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SHORT COMMUNICATION

Evaluation of *Hibiscus sabdariffa* Aerial Parts against Pyloric Ligation-induced Ulcers in Experimental Rats

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Abstract

The sloughing off inflammatory dead tissue is characteristic of ulcers, which are open sores of the skin or mucous membrane. Peptic ulcers are erosive lesions on the stomach or duodenal lining. The drive of the study was to evaluate the effectiveness of Hibiscus sabdariffa aerial parts extracts against pylorus ligation-induced ulcers. The extracts were prepared by maceration using ethanol and double distilled water. In the treatment schedule, Groups A and B were considered negative and standard (Lansoprazole 8 mg/kg b. wt, *p.o.*) controls. Groups C, D, E, and F as low (200 mg/kg b. wt, *p.o.*) and high (400 mg/kg b. wt, *p.o.*) dose treatment groups, received EEHS and AEHS, respectively. Gastric content, pH, free and total acidity, ulcer index, and inhibition of ulcer (%) were assessed. Following administration of both extracts, measurements of stomach content, free and total acidity, and ulcer index decreased. Additionally, pH increased, and a greater percentage of ulcers were inhibited. It was discovered that the ethanolic extract had significantly higher anti-ulcer action than the aqueous extract.

Keywords: Anti-ulcer Activity, Hibiscus sabdariffa, Lansoprazole, Pylorus Ligation, Ulcer Index

1. Introduction

One of the most common diseases in the world, peptic ulcers, affects more than four million individuals annually. The word "peptic ulcer" describes an acid peptic injury to the digestive tract that causes mucosal breakage to the submucosa[1]. Pepsin, acid, and *Helicobacter pylori* are examples of offensive factors, while bicarbonates, prostaglandins, mucin, nitric oxide, and growth factors are examples of defensive factors[2]. Additionally, it has been discovered that peptic ulcer illness has a chronic remitting course and that there is an imperfect association between symptoms and ulcer occurrence[3]. A major contributing factor to primary peptic ulcers is infection with *Helicobacter pylori*. It is linked to 95% of duodenal ulcers and 70% of stomach ulcers[4]. The use of alcohol, cocaine, tobacco, amphetamines, long-term NSAID usage, fasting, Zollinger-Ellison syndrome, and cancer chemotherapy are additional risk factors for developing peptic ulcer disease[5,6].

Several chemically generated medications are used in peptic ulcer treatment with the goals of reducing the proportion of stomach acid secretion, protecting the stomach's mucosa lining and the first portion of the small intestine, or getting rid of *Helicobacter pylori* infection[7]. The current medications have several side effects, whereas native herbal remedies have none and may be a superior option for treating peptic ulcers due to their unwanted effects[8]. Numerous active phytoconstituents found in medicinal plants control a variety of biological processes in the body including ulceration[9,10]. Although the toxicity of herbal pharmaceuticals must be evaluated to identify their safety profile, many studies have found that they are less

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toxic than synthetic drugs[11,12]. In this regard, Natural medicines are a superior option to synthetic medicines for the management and treatment of stomach ulcers[13].

Hibiscus sabdariffa is frequently used to make jams, jellies, and beverages. It is a pricey food item because of its distinctive flavour and beautiful red colour. H. sabdariffa petal is a good colourant and may also be a rich source of antioxidants because of its high anthocyanin content. *H. sabdariffa* was proven to have antioxidant, anti-inflammatory, antibacterial, hepatoprotective, and hypolipidemic properties due to its high levels of anthocyanins and other flavonoids, and polysaccharides[14]. The main objective of the current investigation was to determine the gastric ulcer-preventing properties of aerial portions of *H. sabdariffa* (Malvaceae) in experimental rats.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

The *H. sabdariffa* aerial pieces were collected, washed with water to eliminate any dust, and air-dried under shade. With the use of a hand mill, it was subjected to size reduction to a coarse powder. Dr. K. Madhava Chetty, taxonomist, SV University, Tirupati, Andhra Pradesh, India, verified the plant material's authenticity.

