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Gastroprotective Property of *Psychotria luzoniensis* Leaf Decoction on HCl/Ethanol-Induced Gastric Ulcers in Mice

[1]Angelo Miguel Parungao, [1]Gabriel Louise Sena, [1]Sheilah Zarah Penaranda, [1]Austin Japheth Salvan, [1]Christian Marie Araceli Solis, [1]Phylis C. Rio, [1]Geraldine Susan C. Tengco, [2*]Allan L. Hilario

[1]Department of Biochemistry and Nutrition, College of Medicine, Pamantasan ng Lungsod ng Manila, Philippines.

[2]Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines-Manila, Philippines.

Correspondence Author: Allan L. Hilario, Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines-Manila, Philippines.

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Abstract

Psychotria luzoniensis, or more commonly known as "tagpong gubat", is a speciose *Psychotria* that is believed to have gastroprotective property based on the ethnobotanical knowledge about the plant among Filipino natives. The purpose of this study was to validate the gastroprotective property of *P. luzoniensis* using 15 male Balb/c mice which were randomly divided into three groups wherein each was assigned with different treatments; induction of gastric ulceration was done by giving 0.5 ml of a solution containing 40% of 0.3M HCl and 60% of absolute ethanol. The first group was given 0.5 ml solution of 11.65 mg/ml of sucralfate; the second group received 0.5 ml of the *Psychotria luconiensis* decoction while the third group was given 0.5 ml of distilled water. Specimens were extracted, stained, and evaluated to assess the gastric ulceration. Random sampling and blind testing techniques were used to avoid bias and an arbitrary grading scale of zero to three was used during assessment of ulceration. Results showed significantly lower gastric ulcers in both the sucralfate (1.00 ± 0.71) and *Psychotria luzoniensis* decoction (0.60 ± 0.55) groups than in the distilled water group (2.75 ± 0.50) ($p<0.05$) grade. These findings showed that *Psychotria luzoniensis* decoction has a positive gastroprotective property in HCl/ethanol-induced gastric ulcers in mice. **Keywords:** Gastroprotection, HCl/ethanol-induced gastric ulcers, *P. luzoniensis*

1. Introduction

The pantropical Psychotria L. (Rubiaceae, Rubioideae, Psychotrieae) is the largest member of the Rubiaceae (coffee) family and the third largest angiosperm genus with more than 1,800 species worldwide. There are 112 Psychotria species present in the Philippines and almost all of them are endemic to the country. These endemic species include *Psychotria luzoniensis*, or locally known as tagpong-gubat, a species that is widely distributed throughout the Philippines, specifically in Luzon, Mindoro, Masbate, Leyte, and Panay [1-8]. Some Filipinos are able to utilize parts of the *Psychotria luzoniensis* plant to treat headaches, ulcers, and dysentery due to its phytochemical content. However, these claims are not supported with scientific investigation. The literature about the purported medicinal benefits is lacking [8].

Phytochemicals are various bioactive chemical compounds found in plants, such as antioxidants, that are considered to be beneficial to human health. According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants [9].

According to Boyer and Liu (2004), much of the protective effects of fruits and vegetables have been attributed to phytochemicals, which are plant compounds such as carotenoids, flavonoids, isoflavanoids, and phenolic acids [10].

The young leaves of the *Psychotria luzoniensis* have been claimed to have anti-ulcer activity, but detailed scientific investigation on the anti-ulcer effects of the various extracts of *Psychotria luzoniensis* is lacking. It was therefore the objective of this study to determine the gastroprotective property of the *Psychotria luzoniensis* leaves using acute gastric ulcer model in mice and sucralfate as a reference drug.

2. Materials and Methods

The study was approved by the Pamantasan ng Lungsod ng Manila-College of Medicine Publication and Research Committee and the Pamantasan ng Lungsod ng Maynila-University Research Center. It was conducted at the PLM-Biochemistry Natural Products Laboratory. It was registered to the Research Implementation and Development Office of the College of Medicine, University of the Philippines-Manila.

