AN ASSESSMENT OF ACUTE AND SUBCHRONIC TOXICITY OF GAS ANTA SYRUP IN RODENTS

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Gas Anta Syrup is a formulation derived from herbal ingredients, intended for the supportive treatment of peptic ulcers and gastroesophageal reflux disease. This study was conducted to evaluate the acute and subchronic toxicity of Gas Anta Syrup in Swiss mice. In the acute toxicity test, mice were administered increasing doses of the syrup to determine the minimum dose causing 100% mortality and the maximum dose causing 0% mortality. In the subchronic toxicity study, mice were divided into a control group and two treatment groups receiving the syrup at 4.32 and 12.96 mL/kg/day for 4 weeks. Parameters monitored included general conditions, body weight, hematological and biochemical indices, as well as gross and microscopic examinations of the liver and kidneys. Results indicated that at the maximum feasible dose of 100 mL/kg, no toxic manifestation or mortality was observed in the acute toxicity study. No significant difference was observed in the monitored indices between the treatment groups and the control group in the subchronic toxicity study. In conclusion, Gas Anta Syrup does not induce acute or subchronic toxicity in Swiss mice at the tested dosage levels.

Keywords: Gas Anta Syrup, peptic ulcers, gastroesophageal reflux disease, acute toxicity, subchronic toxicity, *Swiss* mice.

I. INTRODUCTION

Within the digestive system, the stomach and duodenum serve as central organs performing vital mechanical and chemical functions, ranging from food storage and churning to the secretion of enzymes and acid to initiate nutrient breakdown. However, due to frequent direct exposure to harmful agents such as gastric acid, *H. pylori* bacteria, alcohol, and anti-inflammatory drugs, the gastroduodenal mucosa is highly susceptible to injury, leading to chronic pathologies.[1] Currently, peptic ulcer diseases and gastroesophageal reflux disease (GERD) have become prevalent global health challenges, that significantly diminish quality of life, lead to serious clinical complications, and impose a massive economic burden. Furthermore, the prevalence of these diseases exhibits substantial geographical heterogeneity, varying markedly across global regions and even within localized provinces.[2]

Although treatment with proton pump inhibitors (PPIs) is effective, some observational and population-based cohort studies have shown an association between long-term use of PPIs and an increased risk of major adverse drug reactions, such as vitamin B12 deficiency and bone fractures.[3] In recent years, there has been a global resurgence of interest in alternative therapies and herbal products, particularly those derived from standardized botanical sources. Consequently, investigations of the new agents through the screening of different plant extracts led to the discovery of effective and safe drugs with gastroprotective activity.[4]

Gas Anta Syrup is a formulation of herbal ingredients, that *contains Radix et Rhizoma Glycyrrhizae*, *Rhizoma Imperatae cylindricae,*

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Radix Paeoniae lactiflorae, Radix Salviae miltiorrhizae, Radix Clerodendri, Herba Pogostemonis, Radix Bupleuri, Fructus Forsythiae suspensae, Massa Medicata fermentata, Fructus Aurantii immaturus, Fructus Hordei germinatus, and *Rhizoma Curcumae longae*. According to traditional medicine principles, this multi-herbal formulation exerts several therapeutic actions, including soothing the liver, strengthening the spleen, harmonizing the stomach, suppressing gastric acid, and alleviating pain.[5]

While these individual components have a long history of use in medical literature, the selection of a syrup dosage form offers distinct advantages for treating gastric pathologies. Unlike solid tablets, the syrup allows active compounds to be pre-dissolved, ensuring rapid and uniform coverage and adhesion to the damaged gastric mucosa. This mechanism may create a protective "shield" over ulcers, potentially guarding them against gastric acid aggression, providing relief from burning sensations, and possibly enhancing local absorption of the active ingredients. Nevertheless, the synergistic interactions and safety of the final preparation in this liquid form needed to be confirmed by scientific evidence. This study was conducted to determine the acute toxicity and subchronic toxicity of Gas Anta Syrup in *Swiss* mice.