2.2 Preparation of Ethanolic and Aqueous Extracts

The aerial parts of *H. sabdariffa* were first treated with petroleum ether for 18 hours for defatting and removing waxy substances. The marc was used for the preparation of ethanol and aqueous extracts by maceration for seven days with occasional stirring at room temperature. Afterwards, the filtrates were dried in a rotary flash evaporator. Both extracts were stored at 4-8 ºC for screening of anti-ulcer activity.

2.3 Preliminary Phytochemical Screening

The qualitative estimation of various metabolites present in the extracts was carried out as per the protocol specified by Uku *et al*[15].

2.4 Experimental Animals

Male Wistar albino rats of 180-200 g were housed in a maximum of four per cage in a polypropylene cage with standard animal house facility conditions and feed prescribed in CPCSEA guidelines, New Delhi, India. The study protocol was approved by IAEC, CMR College of Pharmacy, Hyderabad, India, with Ethical No: CPCSEA/1657/IAEC/CMRCP/COL-20/82.

2.5 Acute Toxicity Studies

The acute toxicity of aerial parts of *H. sabdariffa* extracts was determined in female Wistar albino mice weighing between 18-22 g. The animals were fasted for 3 hours before performing the acute toxicity study, and the "up and down" (OECD Guideline No. 425) method was followed for the determination of the maximum tolerable dose. $1/10^{th}$ and $1/20^{th}$ of the maximum tolerable dose of individual extracts were considered as treatment doses and used throughout the experimental studies.

2.6 Experimental Design

The rats were randomly divided into six groups of six each. Before the pyloric ligation, animals were fasted for 24 hours with free access to water and measures to avoid coprophagy. Groups A and B served as negative control and standard control receiving Lansaprazole (8 mg/kg b. wt, *p. o.*) respectively. Groups C, D, E, and F as low (200 mg/kg b. wt, *p. o*) and high dose (400 mg/kg b. wt, *p. o*) treatments of EEHS (ethanolic extract of aerial parts of *H. sabdariffa*) and AEHS (aqueous extract of aerial parts of *H. sabdariffa*) respectively. The last treatment was given to the animals one hour before the pylorus ligation.

The abdomen was opened, and the pylorus was ligated and sutured after induction of anaesthesia using ketamine HCL (75 mg/kg b. wt, *i. p*). After 8 hours of ligation, all the animals were sacrificed by the cervical dislocation method, and the stomach was dissected out. Gastric juice was collected into tubes and centrifuged at 1000 rpm for 10 min, and volume was noted. The pH of the gastric juice was recorded by a pH meter. The gastric content was subjected to analysis for free and total acidity[15]. The glandular portion of the stomach was opened along the greater curvature, and the severity of hemorrhagic erosions in the acid-secreting mucosa was assessed on a scale of 0 to 3, i.e., the ulcer index was determined[16]. The stomach samples were stored in 10% formalin for histopathological studies and sections were stained with hematoxylin and eosin.

2.7 Statistical Analysis

The values were presented as Mean ± SEM, and statistical analysis was carried out using one-way ANOVA, followed by post hoc Dunnett's multiple comparison tests with graph pad prism 5.0.

3. Results

3.1 Nature and Percentage Yield of the Extracts

The EEHS and AEHS were semisolid, with both extracts having dark green and dark brown colours respectively. The percentage yields of EEHS and AEHS were found to be 9.50% and 14%, respectively.

3.2 Preliminary Phytochemical Studies

The phytochemical studies of the EEHS found alkaloids, glycosides, sterols, saponins, flavonoids, triterpenes, and tannins. Whereas AEHS was found to be alkaloids, glycosides, saponins, flavonoids, triterpenes, and tannins.

3.3 Acute Oral Toxicity Study

EEHS and AEHS at a dose of 2000 mg/kg b. wt, *p. o.*, was administered to mice, which exhibited normal behaviour, motor activity and secretory signs. So $1/10^{th}$ and $1/5^{th}$ doses of the maximum tolerable dose were selected as low and high doses for screening the anti-ulcer activity, respectively.

