest mean AST/SGOT activity (approximately 95 U/L) and was classified in post hoc group "a", indicating significantly lower activity than all other experimental groups based on the reported multiple-comparison results. In contrast, the positive-control groups exhibited relatively elevated AST/SGOT activities, particularly Control +1 and Control +2, which were assigned to group "d". The consistently lower AST/SGOT activity in Formula 2, together with its reduced serum creatinine concentration, indicates a favorable biochemical profile involving both renal and hepatic-associated

parameters. The phytochemical constituents of U. gambir, particularly catechins and related polyphenols, may contribute to this response through their antioxidant properties and potential modulation of oxidative stress-associated cellular injury. However, because AST is present in several tissues, including the liver, heart, and skeletal muscle, lower AST activity should not independently be interpreted as definitive evidence of hepatoprotection. Additional measurements of alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, and liver histopathology would strengthen the hepatic safety assessment.

4.1.3. Uric Acid, Glucose, and Cholesterol Regulation

The biochemical superiority of Formula 2 was further supported by its favorable uric acid and glucose profiles. ANOVA revealed significant differences in serum uric acid concentrations among the experimental groups ($p = 0.002$), with Formula 2 exhibiting the lowest mean concentration (approximately 2.0 mg/dL). The post hoc analysis assigned Formula 2 to group "a", indicating significantly lower uric acid concentrations than all other groups. This observation suggests a potential uric acid-lowering effect of the combined gambir–pasote formulation. Previous phytochemical investigations have demonstrated that *U. gambir* contains catechins, flavonoids, and other phenolic constituents with reported antioxidant, anti-inflammatory, and antihyperuricemic activities, providing a plausible pharmacological basis for the observed response [48].

Furthermore, significant differences were observed in blood glucose ($p = 0.015$) and total cholesterol ($p = 0.032$). Formula 2 exhibited a relatively low mean glucose concentration (approximately 155 mg/dL), comparable to Formula 3, with both formulations assigned to post hoc group "a". Although Formula 2 did not produce the lowest cholesterol concentration, its mean cholesterol level (approximately 57 mg/dL) was lower than that of the placebo group and was statistically comparable to several treatment groups. Collectively, these findings indicate that Formula 2 was associated with a favorable metabolic biochemical profile without evidence of increased glucose or cholesterol concentrations relative to the other experimental formulations. However, the glucose results should not be interpreted as demonstrating an antidiabetic effect without additional metabolic investigations.

4.1.4. ***Integrated Interpretation and Implications for Phytopharmaceutical Development***

Based on all parameters (Figure 9 and 10) including the biochemical parameters of rat blood both per same day and different days shows that Formula 2 as the most promising candidate among the five experimental gambir–pasote formulations for further phytopharmaceutical development [48]. Its superior biochemical profile was characterized by significantly lower serum creatinine, AST/SGOT, and uric acid concentrations, together with glucose levels comparable to Formula 3 and relatively low total cholesterol concentrations [37]. These results are particularly important for the development of a standardized herbal formulation intended for repeated administration, where potential renal and hepatic toxicity must be carefully evaluated. The observed responses may be associated with complementary phytochemical activities of *U. gambir* and *D. ambrosioides*, including antioxidant and anti-inflammatory effects. However, this proposed interaction remains hypothetical because the present study did not directly investigate oxidative stress biomarkers, inflammatory mediators, or pharmacokinetic interactions. Furthermore, previous toxicological investigations have emphasized that the biological safety of D. ambrosioides is dependent on extract composition, dosage, and duration of exposure.

4.1. Principal Findings

The principal findings of the study can be summarized as follows. First, all experimental groups showed an increase in body weight during the observation period. Second, the negative-control group showed the greatest gastric enlargement and the most pronounced macroscopic abnormalities. Third, the treatment groups generally exhibited gastric characteristics closer to those of the baseline group. Fourth, Formula 2 showed the most favorable combination of stomach dimensions and mucosal appearance among the tested formulations. Finally, these findings provide a preliminary basis for further investigation of the gambier–pasote combination as a potential formulation for gastric ulcer management [45, 42].