II. SUBJECTS AND METHODS

1. Investigational product

Gas Anta Syrup is manufactured by Thanh Phat Pharmaceutical Joint Stock Company and distributed by Hoang Giang Saigon Pharmaceutical Co., Ltd. The product is strictly formulated to meet the manufacturer's internal quality control standards.

Each 3 ml of syrup contains: *Rhizoma Bletillae striatae*: 0.5 g, *Radix Paeoniae lactiflorae*: 0.5 g, *Rhizoma Atractylodis macrocephalae*: 0.5 g, *Radix et Rhizoma Glycyrrhizae*: 0.1 g, *Radix Ginseng*: 2.0 g, *Rhizoma Coptidis*: 2.0 g, *Radix Saussureae lappae*: 1.0 g, *Rhizoma Cyperi*: 0.5 g, *Os sepiae*: 2.0 g

The product is indicated for the supportive treatment of gastric and duodenal ulcers, as well as the management of symptoms associated with gastroesophageal reflux disease (GERD). The recommended therapeutic regimen for adults is 6 mL per dose, administered 2 to 3 times daily via oral intake.

2. Experimental animals

Healthy *Swiss* mice of both sexes, body weight 30 ± 2 g, were supplied by the National Institute of Hygiene and Epidemiology (NIHE). The animals were fed a standard rodent pellet diet and provided with *ad libitum* access to water. They were acclimatized for 7 days prior to and throughout the study at the Laboratory of the Department of Pharmacology, Hanoi Medical University.

3. Methods

Acute toxicity study of Gas Anta Syrup

Acute toxicity study and LD_{50} determination of Gas Anta Syrup was carried out in *Swiss* mice via oral administration.[6,7]

Prior to the study, mice were fasted overnight. Subsequently, they were randomly divided into different groups, each consisting of 10 mice. Gas Anta Syrup was administered at increasing dose levels while maintaining a constant administration volume to determine the minimum dose causing 100% mortality and the maximum dose causing 0% mortality. During the observation period, the general condition of the mice, signs of toxicity (such as vomiting, convulsions, agitation, and changes in excretion), and the number of deaths within 72 hours after administration were recorded. All deceased mice underwent necropsy to evaluate gross pathological damage. Based

on the collected data, a dose-response curve was constructed to determine the LD_{50}. The condition of the remaining mice continued to be monitored until the end of the 7th day following administration.

Subchronic toxicity study of Gas Anta Syrup

The subchronic toxicity study of Gas Anta Syrup was carried out in *Swiss* mice via oral administration. [6,7]

(1) Group allocation and dosage

Mice were randomly divided into three groups (n=20 per group):

- Control group: Administered distilled water at 20 ml/kg/day.

- Treatment group 1: Administered Gas Anta Syrup at 4.32 mL/kg/day (equivalent to the expected human dose, converted using a factor of 12).

- Treatment group 2: Administered Gas Anta Syrup at 12.96 mL/kg/day (equivalent to three times the human dose).

All groups received the administration continuously every morning for 4 weeks.

(2) General Observation and Body Weight Monitoring

Throughout the study, the general condition of the mice was monitored daily, including activity levels, feeding behavior, fur condition, stool consistency, reflexes, and mortality rates. Body weight was recorded at baseline (day 0) and at the end of weeks 1, 2, 3, and 4.

(3) Blood collection and hematological/ biochemical analysis

Blood samples were collected at two intervals: prior to the start of the study (10 mice per group randomly selected to establish baseline values) and after 4 weeks of treatment (the remaining 10 mice per group). Mice were anesthetized via intraperitoneal injection of chloral hydrate at a dose of 350 mg/kg before cardiac puncture for blood collection.

Hematological parameters, including red blood cell (RBC) count, mean corpuscular volume (MCV), hemoglobin, hematocrit, white blood cell (WBC) count, differential WBC count, and platelet count, were quantified using Horiba Medical reagents on an ABX Micros 60 ES hematology analyzer (France).