3.4 Anti-ulcer Activity

The gastric juice, free and total acidity, ulcer index, and percentage ulcers were abnormally increased, and the pH of the gastric juice was decreased in Group A animals. Group B animals treated with standard lansoprazole showed significantly reduced gastric juice, free and total acidity, ulcer index, and percentage ulcers, whereas the pH of gastric juice was increased. The animals in Groups C and D treated with EEHS showed a significant decrease in gastric juice, free and total acidity, ulcer index, and percentage ulcers and an increased pH of gastric juice as compared with Group A. The Group E and F animals administered with AEHS showed decreased gastric juice, free and total acidity, ulcer index, and percentage ulcers, as well as an increased pH of the gastric juice when compared to Group A. The order of potency in anti-ulcer activity was found to be Lansoprazole >EEHS > AEHS. The results are shown in Table 1.

3.5 Histopathological Changes of Stomach

The histopathological changes are represented in Figures 1 and 2. The negative control group animals exhibit substantial inflammatory cell infiltration in the mucosa and submucosa. Diffuse mucosal ulceration with some of the glands exhibited necrosis and acute inflammatory cells were seen. The groups given a low dose of EEHS and AEHS (200 mg/kg) have intact stomach lining with moderate mononuclear inflammatory infiltration in the epithelial cells, and the submucosa exhibits oedema with uncommon congested vascular spaces. The groups given a high dose of EEHS and AEHS (400mg/kg) exhibit mild mononuclear inflammatory infiltration and relatively few clogged vascular spaces can be visible surrounding the epithelial cells. The gastric mucosa in the standard control group treated with

Table 1. Anti-ulcer effect of EEHS and AEHS in pyloric ligation-induced ulcers

Group	Treatment	Gastric Content (ml)	P^H	Free Acidity (meq/L)	Total Acidity (meq/l)	Mean Ulcer Index	Inhibition of Ulcers (%)
A	Negative Control	7.1± 0.55	1.9± 0.08	31.16±2.12	80.33±1.72	4.83±0.21	0 %
B	Standard Lansaprazole (8 mg/kg)	4.6± 0.29*	6.4± 0.35**	15.16±0.83**	38.5± 2.44**	0.91±0.37	81.15 %
C	EEHS (200 mg/kg)	5.7± 0.17*	4.3± 0.17*	24.5±0.671**	57.33±2.04**	2.16±0.30	55.27 %
D	EEHS (400 mg/kg)	4.5± 0.19**	5.8± 0.19**	18.83±0.68**	41.5± 0.10**	1.41±0.23	70.80 %
E	AEHS (200 mg/kg)	6.0± 0.27	6.2± 0.23	28±0.96	53.5± 1.66**	2.66±0.21	44.92 %
F	AEHS (400 mg/kg)	5.7± 0.24**	5.1± 0.24**	25.66±1.36*	43.83±1.38**	1.91±0.15	60.45 %

Values are the Mean ± SEM, Significance at $p < 0.05$*, 0.01** and 0.001*** compared to the negative control group.

