EVALUATION OF ANTIULCER ACTIVITY OF FLOWER EXTRACT OF *HIPPEASTRUM VITTATUM* IN EXPERIMENTAL RATS

Imran Khan*, Dr. Shobhit Prakash Srivastava[1]

1. Dr. M.C. Saxena College of Pharmacy, Lucknow

Abstract

This study investigates the antiulcer activity of the flower extract of *Hippeastrum vittatum* in experimental rats, with the goal of identifying effective alternative treatments for peptic ulcer disease. The ethanolic extract of *Hippeastrum vittatum* flowers was prepared through cold maceration. Wistar albino rats were divided into four groups: a control group (distilled water), a standard group treated with ranitidine (20 mg/kg), and two experimental groups treated with the *Hippeastrum vittatum* extract at dosages of 200 mg/kg and 400 mg/kg, respectively. The antiulcer effects were evaluated using the pylorus ligation model, assessing parameters such as ulcer index, gastric pH, gastric content volume, free acidity, and total acidity. Microscopic examination of gastric tissues was performed to evaluate mucosal integrity, leukocyte infiltration, and edema. The group treated with 400 mg/kg of *Hippeastrum vittatum* extract showed significant reductions in ulcer index, gastric acidity, and volume of gastric content, with results comparable to the ranitidine-treated group. Microscopic analysis further demonstrated reduced mucosal damage and leukocyte infiltration, especially at the higher dosage, supporting the extract's gastroprotective potential. The ethanolic flower extract of *Hippeastrum vittatum* demonstrates considerable antiulcer activity, with efficacy similar to that of ranitidine at higher doses. These findings suggest that *Hippeastrum vittatum* may serve as a promising herbal alternative for managing peptic ulcers, meriting further exploration into its pharmacological properties and mechanisms of action.

Introduction: Peptic ulcer involves acid-induced injury to the intestine that normally occurs in the stomach or upper part of the duodenum; described as having bare mucosa that extends into the submucosa even. Peptic ulcer disease is estimated to affect five to ten percent of the population. Late prevalence research, on the other hand, has revealed a decrease in the rate, frequency of emergency clinic confirmations, and death associated with peptic ulcer. There is no question that the launching of new medicines and improved sanitation contributed to a drop in Helicobacter pylori-related diseases. Mucosal disruption is thought to be caused by the hypersecretory environment of HCl as well as dietary changes or stress in individuals with corrosive peptic disease. Helicobacter pylori infection & NSAIDs use are the two main risk factors in the progression of gastric as well as duodenal ulcers (1).

Epigastric pain in people with a stomach ulcer often begins 15-30 minutes after a meal, but duodenal ulcer pain typically begins 3 hours after a meal is consumed. Lesions in the lining of the stomach caused by gastric acid or pepsin production are characteristic of peptic ulcers. It gets through to the stomach's middle layer. Most cases manifest in the stomach and upper duodenum. The lower esophagus, terminal duodenum, and jejunum may be impacted. As stated by (2).

A total of 23 patients were investigated for UC, resulting in a 44.3% prevalence rate. This incidence was assessed again after a year and reported as 6.02 per 1 lac. According to these studies, UC is frequent in India. India has the highest disease burden. In Asia, the total incidence of IBD, Ulcerative Colitis & CD was 1.37, 0.76, & 0.54/ 100,000, respectively, compared to 23.67, 7.33, and 14.00 in Australia. It was greatest in mainland China- Guangzhou 3.4/ 1 lac, followed by Hong Kong (3.06/ 1 lac) & then Macau (2.2/ 1 lac) among Asian countries (3). PUD diagnosed by a physician had a 1-year prevalence of 0.12–1.5 percent, while PUD was discovered in hospital-administered patients (0.10–0.19%). Sweden includes cross-sectional data reflecting the overall population (4).

Hippeastrum vittatum, commonly referred to as the Amaryllis lily, is a member of the Amaryllidaceae family, a diverse family of flowering plants often appreciated for their ornamental beauty and medicinal value. The taxonomy of *Hippeastrum vittatum* places it within the genus *Hippeastrum*, which comprises about 90 species known for their large, striking flowers. As a treatment for neurological conditions and neurodegenerative diseases, more and more individuals are turning to the plant genus Hippeastrum, which includes several alkaloids (Silva et al. 2008). Its more than 70 species located throughout South America's tropical and subtropical regions (5). Hippeastrum vittatum is the ancestor of the majority of current commercial hybrids, though Hippeastrum plants come in a range of hybrids (6).

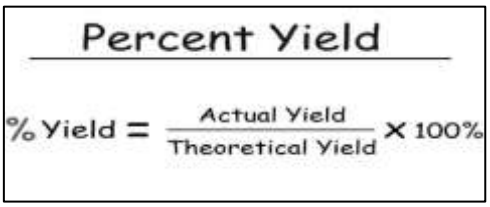
Studies suggest that the antiulcer activity of Hippeastrum vittatum may involve three primary mechanisms: antioxidant action, mucosal protection, and anti-inflammatory effects. The antioxidant properties of its flavonoids help neutralize free radicals and protect the gastric lining from oxidative damage, a common precursor to ulcer formation. The mucosal-protective effects of tannins and other compounds in Hippeastrum vittatum create a protective barrier, reducing acid exposure and enhancing mucosal defenses. Additionally, alkaloids within the plant are thought to lower levels of pro-inflammatory cytokines, which decreases inflammation in the gastric mucosa and promotes healing (7).