Biochemical parameters, including the indices to evaluate liver and kidney function, as well as cellular damage (levels of total bilirubin, albumin, total cholesterol, ALT and AST enzyme activity, and serum creatinine) were measured. These analyses were performed using Erba quantification kits (India) on an Erba semi-automatic biochemical analyzer.

(4) Gross anatomy and histopathological examination

Following blood collection, mice were necropsied for gross examination. Organs including the liver, kidneys, heart, spleen, lungs, brain, and pancreas were harvested and weighed to determine absolute organ weight and calculated as a ratio relative to total body weight (relative organ weight).

Additionally, histopathological evaluations of the liver and kidneys were conducted on a random selection of 30% of the mice from each group. Histopathological specimens were prepared and processed using specialized reagents to observe microscopic structural changes.

Statistical Analysis

Statistical analysis was performed using SigmaPlot 12.0 (SYSTAT Software Inc., Richmond, CA, USA). Results are expressed as mean ± SD. Differences between groups were evaluated using one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant.

III. RESULTS

1. Acute toxicity of Gas Anta Syrup

Mice were administered Gas Anta Syrup at the maximum feasible dose of 0.25 mL/10g, 4 times within 24 hours. At that dose, the mice exhibited no clinical sign of toxicity or abnormal symptom during the 72-hour observation period post-administration. From these data, the maximum tolerated dose of Gas Anta Syrup, which is consistently lower than the LD_{50}, was determined to be 100 mL/kg.

2. Subchronic toxicity of Gas Anta Syrup

Effects of Gas Anta Syrup on mice's general condition, body weight and organ weight

Throughout the experimental period, mice in the biological control group and the two treatment groups exhibited normal behavior. No abnormal manifestation were observed in any of the three groups of mice during the study.

Table 1. Effects of Gas Anta Syrup on mice's body weight

Group	Baseline	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks
Control	30.30 ± 1.25	31.40 ± 1.26	32.50 ± 1.51	34.10 ± 1.79	36.20 ± 1.69#
Gas Anta Syrup (4.32 mL/kg/day)	29.90 ± 1.60	30.60 ± 1.58	31.70 ± 1.89	33.10 ± 1.97	34.50 ± 2.88#
Gas Anta Syrup (12.96 mL/kg/day)	29.70 ± 1.42	30.80 ± 2.15	31.80 ± 2.62	32.90 ± 2.56	34.30 ± 2.71#

 # $p < 0.05$ compared to baseline

** $p < 0.05$ compared to control group*

The results in Table 1 indicate that after 4 weeks of administering Gas Anta Syrup, the body weight of mice in both the control and treatment groups increased significantly compared to baseline values ($p < 0.05$). There were no statistically significant difference in body weight between the control group and the treatment groups.

Moreover, the relative organ weights (liver, kidneys, heart, spleen, lungs, brain, and pancreas) compared to the total body weight in both treatment groups showed no statistically significant differences compared to the biological control group ($p > 0.05$).