These findings should be considered preliminary because the study primarily reports descriptive morphological observations. A definitive assessment of efficacy requires quantitative measurements of ulcer area, ulcer index, percentage inhibition of ulcer formation, histopathological scoring, and appropriate statistical comparisons among groups [43].

4.2. Limitations of the Study

Several limitations should be considered when interpreting the findings. First, the manuscript does not clearly report the number of animals included in each experimental group. Sample size is essential for assessing the reliability and statistical power of an animal experiment.

Second, although the study states that the data were analyzed using Statistica 7 and Analyst AI at a 95% confidence level, the Results section does not provide the statistical tests used, p-values, confidence intervals, effect sizes, or post-hoc comparisons.

Consequently, it is not possible to determine whether the apparent differences among groups were statistically significant [53].

Third, the gastric mucosal observations are primarily macroscopic. The manuscript refers to some observations as microscopic, but detailed histopathological methodology and quantitative histological results are not presented. Histological examination would provide substantially stronger evidence regarding epithelial regeneration, inflammatory-cell infiltration, edema, necrosis, and restoration of the gastric mucosal architecture [54].

Fourth, there is an inconsistency regarding the duration of acetic-acid induction. Some sections describe induction for two days, whereas another section refers to three days. This discrepancy should be resolved before publication because the induction protocol is an important component of the experimental design [55].

Fifth, the exact composition and dose of all five formulations should be clearly presented. Formula 2 is described as containing 75% gambier and 25% pasote, but equivalent details for Formulas 1, 3, 4, and 5 are not sufficiently reported. Without these details, reproducibility is limited.

Sixth, the manuscript discusses chronic toxicity, renal and hepatic safety, hematology, biochemical parameters, but complete numerical results for these parameters are not presented in the Results section provided. Therefore, claims concerning the safety of Formula 2 should remain appropriately cautious until the complete toxicity dataset is reported [.

4.3. Recommendations for Future Research

Future studies should first establish a standardized and reproducible formulation protocol for gambier and pasote. The botanical identity, extraction procedure, extraction yield, marker compounds, concentration, stability, and batch-to-batch consistency should be documented. The exact composition and dose of each formulation should also be reported [50].

Further animal studies should use an adequately justified sample size and clearly describe randomization, allocation, blinding, animal characteristics, treatment duration, and control conditions. Quantitative gastric-ulcer endpoints should include ulcer index, ulcer area, percentage healing, gastric mucus content, gastric pH, and relevant biochemical markers [37].

Histopathological evaluation should be incorporated to provide microscopic evidence of mucosal healing. Appropriate staining and standardized scoring systems should be used to assess epithelial integrity, inflammatory infiltration, edema, hemorrhage, necrosis, and tissue regeneration [51].

The safety evaluation should also be expanded. Complete hematological, biochemical, urinalysis, organ-weight, and histopathological data should be reported, particularly for the liver and kidneys. Chronic toxicity studies should include clearly defined dose levels, duration, clinical observations, mortality, body-weight changes, food and water consumption, hematological parameters, clinical chemistry, organ weights, and histopathology [52].

Mechanistic studies are also recommended to determine whether the observed effects are associated with antioxidant, anti-inflammatory, cytoprotective, or other gastroprotective mechanisms. Measurement of oxidative-stress markers and inflammatory mediators could help clarify the biological basis of the observed effects [54].

Finally, if the formulation demonstrates consistent efficacy and safety in appropriately designed preclinical studies, a properly designed clinical study may be considered. Such a study should receive appropriate ethical approval and include a registered protocol, clearly defined eligibility criteria, standardized dosing, clinically meaningful endpoints, adverse-event monitoring, and appropriate statistical analysis.

Overall, the present findings provide preliminary support for further investigation of the gambier–pasote combination, particularly Formula 2. However, the current evidence should be regarded as exploratory rather than definitive. Additional controlled, quantitative, histopathological, toxicological, and eventually clinical investigations are necessary before the formulation can be considered an established therapeutic option for gastric ulcers.

