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PRECLINICAL EVALUATION OF HYDROALCOHOLIC EXTRACT OF *ENHYDRA FLUCTUANS* LEAVES FOR ITS ANTIULCER ACTIVITY

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ABSTRACT

Numerous plants and herbs are used to treat gastrointestinal disorders in traditional medicine. Peptic ulcer is the major gastrointestinal disorder in clinical practice which occurs due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors consequently, reduction of gastric acid production as well as re-enforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. Phytotherapy is used as an ancient method of using medicinal plants to treat a wide range of disorders. Recently, the usage of herbal medicines (which are derived from various plant sources) and interest in alternative remedies has increased. *Enhydra fluctuans* is one of the valuable plants having different pharmacological activities like anti-diabetics, antimicrobial, and anti-inflammatory. Traditionally this plant is used to treat different physiological problems, ulcer is one of them. To support the traditional use of this plant the antiulcer activity of the hydro-alcoholic extract of fresh leaves of *Enhydra fluctuans* was evaluated by different models (aspirin and stress-induced ulcer model by pyloric ligation method). The 250 mg/kg dose showed prominent antiulcer activity as compared to the control and marketed omeprazole formulation (reduce ulcer formation in aspirin-induced and stress-induced models by *Enhydra fluctuans* were: 58% and 54.15% respectively). The study demonstrated and confirmed the folkloric claim of the benefit of *Enhydra fluctuans* in the treatment of ulcers.

Keywords: *Enhydra fluctuans*, Hydroalcoholic extract, Antiulcer activity, Pyloric ligation method

INTRODUCTION:

One severe gastrointestinal disease is peptic ulcer. At any one time, it impacts 10%–15% of the populace **[1]**. "Disruption of the stomach and/or duodenum mucosal integrity leading to a local defect or excavation due to active inflammation" is the definition of an ulcer **[2]**. An ulcer that appears in the stomach is referred to as a gastric ulcer, while an ulcer that appears in the first segment of the small intestine is known as a duodenal ulcer **[3]**. There are numerous aspects associated with the pathophysiology of stomach ulcers. These include precipitate lesions on the mucosal layer, decreased mucus secretion, increased acid-pepsin secretion, and poor bicarbonate neutralization. Since it might be challenging to differentiate between the individual effects of acid and pepsin secretion in practice, both must be taken into account simultaneously. Medications used to treat peptic ulcers aim to either stimulate the mucosal defenses (mucus, bicarbonate, normal blood flow, prostaglandins, nitric oxide) or counteract aggressive factors (acid plus pepsin, active antioxidants, PAF, leukotrienes, endothelin, bile, or exogenous factors, including NSAIDs) **[4]**. Additionally, many variables are linked to the development of peptic ulcers, such as a stressful lifestyle, alcohol intake, NSAID use, Helicobacter pylori infections (Approximately 70% of affected patients), smoking, a lower socioeconomic position, and family history **[1]**. The drugs used to treat ulcers either neutralize acid secreted from the stomach (antacids) or reduce acid secretion (proton pump inhibitors, H_2 blockers, and prostaglandin analogs) **[5]**. The main goals of peptic ulcer disease treatment are to reduce discomfort, treat the ulcer, and prevent the return of the illness. There is currently no medication that completely satisfies all therapeutic objectives **[4]**.

Because synthetic pharmaceuticals have a lot of side effects, medicinal plants are thought to be a major source of new medications because they are less toxic, more affordable, and have fewer or no adverse effects **[5]**. People have been investigating nature for remedies to their illnesses since the beginning of time. Many biologically active compounds found in plants have been used for countless years to cure a variety of human illnesses. About 80 % of people receive their primary care from traditional medicine, according to WHO estimates. Because of the reason that medicinal plants are the "backbone" of traditional medicine, over 3.3 billion people in less developed nations use them routinely **[6, 7]**. There is a wide variety of chemical substances found in the leaves of *Enhydra fluctuans*, such as alkaloids, carbohydrates, saponins, tannins, gum and mucilage,

protein, and triterpenes. According to research reports, the plant has hepatoprotective, analgesic, CNS depressant, antioxidant, and antidiarrheal properties **[8, 9]**. The purpose of this study was to determine the antiulcer activity of *Enhydra fluctuans* leaves on Albino wister rats.