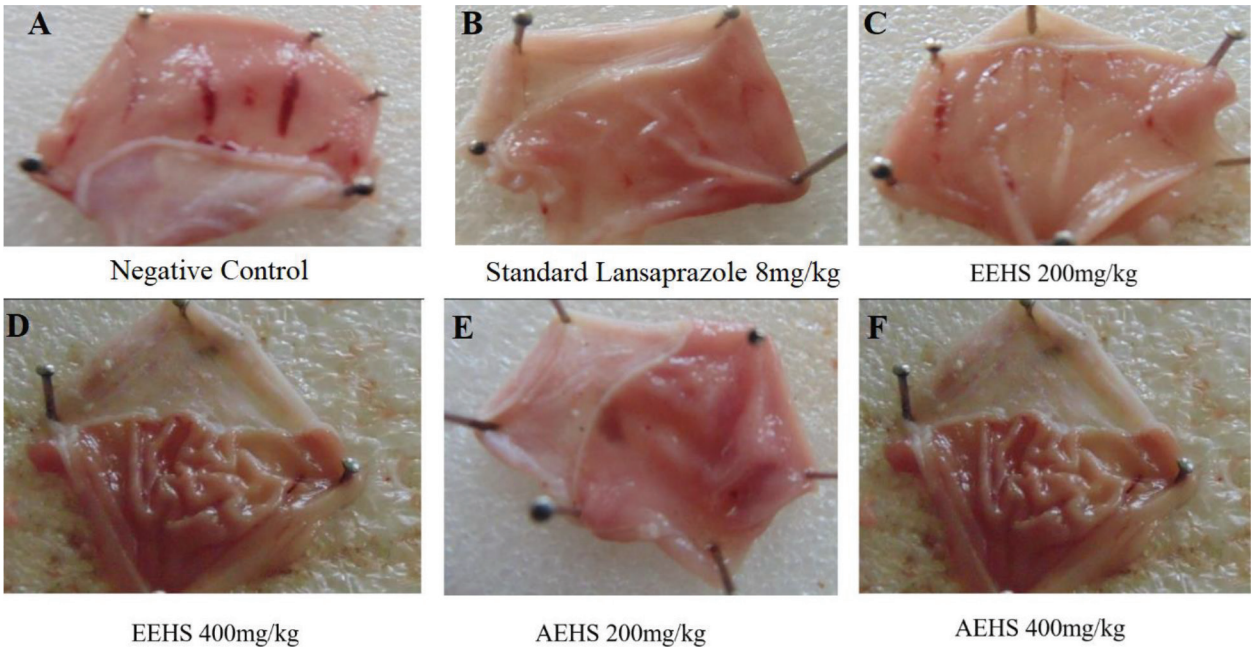

Figure 1. Photographic images of stomachs (Group A – F).

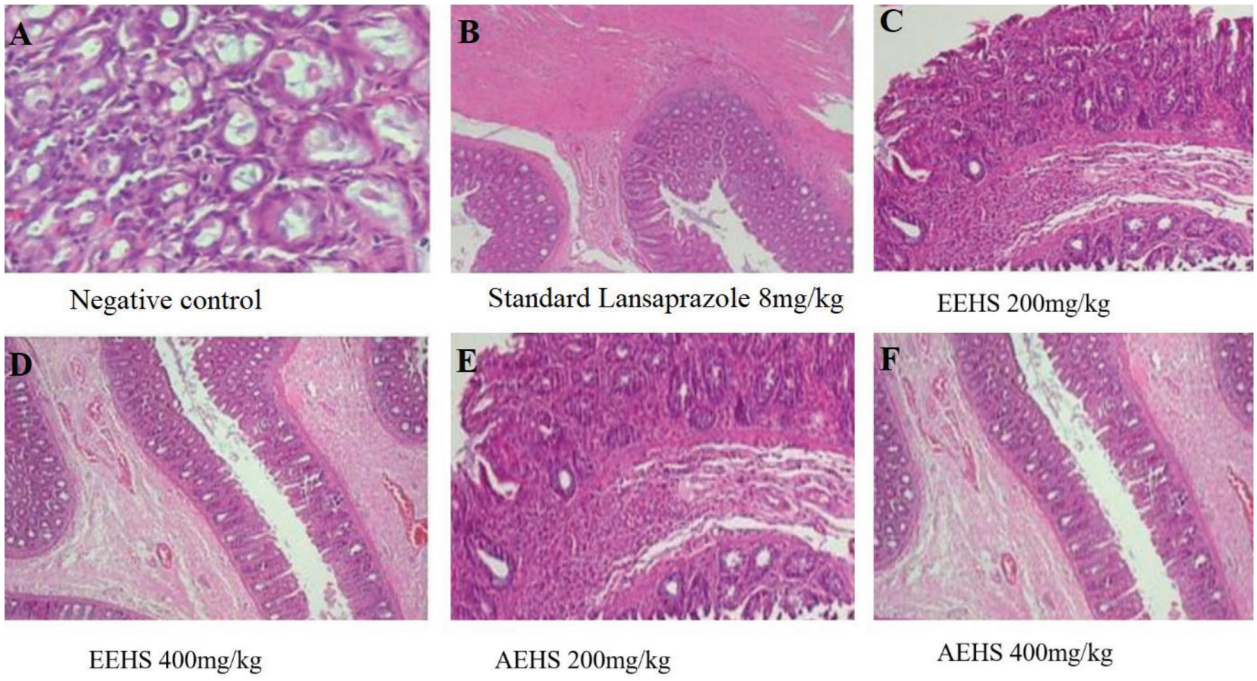

Figure 2. Microscopic images of stomachs (Group A – F).