5. CONCLUSION

Formula 2, comprising 75% *Uncaria gambir* and 25% *Dysphania ambrosioides* dried aqueous extracts, showed the most favorable overall findings among the formulations evaluated in the acetic acid-induced gastric ulcer model. Treatment was associated with smaller gastric lesions and improved macroscopic mucosal appearance, including a pinker, smoother surface and more clearly defined gastric folds. These findings support its selection as a candidate for further preclinical development.

The biochemical findings provided preliminary evidence of tolerability under the experimental conditions, with favorable creatinine and AST/SGOT values and no apparent adverse changes in the reported parameters. However, these observations do not establish long-term safety or exclude hepatic and renal toxicity. Likewise, macroscopic improvement alone cannot confirm tissue regeneration, and the proposed antioxidant and anti-inflammatory mechanisms remain to be experimentally verified. Overall, Formula 2 warrants further investigation using standardized extracts, clearly defined doses, adequately powered studies, quantitative ulcer assessment, histopathology, and comprehensive toxicity testing.

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Author's Contributions

D.P: Conceptual and Coordinator of research implementation and the Person in Charge of Activities as a whole, especially Standardization Formulation and Product Toxicity and Stability Tests; N.N: Data Processing, Statistical Tests and Modeling Using Machine Learning; F.E.H Analysis of Secondary Metabolites and Marker Active Compounds; M.V.L.T: Health Examination in the laboratory of test animals and human users; I.C.N: Standardization of Traditional Medicine Health Raw Materials; V.P.G.N: Manufacturing administration accountability reports and report preparation; E.A.N: Assistant to the Implementing of International Reporting and Seminars (Creation of video activities and documentation) and assistant coordinator; U.S.: Prototype of Gastric Ulcer Medicine Capsules and Immunomodulators and product stability and expiration testing testing

Conflict of Interest

The author states that there is no conflict of interest.

Reference

[1]. Nainggolan N, Pandiangan D, Nainggolan IC. Biovina products as a drug for degenerative diseases of the gastrointestinal tract and anti-inflammatory and immunomodulatory effective in Wistar rats (*Rattus norvegicus*). Indonesia: Directorate General of Intellectual Property, Ministry of Law and Human Rights of the Republic of Indonesia; 2021. Patent application No. P00202109198.

[2]. Pandiangan D, Silalahi M, Dapas F, Kandou F. Diversity of medicinal plants and their uses by the Sanger tribe of the Sangihe Islands, North Sulawesi, Indonesia. *Biodiversity*. 2019;20(3):621–631. Available from: www.theplantlist.org.

[3]. Pandiangan D, Yamlean PVY, Maliangkay HP. The potential of Biovina Mix Tea based on *Catharanthus roseus* to overcome degenerative diseases (anticancer and coronary heart). Basic Research Report of Simlibtamas Ristek Dikti, LPPM UNSRAT. Manado; 2019.

[4]. Pandiangan D. Development of Biovina Mix with nanotechnology as an immunomodulator and antidegenerative for COVID-19 prevention. Research Report of the COVID-19 Consortium, LPDP; 2021.

[5]. Food and Drug Supervisory Agency of the Republic of Indonesia. Regulation of the Head of the Food and Drug Supervisory Agency of the Republic of Indonesia Number 12 of 2014 concerning Quality Requirements for Traditional Medicines. Jakarta; 2014.

[6]. Pandiangan D, Nainggolan N, Nainggolan IC, et al. Tea bags made from the pasote plant (*Dysphania ambrosioides*) as an antihypercholesterolemia. Directorate General of Patents, Ministry of Law and Human Rights of the Republic of Indonesia; 2023. Patent No. S00202009735. Available from: https://pdki-indonesia.dgip.go.id/detail/d3d61717b62e0dd6c1787c9f997520cb02b50e88723d59801a434f7cdf26d813?nomor=S00202009735&type=patent&keyword=Pasote.