MATERIALS AND METHOD:

Experimental Animals: Throughout the research study, 150-200g albino wistar rats of both sexes were used. They have been placed in 32 x 24 x 16 centimetre polypropylene cages. The animals were bought from an approved animal breeder. Within the well-maintained, standard hygienic conditions of the Netaji Subhas Chandra Bose Institute of Pharmacy animal house (approval no: 1502/PO/a/11/CPCSEA), the animals were placed in a 12-hour light and dark cycle with a temperature of 22±2°C and 65% relative humidity. There were commercial food pallets and unlimited tap water available. Every experiment was carried out between the hours of 10 a.m. and 6 p.m.

Drugs and chemicals used: Ethanol (Loba Chemie Pvt. Ltd.), hydrochloric acid (Loba Chemie Pvt. Ltd.), diethyl ether (Merck Specialities Pvt. Ltd., Mumbai.), omeprazole (OMEZ 20 mg Dr. REDDY LABORATORIES LTD.), distilled water.

Preparation of extract formulation: The leaves of *Enhydra fluctuans* were collected from the local market of NSCBIP then washed gently with distilled water and dried under the shed. Leaves crushed by hand. 100 gm of crushed leaves were placed in the percolator with 300 ml of 70% hydro-alcoholic mixture for 72 hours. Then the extract was collected, dried, and stored at 4ºC in the refrigerator.

Preliminary phytochemical investigations of extract: [10, 11, 12] Hydro-alcoholic extract of *Enhydra fluctuans* leaves was evaluated for phytochemical investigation and the presence of phytoconstituents like alkaloid, carbohydrate, saponin, tannins, gum and mucilage, protein, and triterpenes etc. were observed.

Antiulcer activity:[13] The Pyloric ligation method and Stress-induced ulcer by water immersion were used to determine the antiulcer potency of the *Enhydra fluctuans* leave hydro-alcoholic extract.

To determine the potency of our test sample in comparison to the control, commercially available standard drug (omeprazole) research was conducted.

Experimental design: For this study, the animals were divided into three groups of six animals in each group.

- Group I: The animals of this group were untreated and known as control.
- Group II: This group received the treatment of omeprazole 20 mg/kg orally.

• Group III: This group was treated with a 250 mg/kg dose of *Enhydra fluctuans* leave extract orally. This group was known as the test group.

Pyloric ligation method: In this method, the rats are given free access to water but are fasted for 24 hours before the experiment. Create a small cut in the middle of the abdomen to reveal the stomach, then use a surgical thread to ligate the pylorus without hampering the blood vessels. After 45 minutes of treatment, each group of animals received treatment as designated. To produce stomach ulcers, all rats received an oral dose of aspirin (200 mg/kg). The animals were sacrificed after 4 hours by inhaling an anesthetic ether. The stomachs were removed, dissected, and cleansed with distilled water to get clear of any blood and gastric contents. Records were kept of the quantity of ulcers and their severity score. The formulas were used to determine the percentage of ulcer inhibition, and ulcer index.

Formation of any lesion in the stomach wall was observed and examined, and the number of ulcers was recorded and scored as:

0 = No ulcer; 1 = Superficial ulcers; 2 = Deep ulcers; 3 = Perforation.

The mean ulcer score for each animal was expressed as ulcer index (UI):

$UI = UN + US + UP \times 10^{-1}$

UN = Mean value of ulcers/animal

US = Average of severity score

UP = Percentage of animals with ulcers

The acidity and ulcer index of the treated animals were compared with controls. The ulcer inhibition (%) was calculated as:

$$\textbf{Inhibition}(\%) = \frac{\textbf{Mean ulcer index of control} - \textbf{Mean ulcer index of test}}{\textbf{Mean ulcer index of control}} \times \mathbf{100}$$

Stress-induced ulcer by water immersion: The animals in this study were submerged in water for four hours to produce ulcers. Before the experiment started, all of the animals were kept in a state of fasting for a whole day and were allowed free access to drinking water. The animals received care according to their groups, and after 30 minutes, they were put in cages and let to swim in a water tank (20–23°C) for four hours. The animals were removed from the water immersion cage and sacrificed after four hours. Every animal's stomach was removed and mounted, and the quantity of ulcer formation was counted for evaluation. The scores for severity were recorded. The pyloric ligation model's calculations were used for calculating the ulcer index and percentage of ulcer inhibition.

Statistical analysis: The experimental results were shown as mean±SEM for each treatment group. The significance of the

activity was assessed using one-way ANOVA, Dunnett's test between the data of control and treated groups. ***p<0.001 was considered statistically.

