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THE GASTROPROTECTIVE ACTIVITY OF *Citrullus lanatus* (Watermelon) RIND FLAVONOID EXTRACT ON ASPIRIN-INDUCED GASTRIC ACID SECRETION IN MALE SPRAGUE-DAWLEY RATS (*Rattus norvegicus*)

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Abstract: This study determines the potential gastroprotective activity of the watermelon rind flavonoid extract by measuring the volume of gastric acid secreted by Sprague-Dawley rats. Twenty-one rats were utilized in the study and these were randomly assigned into three groups. The rats were weighed and their individual weights were used to calculate the dose of flavonoid extract, aspirin suspension, and omeprazole suspension to be administered. Aspirin suspension was used to induce gastric acid secretion in all three groups. Omeprazole suspension was given to the positive control group, distilled water to the negative control group, and flavonoid extract to the experimental group. After 1 hour, all rats underwent cervical dislocation and their gastric volumes were measured. The experimental group had the lowest mean gastric volume (0.19 mL), the positive control group came second (0.20 mL), and the negative control group had the highest mean gastric volume (0.67 mL). The flavonoid extract had the greatest effect in decreasing the gastric acid secretion of the test animals. The positive control group and experimental group showed no significant difference in gastric acid secretion (p value = 1). This shows that watermelon rind flavonoid extract exhibits an effect on gastric acid secretion similar to that of omeprazole.

Keywords: Gastroprotective activity, flavonoid, watermelon, gastric acid secretion

I. INTRODUCTION

Peptic ulcer disease (PUD) is characterized by discontinuity in the inner lining of the gastrointestinal (GI) tract because of gastric acid secretion (Malik et al., 2022). The most prevalent disease associated with gastric acid secretion in the Philippines is peptic ulcer. People who frequently take pain relievers are more likely to develop ulcers. Two of the most common causes of peptic ulcer disease are bacterial infection and medications. The most common bacteria that may infect the stomach is *Helicobacter pylori.* Peptic ulcer may also be caused by the common pain-relieving nonsteroidal anti-inflammatory drugs (NSAID).

PUD is a global problem with a lifetime risk of development ranging from 5% to 10% thus, it is considered the most common gastrointestinal disorder affecting millions of people (Malik et al., 2022). In an analysis of disease burden conducted by Wong et al. (2018, as cited in Legido-Quigley & Asgari-Jirhandeh, 2018), PUD accounted for 80% of total Daily Adjusted Life Years (DALY) lost in the Philippines. According to the latest WHO data (World Health Rankings, 2020), in 2020 alone, PUD-associated deaths in the Philippines reached 6,865 or 1.02% of total deaths. The age adjusted death rate for PUD in 2020 was 9.95 per 100,000 population making the Philippines rank 12th in the world.

Plants have been used to treat various disease entities (Oteng Mintah et al., 2019). This might be because of their bioavailability, cheaper cost, and the perception that natural remedies are better than synthetic formulations (Kolawole et al., 2016). *Citrullus lanatus*, more commonly known as watermelon and locally as "pakuan," is widely cultivated for its edible fruit, which offers numerous nutritional benefits. The rind, which is the solid mass surrounding the water-logged fruit, is also completely edible. However, it does not appeal to most watermelon consumers and is usually neglected and thrown away. According to Kolawole et al. (2016), this outer covering is rich in alkaloids, saponin, cardiac glycosides, flavonoids, phenol, moisture, lipid, proteins, fiber, and carbohydrates. Different studies have also been conducted for its incredible medicinal value and therapeutic uses.

Flavonoids belong to the group of natural phytochemicals that can be extracted from plant parts. These phytochemicals offer several potential health benefits for humans. They manifest with numerous pharmacological properties in the gastrointestinal tract, including anti-spasmodic, anti-secretory, anti-diarrheal, and anti-ulcer properties. Flavonoids have shown their ability to prevent or reduce gastric lesions induced by different ulcerogenic agents. This can be due to their capability to stimulate the synthesis of mucosubstances in the gastric mucosa, increase gastric blood flow and decrease gastric acid secretion (Mota et al., 2009). Watermelon rind contains a high concentration of flavonoids which have anti-secretory activity (Mota et al., 2009). Therefore, watermelon rind may be utilized as a gastroprotective agent (Kolawole et al., 2016).