Materials: The materials needed are Ranitidine, distilled water, a rotary evaporator, Hippeastrum vittatum flowers, ethanol, Wistar albino rats (either sex), a water bath, and a weighing machine.

Methodology:

Collection, Identification, and Authentication of Plant. The Lucknow region provided the *Hippeastrum vittatum* blooms. A botanist will identify these and verify their authenticity. After

being cleaned to remove dust, the flowers are allowed to dry at room temperature or in the shade. After being dried, the flowers were ground into coarse and then fine powders. After being weighed, the powder is steeped in ethanol for fifteen days while being stirred gradually. A rotary evaporator or water bath is used to partially vacuum-dry the resulting mixed slurry. The following formula was used to determine the extract's % yield of *Hippeastrum vittatum* (8).



Experimental protocols

In the present study, all experimental rats were randomly divided into four groups, each consisting of six animals (n=6). Group 1 served as the normal control and received only distilled water orally each day for a duration of 20 days. Group 2 was administered Ranitidine at a dose of 20 mg/kg body weight orally, once daily for 20 days, serving as the standard treatment group. Group 3 received an ethanolic flower extract of *Hippeastrum vittatum* (EFHV) at a dose of 200 mg/kg body weight orally for 20 consecutive days. Similarly, Group 4 was treated with EFHV at a higher dose of 400 mg/kg body weight orally for the same duration. This experimental design aimed to evaluate the potential dose-dependent anti-ulcer efficacy of the EFHV extract in comparison with the standard anti-ulcer drug, Ranitidine

Methods

Pylorus ligation: All the rats are housed in individual cages and fasted for 24 hours (water *ad libitum*) before performing pylorus ligation. Animals are monitored to avoid coprophagy. Under mild anesthesia driven by ether, a midline abdominal incision is made extending from the xiphoid process (1cm). The pyloric ligature is done with sterilized cotton thread, and care is taken that neither affects the blood supply. The abdominal wound is cleansed thoroughly with normal saline solution, dried, and covered with cotton already soaked in betadine solution. After 19 hours of pyloric ligation, the animals are sacrificed by cervical dislocation. The pyloric segment of the stomach is dissected out by clamping the lower segment of the esophagus. The glandular portion of the stomach is observed for the ulcer index (UI). The length and width of each lesion and the sum of the area of all lesions are expressed as the ulcer area (mm2) (9).

The inhibition percentage was calculated by the following formula-

$$(\%I) = [(UAcontrol - UAtreated) \div UAcontrol] \text{ X } 100$$

The gastric content was poured into tubes, centrifuged, and used for analysis for various biochemical tests.

- pH & volume of gastric juice

- The gastric content is titrated against 0.01N NaOH to determine the 'free & total acidity' (10).

Evaluation of anti-ulcer activity

pH detection: The stomach contents are removed and stored in a contamination-free petri plate. After that, a digital pH meter makes it simple to monitor the pH. It validates the rodent's degree of acidity (10,11).

Gastric Volume Determination: In order to verify the precise amount of gastric fluid, the rat's stomach contents are removed and placed into a measuring cylinder. It validates the drug's impact and the degree of acidity that the rat generated (12-14).

Free & total acidity: The initial step in this process is to remove the stomach contents individually. To ascertain the "free & total acidity," the stomach content is titrated against 0.01N NaOH. It attests to the acidity level and positive effects of the medication.

Microscopical examination: Every rodent group is examined for leucocyte infiltration, submucosal edema, and deep hemorrhagic lesions of the mucosal layer (15-16).

Results and discussion

Percentage yield: The percentage yield of the ethanolic flower extract of the Hippeastrum vittatum was calculated and found to be 62.48%.

Pylorus-ligation-induced ulcer: The effect of ethanolic flower extract of the *Hippeastrum vittatum* on UI and PI was measured across all groups (1-4) in a pylorus-ligation induced ulcer test. Distilled water was used on Group 1, Ranitidine (20mg/kg) was given to Group 2, ethanolic flower extract of the *Hippaestrum vittatum* (200 mg/kg) was given to Group 3, and ethanolic flower extract of the *Hippaestrum vittatum* (400 mg/kg was given to Group 4.

Group 1, which was given distilled water, had a UI of 1.79±0.02 and a PI of 0.00 because there was no change in ulcer ratio. Group 2 received 20mg/kg of ranitidine, resulting in a UI of 0.47±0.04 and a percentage of inhibition of 91.03. Due to its effectiveness in preventing ulcers, it lowered the ulcer index. Ulcer index (UI) and Percentage inhibition were 0.69±0.03and 64.04 for the group administered ethanolic flower extract of the *Hippeastrum vittatum* (200mg/kg). Group 4 ethanolic flower extract of the *Hippeastrum vittatum* 400mg/kg) showed the greatest anti-ulcerogenic effect, with a UI of 0.53±0.06 and a PI of 79.03.