RESULTS AND DISCUSSION:

Extract preparation: *Enhydra fluctuans* leaves were dried. The yield of plant extract was 4.56% after a 72-hour cold percolation extraction process using 70% ethanol.

Preliminary phytochemical investigation: The hydro-alcoholic extract obtained from the leaves of *Enhydra fluctuans* was subjected to preliminary phytochemical investigation and the following observations were found **(Table 1)**.

Antiulcer activity:

Pyloric ligation method:

In this model, the pylorus has been ligated in the rat model, to accumulate secreted acid in the stomach within a predetermined amount of time. In the pyloric ligation anti-ulcer model, omeprazole, EF doses, and the untreated control group all demonstrated considerable antiulcer activity, reducing ulcer formation by 84% and 66%, respectively **(Table 2, Figure 1, 2)**.

Stress-induced ulcer by water immersion: Stress is a state that can interfere with regular physiological processes, including an increase in stomach acid production that leads to the development of ulcers. In the stress-induced anti-ulcer model, omeprazole, EF doses, and the untreated control group all demonstrated considerable antiulcer activity, reducing ulcer formation by 65% and 54%, respectively **(Table 3, Figure 3, 4)**.

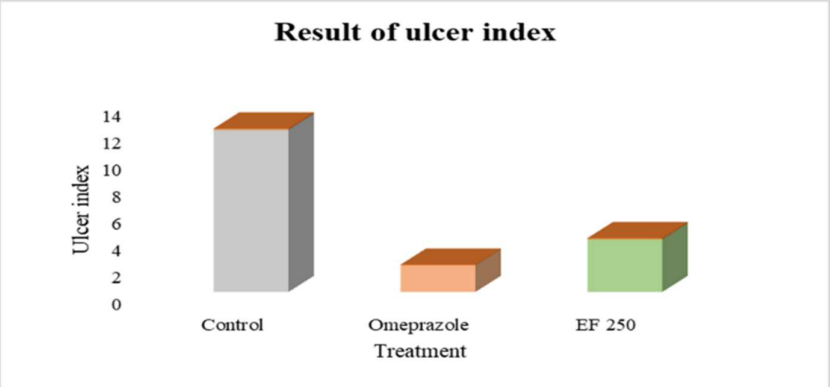
Table 1: Result of preliminary phytochemical investigation

SI. No.	Phyto-constituents	Result
1.	Alkaloid	+
2.	Carbohydrate	+
3.	Glycoside	-
4.	Saponin	+
5.	Tannins	+
6.	Gum and mucilage	+
7.	Protein	+
8.	Triterpenes	+

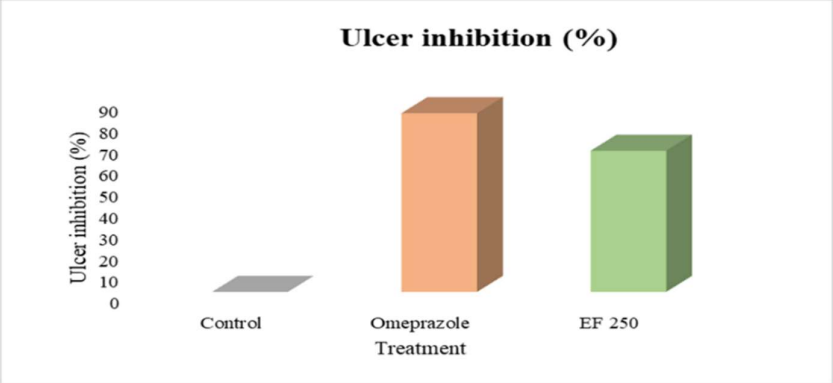
Table 2: Effect of aspirin ulcer model by pyloric ligation method

Treatment	Ulcer index	Ulcer inhibition (%)
Control	12.04±0.096	
Omeprazole	1.98±0.048***	83.55
EF 250	3.91±0.088***	66.01

All the value are mean ± SEM, n=6, ***p<0.001 while compared with untreated control



Results are expressed as mean±SEM, n=6
Figure 1: Impact of buffalo spinach leaves hydro-alcoholic extract on ulcer index