Citrullus lanatus rind possesses potential gastroprotective and antiulcerogenic activities due to its high flavonoid content which inhibits gastric acid secretion in the mucosa (Rabiu & Muhammad, 2015). A study revealed that the rind of *Citrullus lanatus* offered gastroprotective action against aspirin-induced gastric acid secretion in rats (Kolawole et al., 2016). In that study, phytochemicals were found to be present within the rind of *Citrullus lanatus* that showed promise in ulcer treatment. However, that study did not extract the phytochemical flavonoids from the watermelon rind. Thus, this group of

researchers would like to extract the flavonoids from the watermelon rind and establish their gastroprotective activity on aspirin-induced gastric acid secretion in male Sprague-Dawley rats.

II. METHODOLOGY

The study utilized a randomized controlled trial to determine the gastroprotective activity of the *Citrullus lanatus* rind flavonoid extract on aspirin-induced gastric secretion in male Sprague-Dawley rats.

The extraction of flavonoids was conducted in the Pharmaceutical Instrumentation Room of Cebu Doctors' University (CDU), Mandaue City, Cebu, a well-ventilated room with proximity to the stockroom for easy accessibility of materials and reagents. The test animals were housed in the Animal Research Laboratory of CDU, which was equipped with all the necessary and appropriate apparatus and instruments used in the experimentation process. The watermelons were harvested from a plantation located in Esperanza, Sultan Kudarat. No artificial fertilizers were used during the development of the fruits that were chosen for the study. The use of chemical fertilizers has led to several issues such as serious soil degradation, nitrogen leaching, soil compaction, reduction in soil organic matter, and loss of soil carbon which may then affect the plant's growth and development (Lin et al., 2019). It is also of great importance to note that the watermelon rinds utilized for this study were not exposed to any synthetic pesticide that can contaminate soil, water, turf, and other vegetation (Aktar et al., 2009).

A total of 21 male Sprague-Dawley rats with a body weight ranging from 150-250 grams and ages between 8 to 10 weeks were utilized for the experiment. The animal subjects were randomly assigned into three groups: one experimental group, one negative control group, and one positive control group. There were seven animal subjects per group.

Fresh watermelons were harvested early in the morning and these underwent garbling to remove dirt. The rinds were peeled from the whole fruit using a spatula and thoroughly washed with water, dried at 50-60°C, and comminuted into a fine powder. Using the Soxhlet apparatus, 75 g of the powder was subjected to 12 hours of extraction with n-hexane, followed by chloroform, then ethyl acetate, and lastly, methanol. A rotary evaporator was used in each extraction at 40-60°C to concentrate the extract and altogether remove the unnecessary chemicals used.

Three test tubes, each containing 3 mL aqueous solution of the flavonoid extract, were prepared for Shinoda's test, sodium hydroxide test, and ferric chloride test to confirm the presence of flavonoids.

For the preparation of aspirin suspension, an aspirin tablet (80 mg) was pulverized and dissolved in a 5 ml normal saline solution (0.9% sodium chloride). This preparation was used to induce gastric acid secretion for 2 rats. The suspension had an approximate concentration of 1.6% w/v. Similar suspensions were prepared for the rest of the rats. For the preparation of the positive control treatment, the study utilized 40 mg of omeprazole powder dissolved in 30 mL of water, making a 1.33% w/v concentration.

All animal subjects were fasted for 24 hours before the experiment, but drinking water was made accessible up to two hours before the experiment. The first group served as the negative control group and each animal subject in this group received 1 mL distilled water. The second group served as the positive control and each animal subject in this group was given the omeprazole suspension at a dose of 4.11 mg/kg body weight. The third group served as the experimental group and each animal subject in this group was given *Citrullus lanatus* extract with a concentration of

1.2831 mg/mL at a dose of 8.22 mg/kg body weight. After an hour, all animal subjects in the three groups were each given the aspirin suspension at a dose of 200 mg/kg body weight to induce gastric acid secretion. All treatments were administered by oral gavage using a gauge 18 feeding tube. After an hour, the test animals were anesthetized with 5% Isoflurane following the open jar method to render them unconscious. The rats were then sacrificed by cervical dislocation.

The stomachs were opened with the use of surgical scissors and a scalpel. The gastric content was collected using a syringe. The gastric juice volume of each rat in the experimental and control groups were measured.

The watermelon residues were wrapped in plastic and disposed of in a green-colored garbage bin, as those are biodegradable wastes. The used liquid chemical reagents were flushed into the drain using flowing water. The Sprague-Dawley rats were buried at a place chosen by the University Veterinarian.

After gathering the necessary data, the mean and standard deviation were calculated. The significant difference in the mean gastric volume among the three groups was determined using the Kruskal-Wallis test. A pairwise comparison test was utilized to determine the significant difference among each of the three groups. The results were presented in tables and IBM SPSS version 22 was used for data processing and analysis.