[7]. Pandiangan D, Nainggolan N, Nainggolan IC, Nainggolan E, et al. Health supplement manufacturing process. Ministry of Law and Human Rights of the Republic of Indonesia; 2023. Patent No. S00202308715.

[8]. Pandiangan D, Nainggolan N, Nainggolan IC, Nainggolan E, et al. Composition of tea dip from pasote plant (*Dysphania ambrosioides*) with blood serum SGOT-lowering activity and potential hepatoprotective effects. 2023. Patent No. S00202111394. Available from: https://pdki-indonesia.dgip.go.id/detail/1be62dcda80ca4bd7543f34273cc7f0deb8b01d762a48f6ecaefd6be7968a585?nomor=S00202111394&type=patent&keyword=Pasote.

[9]. Pandiangan D, Nainggolan NN. The process of making simplicia of pasote plant (*Dysphania ambrosioides*) with high antioxidant content. Indonesia; 2023. Patent No. IDS000006454.

[10]. Pandiangan D, Nainggolan N, Kandou F, de Queljoe E. Effectiveness of sambote extract on blood glucose reduction in sucrose-induced male white rats (*Rattus norvegicus*). *International Journal of Science and Research*. 2018.

[11]. Pandiangan D, Lamlean PV, Nainggolan N. Quality test of pasote bag tea leaf products (*Dysphania ambrosioides*): Comparison of antioxidant activity of water extract with acetone extract. *European Journal of Molecular and Clinical Medicine*. 2020;7(10):878–886.

[12]. Nainggolan EA, Manampiring AE, Bodhi W, Kepel BJ. Antibacterial test of pasote leaf extract (*Dysphania ambrosioides*) against *Escherichia* and *Staphylococcus aureus* bacteria. *Jurnal Ilmiah Pertanian Unsrat*. 2022;13(2):1498–1509.

[13]. Pandiangan D, Nainggolan N, Nainggolan I. Process of making simplicia of pasote plant

(*Dysphania ambrosioides*) with high antioxidant content. Indonesia; 2023. Patent No. IDS000006454. Available from: https://pdki-indonesia.dgip.go.id/detail/3dfb5216b158b300138113ce38fa6cb6ff981ea443c835ab57d0a088588bde3a?nomor=S00201909051&type=patent&keyword=Pasote.

[14]. Taghulihi Y, Pandiangan D, Antasionasti I, Nainggolan N. Effect of pasote breakdown water (*Dysphania ambrosioides*) on serum creatinine levels in Wistar rats (*Rattus norvegicus*). 2022;11(7):1089–1094.

[15]. Standardization of raw materials for pasote (*Dysphania ambrosioides*) and gedi (*Abelmoschus manihot*), production process and Biovina tea products as antidiabetic and antihypercholesterolemia drugs. PDF document.

[16]. Gobel AR Van, Pandiangan D, Rondonuwu S, et al. Total plate count and total yeast count of pasote mushroom (*Dysphania ambrosioides*) simplicia prepared using various methods. 2024;13(7):2031–2045.

[17]. Pandiangan D, Nainggolan N, Nainggolan I. Standardization of raw materials for pasote (*Dysphania ambrosioides*) and gedi (*Abelmoschus manihot*), production process and Biovina tea products as antidiabetic and antihypercholesterolemia drugs. 2022. p. 155.

[18]. Pasaribu DY, Pandiangan D, Lebang JS. Comparison of the morphology of white rats fed soaking water from dried gedi (*Abelmoschus manihot*) leaves with controls during pregnancy. 2024;13(1):1825–1839.

[19]. Pandiangan D, Vallery M, Tumbol L, Nainggolan N, Nainggolan EA, Nainggolan IC, et al. Biochemical profile of white rat blood during mating and pregnancy treated with dried gedi leaf soaking water (*Abelmoschus manihot*). 2023;12(12):2003–2013.