Table 1: Ulcer Index & Percentage Inhibition Role of ethanolic flowers extract of the *Hippeastrum vittatum*

Treatment	UI	PI
Vehicle	1.79±0.02	0.00

Ranitidine (20mg/kg)	0.47±0.04	91.03
EFHV (200mg/kg)	0.69±0.03	64.04
EFHV (400mg/kg)	0.53±0.06	79.03

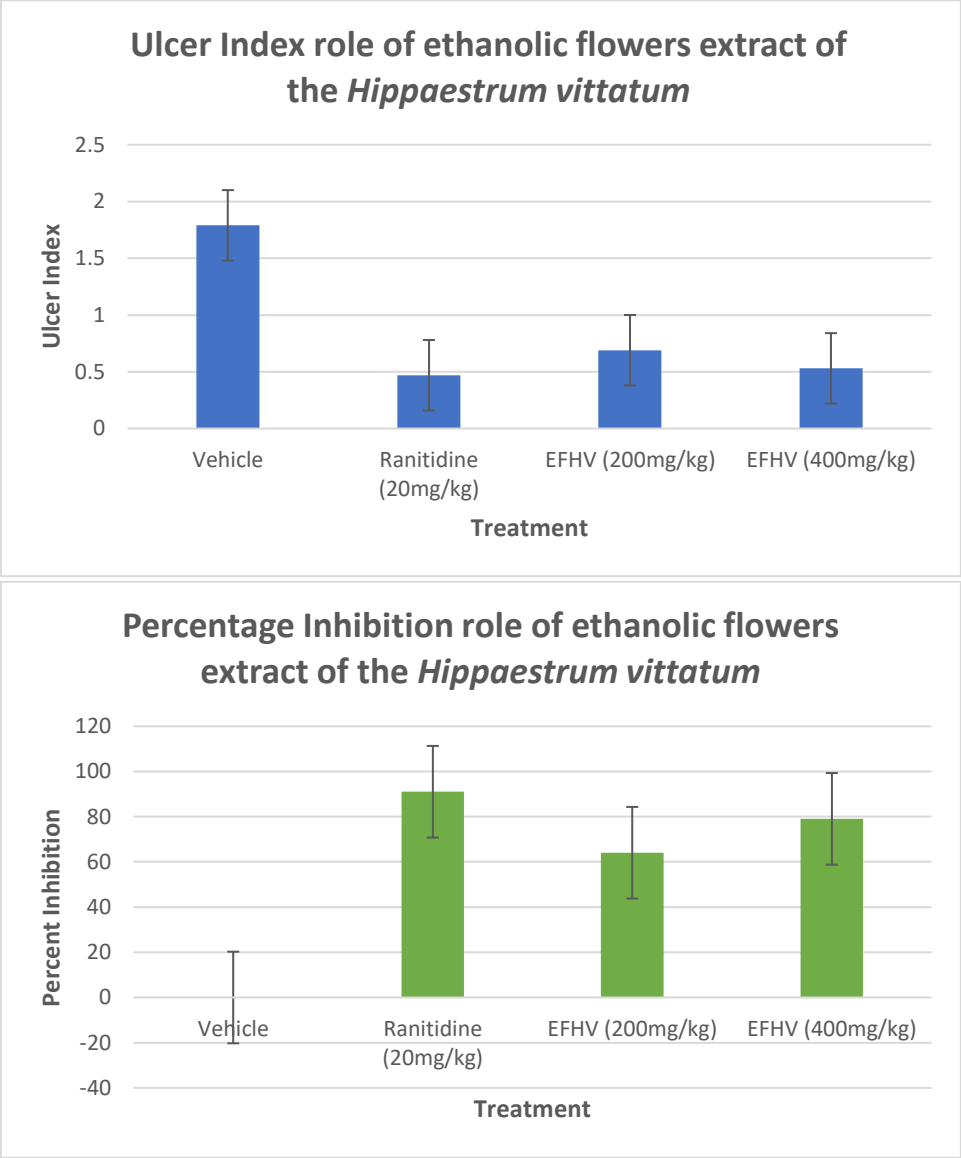


Figure 1 Graphical data of PI of ethanolic flower extract of the *Hippeastrum vittatum*

pH detection: After subjecting all groups (1-4) to a forced swimming-induced ulcer. The pH of the ethanolic flower extract of the *Hippeastrum vittatum* was calculated. The ethanolic flower extract of the *Hippaestrum vittatum* was supplied to Group 3 at 200mg/kg; Group 1 received

vehicle, Group 2 received Ranitidine (20mg/kg), and Group 4 received ethanolic flower extract of the *Hippaestrum vittatum* (400 mg/kg).

After 15 days of once-daily dosage with distilled water, the pH of the water in Group 1 was measured at 4.26±0.23. The optimal and raised pH in group 2 was 5.12±0.17 after receiving 20mg/kg of ranitidine. The pH in Group 3 was 3.58±0.34 after 15 days of continuous exposure to the ethanolic flower extract of the *Hippaestrum vittatum*, while in Group 4 it was 4.76±0.42 after receiving the ethanolic flower extract of the *Hippaestrum vittatum* once daily for 15 days. Thus, a greater dose of the common medicine Ranitidine (20mg/kg) was required to achieve the same pH-raising effects.