Effects of Gas Anta Syrup on hematological parameters in mice

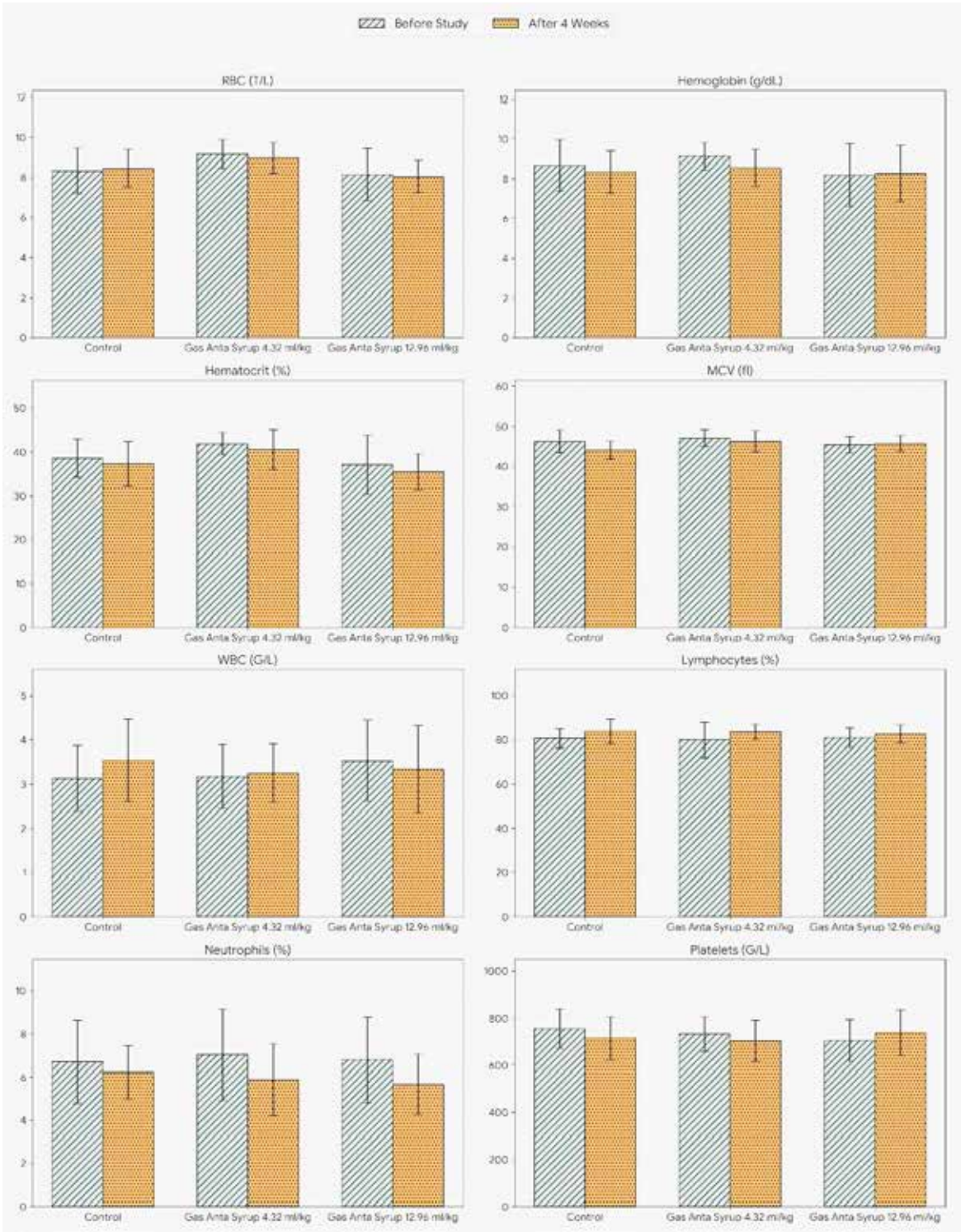

Figure 1. Effects of Gas Anta Syrup on mice's hematological parameters

As shown in Figure 1, both at baseline and after 4 weeks of Gas Anta syrup administration, the hematological parameters-including red blood cell (RBC) count, hemoglobin content, hematocrit, mean corpuscular volume (MCV), white blood cell (WBC) count, differential WBC count, and platelet count-in the blood of mice in both treatment groups showed no statistically significant difference compared to the biological control group ($p > 0.05$).