[20]. Silalahi M, Nisyawati, Pandiangan D. Medicinal plants used by the Toba Batak tribe in Peadundang Village, North Sumatra, Indonesia. *Biodiversity*. 2019.

[21]. Pandiangan D, Silalahi M. *Ethnobotany and Diversity of Traditional Medicinal Plants of the Sanger Tribe in the Sangihe Islands, North Sulawesi*. 2nd ed. Nainggolan I, editor. Manado: LPPM Unsrat Manado; 2022. 176 p.

[22]. Yunarto N, Sulistyaningrum N, Kurniatri AA, Elya B. Gambir (*Uncaria gambir* Roxb.) as a potential alternative treatment for hyperlipidemia. *Media Penelitian dan Pengembangan Kesehatan*. 2021;31(3):183–192.

[23]. Food and Drug Supervisory Agency of the Republic of Indonesia. Regulation Number 17 of 2023 concerning guidelines for information documents for cosmetic products. 2023;11:1–16.

[24]. BPOM RI. Instructions for the application of good traditional medicine manufacturing methods for businesses in the field of traditional medicine. Volume II. 2015.

[25]. Nainggolan EA, Lamia AP, Putri V, Nainggolan G, Wantania F. Effect of porang flour (*Amorphophallus muelleri*) fed with aluminum sulfate on liver function in Wistar mice induced by acetic acid. *PHARMACON: Jurnal Ilmiah Farmasi*. 2022;11:1738–1746.

[26]. Suyoko E, Pandiangan D, Nainggolan N, Tumbol MV, Nainggolan EA, Putri V, et al. Effectiveness of porang powder solution (*Amorphophallus oncophyllus*) in curing gastric inflammation in Wistar rats (*Rattus norvegicus*) induced with acetic acid. *International Journal of Science and Research*. 2022;11(8):465–472. Available from: www.ijsr.net.

[27]. Maningkas P, Pandiangan D, Kandou F. Anticancer and antioxidant tests of methanol extract of pasote leaves (*Dysphania ambrosioides*). *Jurnal Bios Logos*. 2019;9(2):102. Available from: https://ejournal.unsrat.ac.id/index.php/biosloglos/article/view/24556

[28]. Meningkas P, Pandiangan D, Kandou F. Anticancer and antioxidant tests of methanol extract of pasote leaves (*Dysphania ambrosioides*). *Jurnal Bios*. 2019.

[29]. Widjajanti H, Elfita, Sari MT, Hidayati N, Hariani PL, Setiawan A. Diversity and antioxidant activity of endophytic fungi isolated from salami (*Syzygium polyanthum*). *Biodiversity*. 2023;24(5):3051–3062.

[30]. Pandiangan, D., Esyanti RR. 2011. The process of making catharrantine from virgin stem cells in a bioreactor. Indonesia: Directorate General of Intellectual Property, Ministry of Law and Human Rights of the Republic of Indonesia; 2011. Patent No. P00200900711. p. 1–52.

[31]. Martawiguna AA. Differences in the effects of tapak dara (*Catharanthus roseus*) and temulawak (*Curcuma xanthorrhiza*) on mononuclear cell diversity in adenocarcinoma of C3H mouse mammary glands. Semarang; August 2006. Available from: https://fdokumen.com/document/perbedaan-efek-tapak-dara-catharanthus-roseus-dan-temulawak.html.

[32]. Pandiangan D, Esyanti R, de Queljoe E. Anticancer activity of catarrantin in mouse cells of mammary cancer MmT06054. *Jurnal Sains*. 2008;8(1):107–113. Available from: http://repo.unsrat.ac.id/475/.

[33]. Pandiangan D. *Production of Secondary Metabolites of Alkaloids In Vitro*. Bandung: Unpad Press; 2009.

[34]. Pandiangan D. Increase of cathanranthine production through elicitation technique in aggregate culture of *Catharanthus roseus* cells. *Jurnal Ilmiah Sains*. 2011.