Table 3: Estimation of pH range

Treatment	pH range
Vehicle	4.26±0.23
Ranitidine (20mg/kg)	5.12±0.17
EFHV (200mg/kg)	3.58±0.34
EFHV (400mg/kg)	4.76±0.42

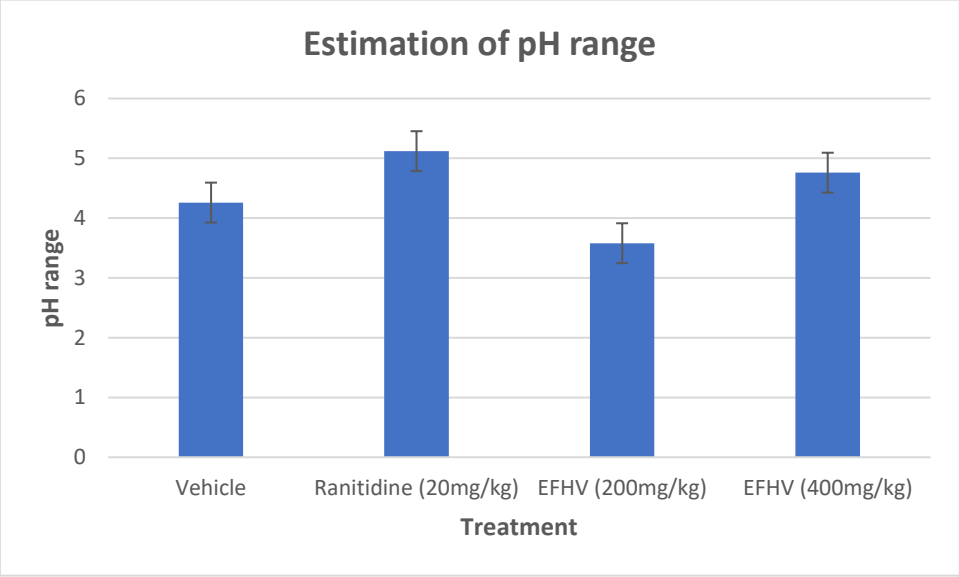


Fig. 3 Graphical data of pH range

Volume of gastric content: After subjecting all groups (1-4) to the pylorus ligation-induced ulcer test, the gastric content of ethanolic flower extract of the *Hippeastrum vittatum* was calculated. The ethanolic flower extract of the *Hippaestrum vittatum* was supplied to Group 3

at 200 mg/kg; Group 1 received distilled water, Group 2 received Ranitidine (20 mg/kg), and Group 4 received ethanolic flower extract of the *Hippaestrum vittatum* (400 mg/kg).

The total volume of stomach contents measured after 15 days of treatment for Group 1 was 11.34±0.18 ml. The total volume of stomach contents for the group given 20 mg/kg of ranitidine was 6.16±0.27 ml. Group 3, which received ethanolic flower extract of the *Hippaestrum vittatum* showed an increase in gastric content (ml) of 8.23±0.38 whereas Group 4 (higher dose), which received ethanolic flower extract of the *Hippaestrum vittatum*, showed a decrease in secreted volume of gastric content (ml) of 7.10±0.39 which is highly significant and equivalent to the standard group.

The following table demonstrates the decreased volume of gastric content (ml)-

Table 4: Effect on the volume of gastric content

Treatment	Volume of gastric content (ml)
Vehicle	11.34±0.18
Ranitidine (20mg/kg)	6.16±0.27
EFHV (200mg/kg)	8.23±0.38
EFHV (400mg/kg)	7.10±0.39

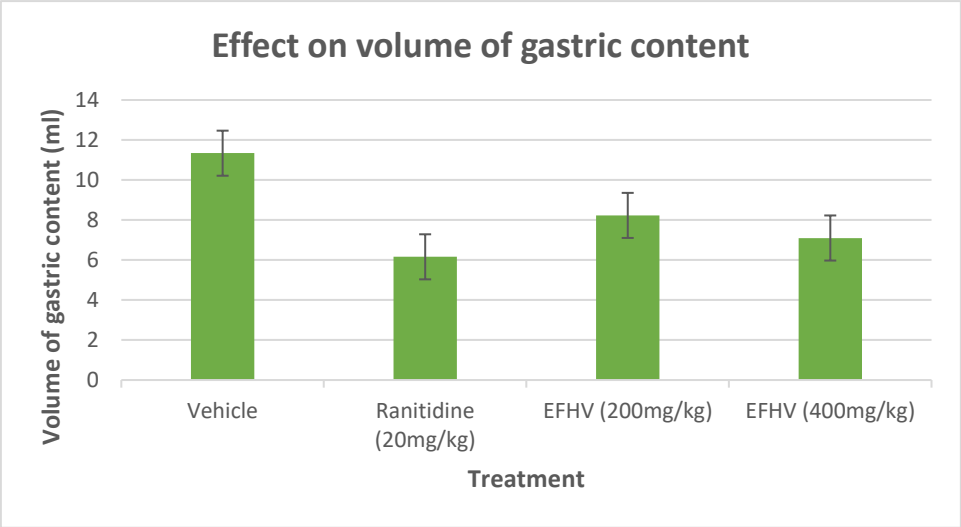


Figure 4 Graphical data of volume of gastric content

Free acidity: In all groups (1-4) of pylorus ligation-induced ulcer test, the free acidity of ethanolic flower extract of the *Hippeastrum vittatum* was calculated. The ethanolic flower extract of the *Hippaestrum vittatum* was supplied to Group 3 at 200 mg/kg; Group 1 received

distilled water, Group 2 received Ranitidine (20mg/kg), and Group 4 received ethanolic flower extract of the *Hippaestrum vittatum* (400mg/kg).