lansoprazole (8mg/kg) had shown as normal with less mononuclear inflammatory infiltrate and clogged vascular spaces in the epithelial cells. Vascular areas in the submucosal connective tissue stroma are dilated and densely packed. The muscularis propria appears typical.

4. Discussion

Ulceration happens when either more aggressive behaviour or lowered mucosal resistance breaks the natural balance. The stomach mucosa is continuously exposed to elements that can be hazardous, such as

medications, dietetic components, alcohol, microbial byproducts, acid, pepsin, and bile acids. These elements have been connected to the aetiology of gastric ulcers, and they include increased secretion of pepsin and stomach acid, decreased blood flow and motility, suppression of prostaglandin synthesis, and altered cell proliferation[17,18].

Prostaglandin synthesis, stress resistance, the production of antioxidant enzymes, and wound healing characteristics are all enhanced by phenolic compounds and flavonoids[8]. Flavonoids also improve microcirculation and capillary resistance. Tannins directly shield the mucosa's top layer and alter its structure to make it more resistant to chemicals and mechanical harm[19]. Compounds linked to saponins, and triterpenoids promote the production of mucus[20]. As a result, the presence of active secondary metabolites such as polyphenolics, terpenes, saponins, and tannins may be related to their strong anti-ulcer activity[21]. The phytochemical investigation of the current study indicated that ethanolic and aqueous extracts of *H. sabdariffa* were sources of alkaloids, polyphenols, saponins, tannins, and glycosides.

Acute toxicity of the EEHS and AEHS showed no morbidity or mortality at a dose of 2000 mg/kg b. wt. Based on that, $1/10^{th}$ and $1/5^{th}$ of the tolerable dose were chosen for further research. Ligation of the pyloric part is one of the approaches to induce the gastric ulcer. Ligation of the pyloric portion causes a collection of gastric acid and pepsin activation, which leads to the formation of ulcers[22]. Additionally, mucosal digestion affects the production of prostaglandins E2 and I2, which are critical regulators of mucus, bicarbonate, and phospholipid, as well as the suppression of gastric acid secretion and stimulation of mucus secretion in the stomach epithelial cells[23].

Pylorus ligation is a crucial technique that demonstrates potential modifications to the factors governing stomach content, such as the volume of gastric juice, overall acidity, and pH. The increased production of pepsin and gastric acid, which results in the auto-digestive process of the gastric mucosa, is what causes ulcers brought on by pyloric ligation. Since high stomach acidity overwhelms the mucosal defence mechanisms and causes ulcer formation, inhibition of raised gastric acidity is one of the key preventive factors[24].

The present study assessed all the potential parameters that may be used to degree the overall anti-gastric ulcer activity of EEHS and AEHS such as gastric volume, pH, free and total acidity, ulcer index, and percentage of inhibition. A significant increase in the pH of gastric juice and decrease in free and total acidity, and ulcer index after treatment with both extracts were observed. The results were correlated with the results of Ahmed *et al.*,[24] and a significant protective effect was observed with EEHS. Histopathological studies also further support the protective effect of aerial parts of *H. sabdariffa* against pylorus ligation-induced ulcers.

5. Conclusion

The ethanolic and aqueous extracts of *H. sabdariffa* aerial parts have significant anti-ulcer activity, which upholds the traditional claim of the plant. In addition to its safety record, *H. sabdariffa* aerial portions have been proven to be effective in treating gastric ulcers in people. However, further detailed studies are required to determine the molecular mechanism involved in the anti-ulcer activity.

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