2.1 Plant Identification and Authentication

The plant was obtained from a refutable farm in Alaminos, Laguna, Philippines. It was identified as *Psychotria luzoniensis* of the family Rubiaceae by the Botany Division of the National Museum, Manila, Philippines.

2.2 Plant Preparation

The fresh leaves of the plant were cut into one-inch lengths. Twenty (20) grams of cut fresh leaves (by fresh weight) were obtained and put into 50 mL of boiling water for five minutes. The resulting decoction was cooled and stored inside a plastic container until further use.

2.3 Acclimatization of Experimental Animal

Fifteen male Balb/c mice (*Mus musculus*) of the same age (12-14 weeks) and more or less of the same weight (22-24 gms) were obtained from the Department of Pharmacology and Toxicology, College of Medicine, University of the Philippines Manila. These mice were caged separately at the Biochemistry Laboratory of the College of Medicine in Pamantasan ng Lungsod ng Maynila. The mice were kept at standard relative humidity (50±5%), temperature (28±2^{O}C), and light intensities (12-hour light and dark cycles). The animals were fed with standard pellet diet for rodents and water *ad libitum*.

2.4 Induction of Gastric Ulceration and Treatments

The fifteen acclimatized mice were randomly divided into three groups that each received a different treatment before the induction of ulceration; both were administered by oral gavage. The first group received 0.5 mL solution of 11.65 mg/ml of sucralfate, which served as the reference drug (positive control). The second group, on the other hand, received 0.5 mL of the *Psychotria luzoniensis* decoction. The negative control group was given 0.5 mL of distilled water.

After 30 minutes, gastric ulceration was induced in all groups. The solution for ulceration was composed of 40% of 0.3M HCl/ethanol and 60% ethanol [11].

2.5 Specimen Preparation

An hour after the second treatment, the mice were sacrificed and were dissected. Briefly, all mice were given general anesthesia through cardiac puncture under

anesthesia using ketamine HCl (Etamine®, International Apex Pharmaceutical, Inc., Pasig City, Philippines) in combination with xylazine (Sedazine®, MO, USA) at 10/0.5 mg/Kg BW. A vertical ventral abdominal laparotomy was though the wall if the abdomen. Abdominal cavity was entered and the stomach was identified and isolated. The stomachs that were isolated were then briefly washed in 0.9% NaCl solution to eliminate debris and other contaminants. After extracting all the needed specimens from the mice, the samples were placed in individual test tubes with 10% buffered formaldehyde for preservation overnight. The ratio of 10% buffered formaldehyde to the sample was approximately nine parts to one part sample.

2.6 Histopathologic and Gastric Ulceration Grading

The stomach samples were brought to the Department of Pathology of the Ospital ng Maynila Medical Center where they were stained with hematoxylin and eosin dye to evaluate histological gastric mucosal degeneration. The slides were then brought to the Biochemistry Laboratory of the College of Medicine, Pamantasan ng Lungsod ng Maynila where they were viewed under a computer-assisted light microscope equipped for photography. The software used to visualize and photograph the specimens was Metamorph Microscropy Automation and Image Analysis Software 2013 (Molecular Devices, LLC).

Table 1. Grading Scale used to assess gastric ulceration

Grade	Degree of Ulceration
0	Absence of ulceration
1	Mild degree of ulceration
2	Moderate degree of ulceration
3	Severe degree of ulceration

Using an arbitrary scale of 0 to 3 as seen in Table 1, the grading of the degree of ulcerations was determined. Grading the degree of ulcerations was done blindly. The person grading the slides was unaware of the treatment used on the sectioned stomach. The grading was done twice by blinded pathologist. The average reading was used for that sample.