The free acidity value of 36.33±2.45 in Group 1 after receiving 20mg/kg of distilled water proved fully ineffective. All of the animals in Group 2 (ranitidine 20mg/kg) had a free acidity value of 24.56±2.29 on average. The free acidity of animals given ethanolic flower extract of the *Hippeastrum vittatum* (200mg/kg) was 31.40±1.55, whereas the free acidity of animals given the same dose (400mg/kg) was 26.39±1.24, putting it within striking distance of the standard medication, ranitidine.

Table 5 Effect on free acidity (mEq/l)

Treatment	Free acidity (mEq/l)
Vehicle	36.33±2.45
Ranitidine (20mg/kg)	24.56±2.29
EFHV (200mg/kg)	31.40±1.55
EFHV (400mg/kg)	26.39±1.24

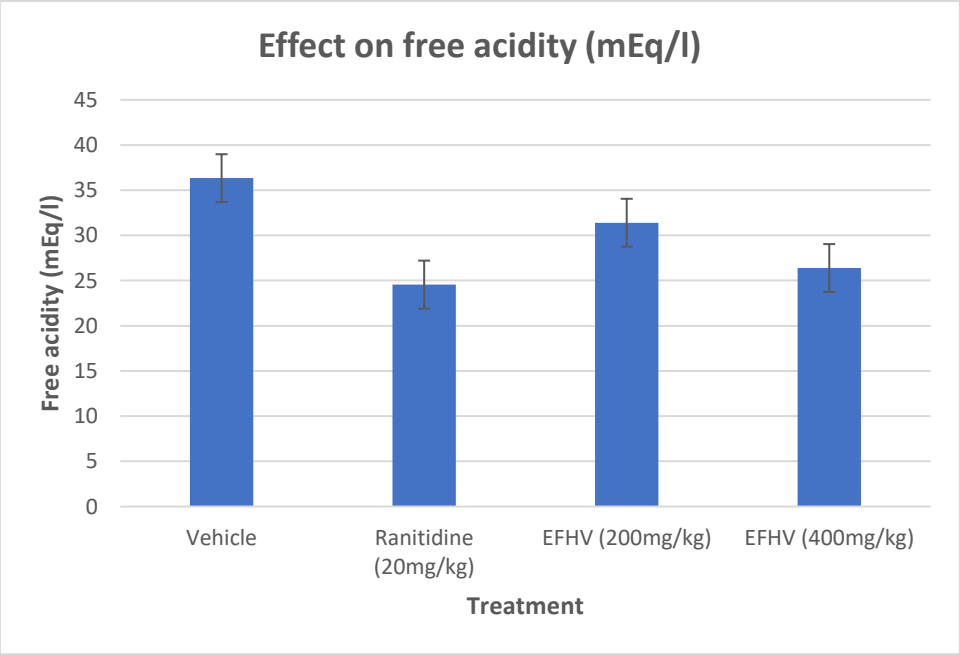


Figure 5 Graphical data of *Hippeastrum vittatum* on free acidity

Total acidity: In all treated groups (1-4) of the pylorus ligation-induced ulcer test, the total acidity of ethanolic flower extract of the *Hippeastrum vittatum* was calculated. The ethanolic flower extract of the *Hippaestrum vittatum* was supplied to Group 3 at 200 mg/kg; Group 1 received distilled water, Group 2 received Ranitidine (20mg/kg), and Group 4 received ethanolic flower extract of the *Hippaestrum vittatum* (400mg/kg).

In group 1, which was given distilled water (20 mg/kg), the total acidity was measured at 73.54±1.56 (mEg/l). Total acidity was 47.20±2.39 in group 2 after 20mg/kg Ranitidine treatment. In contrast, after 20 days of continuous dosing with 200mg/kg of ethanolic flower extract of the *Hippeastrum vittatum*, group 3's total acidity was 63.16±1.42. Finally, total acidity was measured, and it was found to be 54.22±1.28 in group 4 after ethanolic flower extract of the *Hippeastrum vittatum* (400mg/kg) was administered.

Table 6: Effect on total acidity

Treatment	Total acidity (mEq/l)
Vehicle	73.54±1.56
Ranitidine (20mg/kg)	47.20±2.39
EFHV (200mg/kg)	63.16±1.42
EFHV (400mg/kg)	54.22±1.28