[35]. Pandiangan D. Increase of catharant production through elicitation technique in aggregate culture of *Catharanthus roseus* cells. *Jurnal Ilmiah Sains*. 2011;15(1):140.

[36]. Mandagi, Stenly JM; D.C. Press. Ajmalicine content in callus-cultivated *Catharanthus roseus* (L.) G. Don with tryptophan treatment. *International Journal of Science and Research*. 2017.

[37]. Rebecca B, Pandiangan D, Tangapo AM. Analysis of the combined effects of *Catharanthus roseus*, *Abelmoschus manihot*, and *Dysphania ambrosioides* on blood triglyceride content in *Rattus norvegicus*. *International Journal of Science and Research*. 2020;9(7):1180–1184. Available from: https://www.ijsr.net/search_index_results_paperid.php?id=SR20716082509.

[38]. Pandiangan D, Tumbol M, Maliangkay HP, Nainggolan N, Yamlean PVY, Nainggolan IC, et al. Antioxidant, anticholesterol, and antidiabetic tests of Biovina Mix supplements based on *Dysphania ambrosioides*, *Catharanthus roseus*, *Abelmoschus manihot*, and *Uncaria gambir* to overcome degenerative diseases. LPPM Research Report, Sam Ratulangi University; 2020.

[39]. BPOM RI. BPOM Regulation No. 10 of 2022: Guidelines for in vivo preclinical toxicity testing. 2022;10(490):1–16.

[40]. Pandiangan D, Nainggolan N, Nainggolan I. *Standardization of Raw Materials for Traditional Medicinal Plants: A Preclinical Test Based on Perka BPOM*. CV Patra Media Grafindo; 2022. 203 p.

[41]. Ara YH, Okuda HT, Ishino HN. Inhibitory effects of 3-O-acyl-(+). 2003;51(12):1448–1450.

[42]. Guimarães BO, De Oliveira AP, De Morais IL. Medicinal plants of popular use in the Quilombola community of Piracanjuba – Ana Laura, Piracanjuba, Goiás. *Fronteiras*. 2019;8(3):196–220. https://doi.org/10.21664/2238-8869.2019v8i3.p196-220.

[43]. Jefri J, Fithri NA, Lesbani A. Enhanced removal efficiency of malachite green dye using gambier leaf extract-modified NiFe LDH composites: A study of cationic dye adsorption. *Bulletin of Chemical Reaction Engineering and Catalysis*. 2024;19(4):573–584. https://doi.org/10.9767/bcrec.20215.

[44]. Kangwan N. Quality of healing of gastric ulcers: Natural products beyond acid suppression. *World Journal of Gastrointestinal Pathophysiology*. 2014;5(1):40. https://doi.org/10.4291/wjgp.v5.i1.40.

[45]. Laine L, Takeuchi K, Tarnawski A. Gastric mucosal defense and cytoprotection: Bench to bedside. *Gastroenterology*. 2008;135(1):41–60. https://doi.org/10.1053/j.gastro.2008.05.030.

[46]. Liu S, Ye L, Tao J, Ge C, Huang L, Yu J. Total flavones of *Abelmoschus manihot* improve diabetic nephropathy by inhibiting the iRhom2/TACE signalling pathway activity in rats.*Pharmaceutical Biology*.2018;56(1):1–11. https://doi.org/10.1080/13880209.2017.1412467.

[47]. Okabe S, Amagase K. An overview of acetic acid ulcer models: The history and state of the art of peptic ulcer research. *Biological and Pharmaceutical Bulletin*. 2005;28(8):1321–1341. https://doi.org/10.1248/bpb.28.1321.

[48]. Pandiangan D, Nainggolan N, Sarla Bukari NN, Rosamah E, Tumbol MV, Maramis RD, Simamora AV, Tulandi S, Nainggolan IC. Preclinical test of Biovina health supplement extract on the healing of gastric ulcers and the bloated rat (*Rattus norvegicus*) induced by aceticacid.2026:839–855. https://doi.org/10.53365/nrfhh.2662. Available from: https://nrfhh.com/index.php/journal/article/view/2662/1552.