Effects of Gas Anta Syrup on biochemical parameters in mice

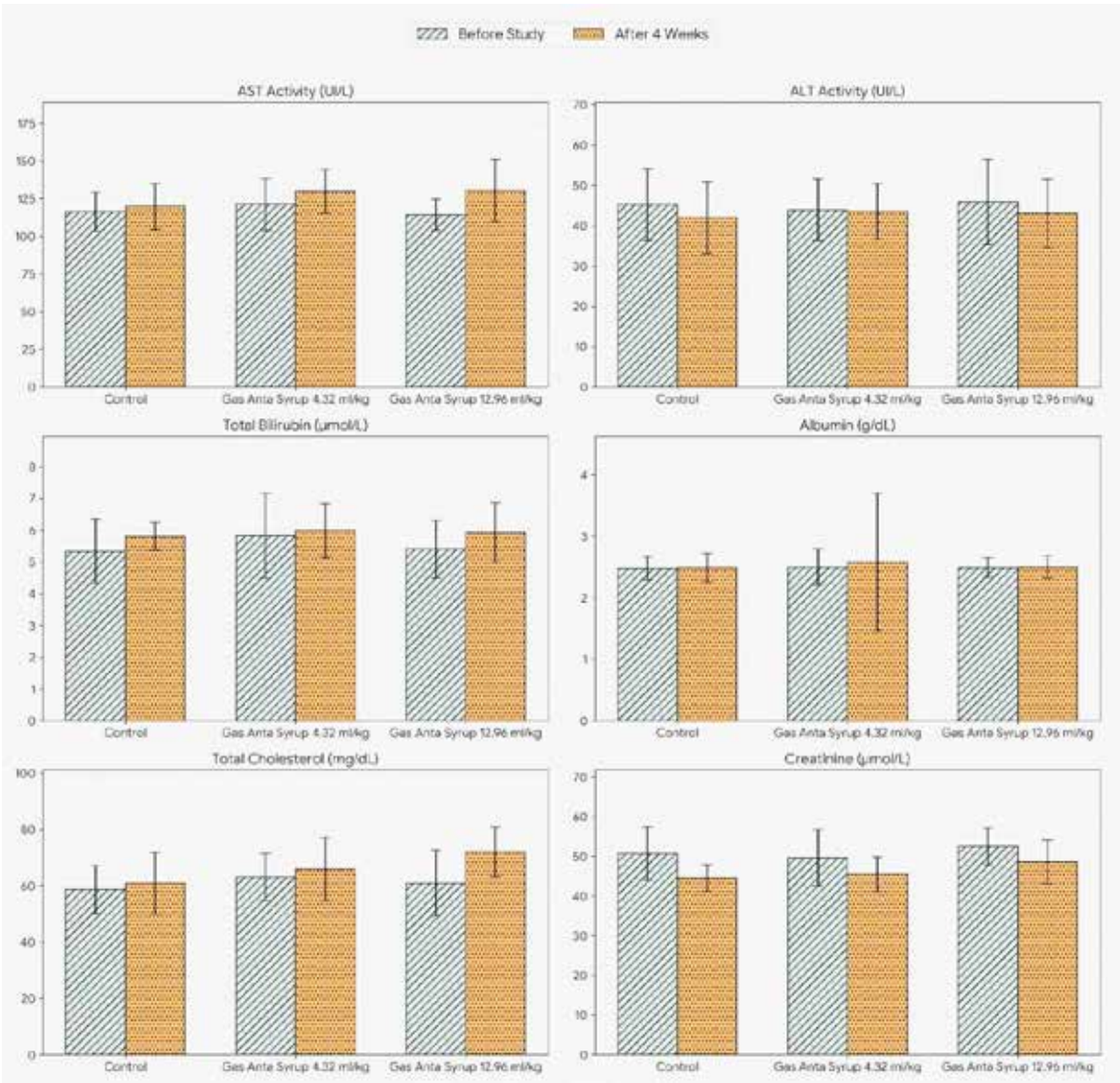

Figure 2. Effects of Gas Anta Syrup on mice's biochemical parameters

As shown in Figure 2, both at baseline and after 4 weeks of Gas Anta Syrup administration, parameters assessing liver cell damage (AST, ALT), liver function (including total bilirubin, albumin, total cholesterol), and kidney function (creatinine) in the blood of mice across both treatment groups showed no statistically significant differences compared to the biological control group ($p > 0.05$).

Effects of Gas Anta Syrup on the morphology of organs and microstructure of liver and kidneys in mice

Macroscopic observation: In all experimental mice (including the control group and the two treatment groups), no pathological change was observed in the gross morphology of the heart, lungs, liver, spleen, pancreas, kidneys, or digestive system.

Microstructure: After 4 weeks of continuous administration, the microscopic structures of the liver and kidneys in the mice treated with Gas Anta Syrup showed no distinct difference compared to those of the biological control group.