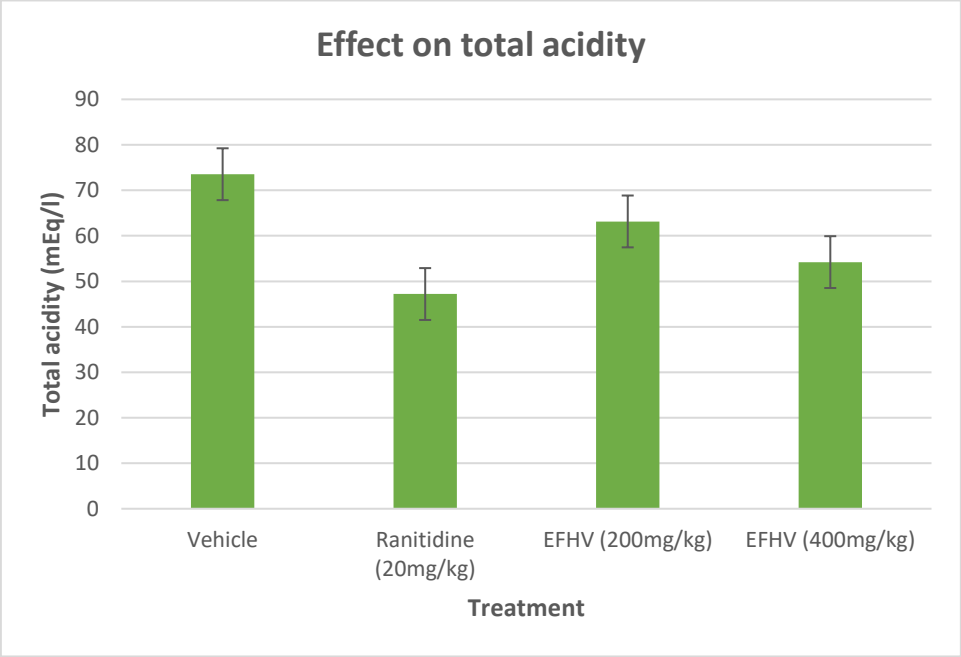


Fig. 6 Graphical data of the effect on total acidity

Microscopic studies: The stomach samples were studied under a compound microscope with a 100x magnification power. Before anything more could be done, the stomach had to be dissected and cleaned with saline solution to remove all traces of fat and gastric content. After that, it was preserved in a saline solution till the high-powered compound microscope investigation was finished. The infiltration and streaking of ulcer perforations by leucocytes are seen in the next figure.

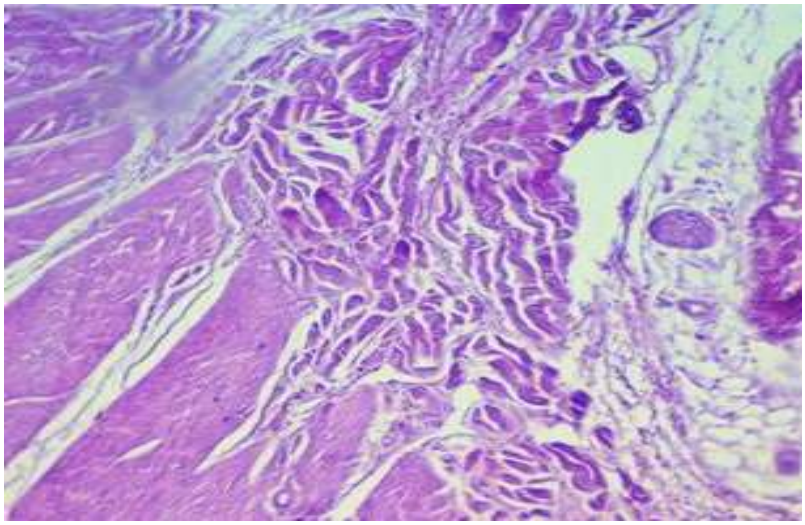


Fig. 6 Leucocyte infiltration treated with EFHV (200mg/kg)

The mucosal edema and streaks are depicted in the accompanying image. The degree of swelling in Group 4 is verified.

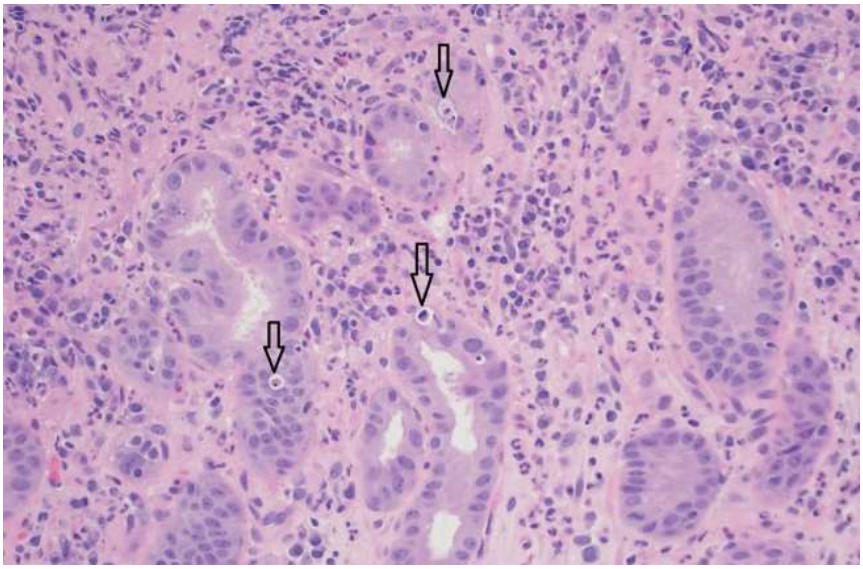


Fig. 7 Mucosal edema treated with EFHV (400mg/kg)

For 20 days, every single rat received treatment on a once-daily schedule. Group 4 received ethanolic flower extract of the *Hippeastrum vittatum* at a dose of 400mg/kg, and results from all microscopical analyses demonstrated negligible mucosal edema and leucocyte infiltration. As a result, the observed reaction was shown to be dosage-dependent. Group 3 received ethanolic flower extract of the *Hippeastrum vittatum* (200mg/kg), which resulted in fewer anti-ulcer effects.The standard group, shown in the next figure, had mild ulceration.