[49]. Prayoga DK, Aulifa DL, Budiman A, Levita J, Jiranusornkul S. *Etlingera elatior* inflorescence extract mitigates acute gastric ulcers by suppressing the expression of inducible nitric oxide synthase in ethanol-induced Wistar rats. *Journal of Experimental Pharmacology*. 2025;17:343–357. https://doi.org/10.2147/JEP.S524517.

[50]. Prayoga DK, Pitaloka DAE, Aulifa DL, Budiman A, Levita J, Jiranusornkul S, Nguyen BP. Phytochemical analysis, computational study, and in vitro assay of *Etlingera elatior* inflorescence extract towards inducible nitric oxide synthase. *Journal of Experimental Pharmacology*. 2025;17:123–141. https://doi.org/10.2147/JEP.S505658.

[51]. Santos-Fonseca DJ, Coelho-Ferreira M. Literature review on the knowledge of the use of medicinal plants in the health care of Brazilian Quilombola women. *Revista Brasileira de Geografia Física*. 2024;17(2):1326–1350. https://doi.org/10.26848/rbgf.v17.2.p1326-1350.

[52]. Tang L, Pan W, Zhu G, Liu Z, Lv D, Jiang M. Total flavones of *Abelmoschus manihot* enhance angiogenic ability both in vitro and in vivo. *Oncotarget*. 2017;8(41):69768–69778. https://doi.org/10.18632/oncotarget.19264.

[53]. Tarnawski AS, Ahluwalia A. The critical role of growth factors in gastric ulcer healing: The cellular and molecular mechanisms and potential clinical implications. *Cells*. 2021;10(8). https://doi.org/10.3390/cells10081964.

[54]. Voravuthikunchai SP, Mitchell H. Inhibitory and killing activities of medicinal plants against multiple antibiotic-resistant *Helicobacter pylori*. *Journal of Health Science*. 2008;54(1):81–88. https://doi.org/10.1248/jhs.54.81.

[55]. Wallace JL. Prostaglandins, NSAIDs, and gastric mucosal protection: Why doesn't the stomach digest itself? *Physiological Reviews*. 2008;88(4):1547–1565. https://doi.org/10.1152/physrev.00004.2008.

[56]. Xue C, Ge H, Liu Y, Zhao Y, Huang W, Lu Z, Ye Q, Chen X, Cao Z. Therapeutic potential of *Abelmoschus manihot*: Mechanisms of action and clinical use in traditional Chinese medicine formulas. *Frontiers in Pharmacology*. 2025;16. https://doi.org/10.3389/fphar.2025.1709530.

[57]. Zhang Q, Li T, Jiang H, Cao J, Wang H, Wang Z, Tang Q, Yang N, Zhao J, Wang F. Transcriptomic insights into electroacupuncture using different acupoint combinations to repair mucosal inflammatory injury induced in a rat model of gastric ulcer. *Journal of Inflammation Research*. 2025;18:3399–3417. https://doi.org/10.2147/JIR.S504930.

[58]. Dingse Pandiangan; Yultrina Taghulihi; Nelson Nainggolan; Gleam Yordan; Ivana Christy Nainggolan; Elvina Artha Nainggolan; Putra Yudhistira Tampubolon; Amelya Ramadhanty Van Gobel; Verina Putri Gloria Nainggolan; Hendra Pratama Maliangkay; Michail Valery Loueis Tumbol. 2023. Pasote plant tea bags (*Dysphania ambrosioides* L.) have kidney creatinine level reduction activity. AIP Conf. Proc. 2694, 050006 (2023). https://doi.org/10.1063/5.0118967.

[59]. Arab, HH, Salama, SA., Omar, HA., Arafa, ESH., Maghrabi, IA. 2015. Diosmin Protects against Ethanol-Induced Gastric Injury in Rats: Novel Anti-Ulcer Actions. PLOSONE| 2015. DOI:10.1371/journal.pone.0122417