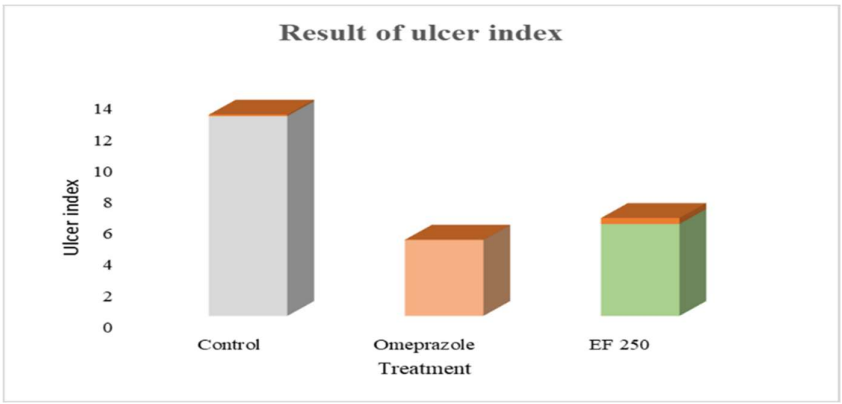


Results are expressed as mean±SEM, n=6
Figure 2: Impact of buffalo spinach leaves hydro-alcoholic extract on ulcer inhibition (%)

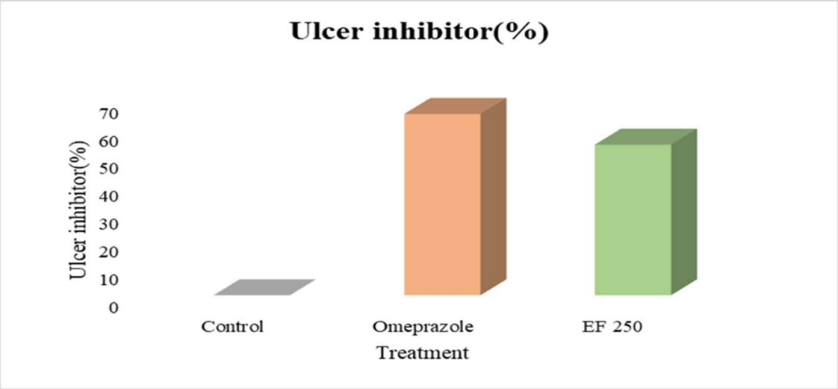
Table 2: Effect of Stress-induced ulcer water immersion model by pyloric ligation method

Treatment	Ulcer index	Ulcer inhibition (%)
Control	12.78±0.090	
Omeprazole	4.86±0.0126***	65.21
EF 250	5.88±0.382***	54.15

All the value are mean ± SEM, n=6,*p<0.001 while compared with untreated control**



Results are expressed as mean±SEM, n=6
Figure 3: Impact of buffalo spinach leaves hydro-alcoholic extract on ulcer index



Results are expressed as mean±SEM, n=6

Figure 4: Impact of buffalo spinach leaves hydro-alcoholic extract on ulcer inhibition (%)

The anti-ulcer activity of *Enhydra fluctuans* leaves expression against aspirin and the stressed-induced ulcer was established in this study. An early study has suggested the individual plant's ability to protect against peptic ulcers. However, an anti-secretory effect of herbal formulation might be indicated as the extract protects the stomach mucosa from NSAIDS and stress-induced damage. The damage is elicited by the inhibition of prostaglandin synthesis, which is essential for mucosal integrity and regeneration. This results in to sustained reduction in mucosal blood flow and a subsequent generation of ulcers. Omeprazole was employed in this study for cytoprotective but not anti-secretory effect and its effectiveness against experimentally induced stress ulcers omeprazole exhibits an anti-secretory and protective effect against ulcers and agents providing ulcer healing against aspirin-induced ulcers may provide a similar effect.

The presence of like alkaloid, carbohydrate, saponin, tannins, gum and mucilage, protein, and triterpenes in the juice has been seen and reported in studies. Ulcer protection may be attributed to these. This study has demonstrated that the *Enhydra fluctuans* leaves have an ulcer healing property against experimentally induced ulcers in rats and this study confirms folkloric claims of the benefits of the plant in the treatment of ulcers.

CONCLUSION:

The current research assessed the hydroalcoholic extract of *Enhydra fluctuans* leaves' antiulcer activities. The findings suggest that *Enhydra fluctuans* leaf hydroalcoholic extract has potential applications as a natural agent antiulcer. Before the extract is suggested for clinical usage, more research is required to clarify its mode of action and safety. In summary, this research emphasizes the need to investigate natural compounds as possible sources of antiulcer medications, particularly in the

context of the increasing need for alternative therapy and drug resistance.

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