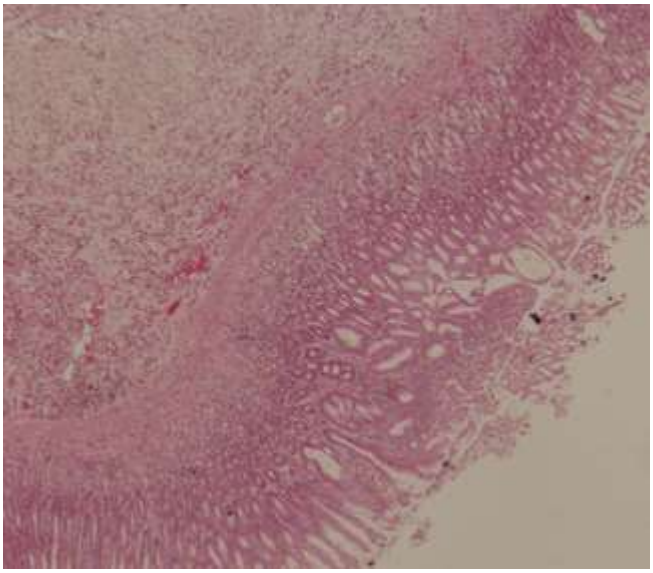


Fig 8 Mucosal edema treated with Ranitidine (20mg/kg)

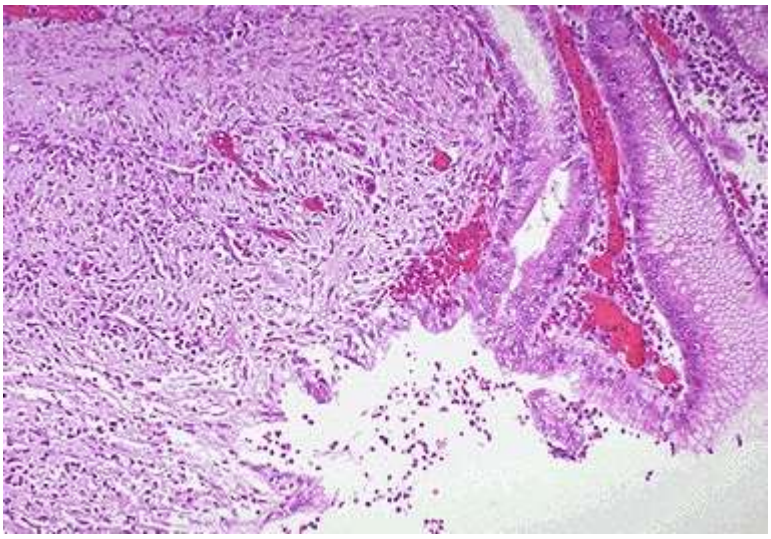


Fig. 9 Mucosal edema of control

The pH level was measured to be below the reference and comparison ranges. Decreases in pH are a well-supported treatment for ulcers, as hyperacidity is the primary determinant in their anti-ulcerogenic potential. Because of its acid-neutralizing properties, it can boost the release of alkaline bicarbonate ions and possibly even more. Group 2 received ranitidine (20mg/kg), while

group 4 received ethanolic flower extract of the *Hippeastrum vittatum* (400mg/kg), resulting in the greatest reduction in pH.

The quantity of stomach contents was found to be lower than in the control group. One possible explanation for group 4's success in reducing gastric content volume is its ability to block Histamine receptors in the stomach's lining. This, in turn, reduces the production of gastrin and stomach acid. The effect of ethanolic flower extract of the *Hippeastrum vittatum* at a dose of 400mg/kg was quite similar to the control group.

After being exposed to the ethanolic flower extract of the *Hippeastrum vittatum* for 20 days straight, it was discovered that the free acidity had decreased. It could work to neutralize free acid, reducing the potentially devastating effects of free acid on the development of stomach ulcers and perforations. Perhaps this is the case because the pharmacological effect is induced and accommodated by the plant extract over time.

After treatment, the total acidity of the animals was also determined to be lower than before. It showed that in groups 2, 3, and 4, overall acidity decreased as well. No clear mechanism for this pharmacological effect has been identified.

The anti-ulcerogenic activity of ethanolic flower extract of the *Hippeastrum vittatum* was much higher than that of the reference medication, ranitidine, across all protocols. There appeared to be a dosage response.

The anti-ulcer potential was seen in all groups when we compared data from pylorus ligation, cold-restraints, and the forced swimming test, although the impact was most pronounced in pylorus ligation when examined under the microscope.

Because it reduces gastrin release and, in consequence, gastric juice (hydrochloric acid) production, ranitidine (20mg/kg) treatment in a standard group of rats exhibited the greatest anti-ulcerogenic potential. Leukocyte infiltration and mucosal injury were found to be minimal. Comparing the results of animals given either ethanolic flower extract of the *Hippeastrum vittatum* or distilled water at a dose of 200mg/kg reveals that both treatments are equally effective. In contrast, animals given a higher dose showed much more anti-ulcerogenic activity than those given the conventional medication.