IV. DISCUSSION

The increasing prevalence of peptic ulcers and gastroesophageal reflux disease has intensified the search for herbal formulations that offer effective symptomatic relief with high long-term safety profiles. Unlike synthetic antacids or proton pump inhibitors which may cause adverse effects upon prolonged use, polyherbal remedies like Gas Anta Syrup aim to restore the balance between aggressive and protective factors in the gastric mucosa. Establishing the safety of this formulation is a fundamental step in validating its clinical utility for chronic gastrointestinal conditions.

In the acute toxicity study, the results demonstrated that even at the maximum feasible dose of 100 mL/kg, no mortality or clinical signs of toxicity were recorded in mice. Although the LD_{50} could not be determined, it is evident that the lethal threshold exceeds 100 mL/kg. Based on body surface area conversion, this dose is approximately 23 times higher than the maximal recommended human therapeutic dose (calculated for a 50 kg adult with an extrapolation factor of 12). These findings classify Gas Anta Syrup as a product with low acute toxicity. This high safety profile is consistent with toxicological studies of its individual herbal components, which have also demonstrated remarkably low acute toxicity and high LD_{50} values. For instance, *Panax ginseng* exhibits low acute oral toxicity, with LD_{50} values exceeding 5000 mg/kg in rats and mice and no toxic effects observed in mini pigs at 2000 mg/kg.[8] Similarly, acute toxicity studies in *Kunming* mice established the oral LD_{50} of the fibrous root of *Rhizoma Coptidis* at greater than 7000 mg/kg.[9] Furthermore, a single oral administration of *Radix Saussureae lappae* ethanolic extract at doses up to 5000 mg/kg caused no mortality in male *Wistar* rats, indicating an LD_{50} greater than 5000 mg/kg.[10]

Regarding the subchronic toxicity study, repeated oral administration of Gas Anta Syrup at 4.32 mL/kg/day and 12.96 mL/kg/day for 28 days did not induce significant changes in general conditions, body weight or the relative organ weights of the major organs. Hematological and biochemical parameters remained within physiological limits and showed no statistical significance compared to the control group. The integrity of the gross morphology of major organs and histological structures of the liver and kidneys further confirms that repeated dosing does not cause systemic structural damage.

These findings align with existing toxicity data on the product's constituent herbal ingredients. Recent studies demonstrate that systemic administration at doses of 0.1, 0.2, and 0.4 g/kg of *Bletilla striata* polysaccharides has a metabolic safety, as evidenced by the lack of significant changes in hepatic weight and serum biomarkers such as ALT, TC, and TG.[11] Subchronic evaluations of *Panax ginseng* established its safety thresholds,

showing no adverse effect in rats at 4000 mg/kg for 20 days, or at 15 mg/kg in 90-day dog.[8] In a 13-week study, *Sprague-Dawley* rats received *Rhizoma Coptidis* via oral gavage at dose levels ranging from 25 to 2000 mg/kg/day, administered five times per week. The results demonstrated no adverse effect on mortality, body weight, hematology, serum chemistry, organ weights, or histopathology at any dosage level.[12] Furthermore, subchronic oral administration of *Saussurea lappa* root ethanolic extract at 200, 400, and 800 mg/kg for 28 days resulted in no significant change in hematological or biochemical parameters, nor any histological abnormality observed in the liver and kidneys.[10] In a 13-week sub-chronic study, male and female F344 rats received daily oral administration of *Cyperi rhizoma* aqueous extract at dosages ranging from 125 to 2000 mg/kg/day. The evaluation revealed no treatment-related abnormality in mortality, hematology, serum chemistry, or histopathologic examination, leading to a determined NOAEL of more than 2000 mg/kg/day.[13]

In conclusion, Gas Anta Syrup demonstrates a favorable safety profile in both acute and subchronic preclinical models. Notably, the polyherbal combination within the formulation showed no evidence of synergistic toxicity or potentiation of adverse effect compared to the safety profiles of its individual components.