III. RESULTS AND DISCUSSION

Table 1. Mean Gastric Volumes of Male *Rattus norvegicus* Among the Three Groups

Treatment group	n	Mean (mL)
Positive Control Group (Omeprazole at 4.11 mg/kg)	7	0.20
Experimental Group (Watermelon rind extract at 8.22 mg/kg)	7	0.19
Negative Control Group (Distilled water)	7	0.67

Table 1 presents the mean gastric volumes of the animal subjects from the three groups. It was evident that the mean gastric volumes of the experimental and positive control groups were closer to each other at 0.19 mL and 0.20 mL, respectively, with a difference of only 0.01 mL. In comparison, it was also clear that there was a greater difference between the positive and negative control groups, which was 0.47 mL. The experimental group had a more significant effect in decreasing the gastric secretion of the test animals. This might also support the study of Rabiu and Muhammad (2015), which states that the *Citrullus lanatus* rind possesses high flavonoid contents that inhibit gastric acid secretion in the mucosa.

Table 2. Kruskal-Wallis Test for the Difference in the Gastric Volumes Between Groups

H value	df	*p* value	Decision on Ho	Conclusion
9.702	2	0.008	Reject H0 at 0.05 alpha levels.	There is a significant difference across groups.

The Kruskal-Wallis test is a rank-based nonparametric test used to determine the difference in the gastric volumes of the animal subjects in the experimental and control groups. Table 2 presents the conclusion of the test across all groups. The pairwise comparison test was utilized to present the possible significant differences among the three groups. Table 3 shows the resulting comparison among the groups.

Kruskal-Wallis test revealed that overall, there was a significant difference in the post-treatment median gastric volume (ml) of male Sprague-Dawley rats among the experimental and control groups, $H(2) = 9.702$, $p < 0.05$. The groups' post-treatment mean gastric volumes (ml) were not the same. The *p* value of .008 indicated the rejection of the null hypothesis, thus establishing a significant difference among the groups.

Table 3. Pairwise Comparison Test for the Differences in the Gastric Volumes

Treatment group	Median difference	*p* value	Interpretation
Positive control group vs. Experimental group	0.04	1.000	There is no significant difference between the two groups.
Negative control group vs. Experimental group	0.27	.014	There is a significant difference between the two groups.
Positive control group vs. Negative control group	0.31	.033	There is a significant difference between the two groups.

Table 3 shows the pairwise comparison test between the positive control group and the experimental group, between the negative control group and the experimental group, and between the positive control group and the negative control group. Between the positive control group and the experimental group, no significant difference was noted in median difference in gastric volume; therefore, the watermelon rind extract had an effect comparable to that of omeprazole, preventing gastric acid secretion. Furthermore, it is reflected in the table that the median difference in the gastric volume between the experimental and the positive control groups was only 0.04 mL which is negligible. Thus, the flavonoid extracted from the watermelon rind had a gastroprotective activity.

The gastric volumes of the experimental and negative control group showed a significant difference, with a median difference of 0.27 mL. The watermelon rind extract had a better effect in preventing gastric acid secretion than distilled water. The same was true between the positive and negative control groups. There was a significant difference in their gastric volumes, about 0.31 mL.

According to Rabiu and Mohammad (2015), *Citrullus lanatus* rind has potential gastroprotective and antiulcerogenic properties. An explanation hypothesized is the high flavonoid content of watermelon rind which inhibits gastric acid secretion in the mucosa. Similar results were shown in the study of Kolawole et al. (2016) about the effects of watermelon rind methanolic extract in aspirin-induced gastric ulceration in male Wistar rats. The study showed that the experimental and positive control groups had no significant difference in the gastric volumes collected. In contrast, the gastric volumes of the negative control group

significantly differed from those of the positive and experimental groups.

Shinoda's test yielded a pink-red colored solution; the Sodium hydroxide test yielded a yellow solution; and the Ferric chloride test yielded a greenish-black colored solution. All these results were indications that flavonoids were present in the *Citrullus lanatus* rind extract.

IV. CONCLUSION

Based on the study's findings, the researchers conclude that the flavonoid extract from watermelon rind exhibits gastroprotective activity by reducing the gastric acid volume secreted by the Sprague-Dawley rats. The researchers recommend further studies to determine the specific flavonoid responsible for the gastroprotective effect, do toxicity testing, and determine the mechanism of action for the gastroprotective activity of the watermelon rind flavonoid extract.

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