Conclusion

The health benefits of ethanolic flower extract of the *Hippeastrum vittatum* have been linked to the plant's high concentration of beneficial phytochemicals, essential oils, and biologically active components. Based on its beneficial properties, ethanolic flower extract of the *Hippaestrum vittatum* and its bioactive components can be employed in the manufacturing of pharmaceuticals and functional foods.

The results show that both 200mg/kg and 400mg/kg of ethanolic flower extract of the *Hippeastrum vittatum* have significant anti-ulcer potential. After successful clinical trials, it may be administered to humans. When compared to the control group ethanolic flower extract of the

Hippeastrum vittatum considerably reduced both the total acidity and volume of stomach content. The extract was given through the oral route, and the standard drug ranitidine was given intraperitoneally.

Thus, it might be given in the cure of gastric ulcers and other gastric-related problems that were effective in relation to ranitidine-administered rats. However, at the dose of 400mg/kg, the effect was near to ranitidine-treated rats.

Conflict of Interest: The Author has declared no conflict of interest

Reference:

1. Abdallah E.M. Plants: An alternative source for antimicrobials. J. Appl. Pharm. Sci. 2011;1:16–20.
2. Abdulla Mahmood Ameen, Hapipah Mohd Ali, Khaled Abdul-Aziz Ahmed, Suzita Mohd Noor, Salmah Ismail. Evaluation of the anti-ulcer activities of Morus alba extracts in experimentally-induced gastric ulcer in rats. Biomedical Research, 2009; 20 (1): 35-39.
3. Andoh A, Imaeda H, Komatsu T, et al: Comparison of the fecal microbiota profiles between ulcerative colitis and Crohn's disease using terminal restriction fragment length polymorphism analysis. J Gastroenterol 2011;46:479-486.
4. Asher G.N., Corbett A.H., Hawke R.L. Common herbal dietary supplement-drug interactions. Am. Fam. Physician. 2017;96:101–107
5. Attia Eman Zekry, Marwa Fathy Khalifa John Refaat Fahim Mohamed Salah Kamel. The anti-diabetic potential of mucilage from Hippeastrum vittatum bulbs in streptozotocin-induced diabetic rats. South African Journal of Botany, 2021; 136: 100-104.
6. Aubert J., Bejan-Angoulvant T., Jonville-Bera A.P. Pharmacology of misoprostol pharmacokinetic data, adverse effects and teratogenic effects J. Gynecol. Obstet. Biol. Reprod. (Paris) 2014;43:114–122.
7. Bhajoni et al. Evaluation of the Antiulcer Activity of the Leaves of Azadirachta indica: An Experimental Study. Integrative Medicine International, 2016; 3:10–16.
8. Bhala N., Emberson J., Merhi A., Abramson S., Arber N., Baron J.A., Bombardier C., Cannon C., Farkouh M.E., FitzGerald G.A., et al. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: Meta-analyses of individual participant data from randomized trials. Lancet. 2013;382:769–779.
9. Bhatnagar M and Sisodia SS: Antisecretory and antiulcer activity of Asparagus racemosus against indomethacin plus pyloric ligation-induced gastric ulcer in rats. J Herb Pharmacother 2006; 6(1): 13-20.
10. Bjarnason I., Scarpignato C., Takeuchi K., Rainsford K.D. Determinants of the short-term gastric damage caused by NSAIDs in man. Aliment. Pharmacol. Ther. 2007;26:95–106.
11. Chandra P, Kamal Kishore, Ashok K G. Effect of Ethanol Extract from Morus Alba Leaves Supplementation on Gastric Tissue Glutathione Level in Indomethacin Induced Ulcers in Rats. IJPSR, 2015; 6(12): 5308-14.
12. Charpignon C., Lesgourgues B., Pariente A., Nahon S., Pelaquier A., Gatineau-Sailliant G., Roucayrol A.M., Courillon-Mallet A., Group de l'Observatoire National des Ulcères de l'Association Nationale des HépatoGastroentérologues des Hôpitaux Généraux (ANGH) Peptic ulcer disease: One in five is related to neither Helicobacter pylori nor aspirin/NSAID intake. Aliment. Pharmacol. Ther. 2013;38:946–954.

13. Chatterjee T K, Chakraborty A, Pathak M, Sengupta G C. Effects of plant extract Centella Asiatica (Linn.) on cold restraint stress ulcer in rats. Indian Journal of Experimental Biology, 1992; 30(10): 889-891.
14. Chattopadhyay I, Nandi B and Chatterjee R: Mechanism of the antiulcer effect of neem (Azadirachta indica) leaf extract: effect on H+ -K + -ATPase, oxidative damage and apoptosis. Inflammo Pharma 2004; 12(2): 153-76.
15. Chaves K. M, Soane; M. Feitosa, Chistiane; Da S. Araújo, Lidiane. Alkaloids Pharmacological Activities - Prospects for The Development of Phytopharmaceuticals for Neurodegenerative Diseases. Current Pharmaceutical Biotechnology, 2016; 17(7): 2016, 629-635.
16. Chen P.Y., Wu M.S., Chen C.Y., Bair M.J., Chou C.K., Lin J.T., Liou J.M., Taiwan Gastrointestinal Disease and Helicobacter Consortium Systematic review with meta-analysis: The efficacy of levofloxacin triple therapy as the first- or second-line treatments of Helicobacter pylori infection. Aliment. Pharmacol. Ther. 2016;44:427–437.