V. CONCLUSION

The research results indicate that Gas Anta Syrup does not induce acute toxicity in *Swiss* mice at the maximum feasible dose of 100 mL/kg via oral administration. Consequently, the median lethal dose of Gas Anta Syrup could not be determined. Furthermore, Gas Anta Syrup does not exhibit subchronic toxicity in *Swiss* mice when administered orally at 4.32 mL/kg/day and 12.96 mL/kg/day for 28 consecutive days. To further validate the therapeutic potential of the product, it is recommended that subsequent studies be conducted to evaluate the efficacy of Gas Anta Syrup in experimental models of gastroesophageal pathologies.

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REFERENCES

1. Schubert ML. Gastric physiology. In: Reinus JF, Simon D, eds. *Gastrointestinal Anatomy and Physiology*. Wiley-Blackwell; 2014: chap 5. doi:10.1002/9781118833001.ch5.

2. Wickramasinghe N, Devanarayana N. Insight into global burden of gastroesophageal reflux disease: Understanding its reach and impact. *World J Gastrointest Pharmacol Ther*. 2025; 16(1). doi:10.4292/wjgpt.v16.i1.97918.

3. Bhatnagar MS, Choudhari S, Pawar D, Sharma A. Long-Term Use of Proton-Pump Inhibitors: Unravelling the Safety Puzzle. *Cureus*. 2024; 16(1): e52773. Published 2024 Jan 23. doi:10.7759/cureus.52773.

4. Kuna L, Jakab J, Smolic R, Raguz-Lucic N, Vcev A, Smolic M. Peptic Ulcer Disease: A Brief Review of Conventional Therapy and Herbal Treatment Options. *J Clin Med*. 2019; 8(2): 179. Published 2019 Feb 3. doi:10.3390/jcm8020179.

5. Loi DT. *Medicinal Plants and Ingredients of Vietnam*. Hanoi: Science and Technology Publishing House; 1991.

6. Gerhard Vogel H. *Drug discovery and evaluation Pharmacological assays*. Springer. 2016.

7. World Health Organization. *Working group on the safety and efficacy of herbal medicine*. Report of regional office for the western pacific of the World Health Organization. 2013.

8. Burdock, George & Chatzidakis, Chris. Safety Assessment of *Panax Ginseng*. *Int J Toxicol*. 2000; 19(4). 293-301. doi: 10.1080/10915810050202105.

9. Ning N, Wang YZ, Zou ZY, Zhang DZ, Wang DZ, Li XG. Pharmacological and safety evaluation of fibrous root of *Rhizoma Coptidis*. *Environ Toxicol Pharmacol*. 2015 Jan; 39(1): 53-69. doi: 10.1016/j.etap.2014.11.006.

10. Khalid AS, Momodu I, Iduh MU, Sirajo BS, Khalid HS, Wasagu HI. Toxicity assessment of ethanol extract of *Saussurea lappa* (costus) root in male *Wistar* rats. *Afr J Biol Med Res*. 2024; 7(4): 79-89. doi:10.52589/AJBMR-0ICVBWCK.

11. Wang B, Wu C, Ma Y, et al. Recent advances in Bletilla striata polysaccharide research: extraction methodologies, structural elucidation, pharmacological mechanisms, structure-activity relationships, and therapeutic delivery applications. *Front Pharmacol*. 2025; 16: 1688676. Published 2025 Nov 5. doi:10.3389/fphar.2025.1688676.

12. Lee YH, Kim D, Lee MJ, Kim MJ, Jang HS, Park SH, Lee JM, Lee HY, Han BS, Son WC, Seok JH, Lee JK, Jeong J, Kang JS, Kang JK. Subchronic toxicity study of *Coptidis Rhizoma* in rats. *J Ethnopharmacol*. 2014; 152(3): 457-463. doi:10.1016/j.jep.2014.01.011.

13. Han SR, Han HY, Park HJ, Mi BS, Chung MK, Moon KS, Jeong JY, Roh HS, Seok JH, Kim SK. Toxicity assessment of *Cyperi rhizoma* aqueous extract orally administered to rats for 13 consecutive weeks. Yakhak Hoeji. 2013; 57(4): 258-264.