2.7 Statistical Analysis

The data were presented in mean ± SD and subjected to one-way analysis of variance using STATA/SE 12.0 for Windows (StataCorp LP, Texas, USA). The treatments of *Psychotria luzoniensis* decoction, sucralfate, and distilled water served, as the independent variables in each group, while the quantified values of gastric ulceration were the dependent variables. Bonferroni was used in the post hoc analysis for multiple comparisons. The level of significance for all analyses was set at five percent ($p < 0.05$).

3. Results and Discussion

Gastric ulcer is a condition caused by the disruption of the balance between aggressive and protective factors in the gastric mucosa. It arises from multifactorial origins and has since affected millions of people around the world [16,17]. In most studies, hydrochloric acid in ethanol is generally utilized in inducing the ulcer formation of mice since it can generate necrotic lesions in the gastric mucosa through its irreversible toxic effects [18].

Various drugs, such as sucralfate, are usually used for the treatment of gastric ulcer. Sucralfate is a sucrose sulfate-aluminium complex that protects that gastrointestinal tract from stomach acid by binding to the ulcer to create a physical barrier. It also prevents the degradation of mucus, which is natural protection of the gastric mucosa from the highly acidic environment in the gastric lumen [19]. However, with its adverse side effects such as constipation, flatulence and xerostamia [17, 20, 21], the gastroprotective property of *P. luzoniensis* extract, which is a non-toxic alternative herbal drug with fewer side effects, was therefore evaluated in this study [22].

Table 2. Means ± SD of Gastric Ulcer in Different Groups

Group	Means ± SD	P-value
Positive	1.00 ± 0.71	>0.05*
Treatment	0.60 ± 0.55	<0.05**
Negative	2.75 ± 0.50	<0.05**

*Treatment vs. Positive

**Positive and Treatment vs. Negative

As the negative control in the experiment, water-treated mice are expected to yield a high degree of ulceration since water has no clinical cytoprotective effect on the gastric mucosa aside from a possible dilution of acidity. In the study, the results revealed an insignificant difference (p-value<0.05) between the effects of *P. luzoniensis* extract and sucralfate solution, supporting the gastroprotective effect of the former. As the positive control in the experiment, sucralfate-treated mice were expected to yield minimal gastric ulceration. As seen in table 2, the resulting mean value of sucralfate-treated mice was consistent with the cytoprotective property of the drug (Nagashima, 1981).

Overall, Table 2 shows that the initial analysis and comparison of mean values of HCl/ethanol-induced gastric ulcers in P. luzoniensis decoction, distilled water (negative control), and sucralfate solution (positive control)-treated mice revealed that P. luzoniensis possesses a gastroprotective property.

The results of the study also revealed a significant difference (p-value<0.01) between the gastric ulcers treated with P. luzoniensis and distilled water, indicating a gastroprotective effect against HCl/ethanol on Ethanol-induced gastic ulcer in mice that is comparable with sucralfate. These findings therefore validate the ethno-botanical use of *P. luzoniensis* in the Philippines as a gastroprotective herbal drug.

4. Conclusion

This study showed that *P. luzoniensis* has gastroprotective property against HCl/ethanol/ethanol-induced gastric ulcer, validating the ethnobotanical use of the plant. The gastroprotective property of the *P. luzoniensis* decoction is similar to sucralfate as gastric mucosa protectant from increased acid environment of the stomach. This study also used the method of preparing medicinal plant, which would appeal to the community due to its simple method of preparation and decoction. This method is readily available in communities with poor access to health care.

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Authors

Angelo Miguel Parungao, Gabriel Louise Sena, Sheilah Zarah Penaranda, Austin Japheth Salvan, and Christian Marie Araceli Solis are post-graduate interns at the hospital base of the College of Medicine, Pamantasan ng Lungsod ng Maynila.

Phylis C. Rio, MD and Geraldine Susan C. Tengco, MD are full time-faculty members of the Department of Biochemistry and Nutrition, College of Medicine, Pamantasan ng Lungsod ng Maynila.

Allan L. Hilario, MD, MHA, MSc is the corresponding author and presently a full-time Associate Professor at the Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines-Manila.