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Exploring Diverse Approaches in Screening Antiulcer Drugs: A Comprehensive Review

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ABSTRACT

Peptic ulcers stem from an imbalance between gastro-duodenal mucosal defensive factors, including bicarbonate and mucus, versus aggressive factors like acid and pepsin secretion. The advent of H2 blockers and proton pump inhibitors in drug treatments has notably reduced morbidity and mortality, minimizing the necessity for surgical interventions. Symptoms often manifest several hours post-meal, concurrent with elevated acid production after food has exited the stomach. Instead of pain, some individuals report intense hunger or bloating. Various animal models, such as Pylorus-ligated rats, stress-induced ulcers, histamine-induced gastric ulcers, cysteamine-induced duodenal ulcers, and others, are employed to induce ulcers, aiding in the identification of antiulcer properties in both new and existing drugs. These induced types serve as valuable experimental models for research in this domain. In the realm of research, various animal models serve as invaluable tools for inducing ulcers and assessing the antiulcer properties of both novel and established drugs. These models encompass a spectrum, including Pylorus-ligated rats, stress-induced ulcers, histamine-induced gastric ulcers, cysteamine-induced duodenal ulcers, and others. Each model offers unique insights, contributing to a comprehensive understanding of the pathophysiology of peptic ulcers and the efficacy of therapeutic interventions. These experimental approaches play a pivotal role in advancing our knowledge and refining treatment strategies for this gastroenterological condition.

INTRODUCTION

Gastrointestinal disorders stand as a formidable category of human afflictions, precipitating substantial discomfort, morbidity, and impaired mobility [1]. Among these disorders, peptic ulcer, a lesion affecting the gastric or duodenal mucosa, manifests as a significant gastrointestinal ailment [1]. The condition arises at sites where the mucosal epithelium is exposed to acid and pepsin, leading to a benign ulceration. Various factors contribute to the development of peptic ulcers, including stress, alcohol consumption, cigarette smoking, H. pylori infection, and the ingestion of drugs and chemicals. Prolonged consumption of alcohol, chronic cigarette smoking, and the regular use of NSAIDs have been identified as key contributors to peptic ulcer formation [1]. The established role of free radicals in the pathogenesis of peptic ulcers is linked to mucosal damage. Common symptoms encompass severe pain and irritation in the upper abdomen. Without proper intervention, peptic ulcers may progress to perforations in the gastrointestinal tract wall, underscoring the importance of timely and effective treatment strategies. [1]

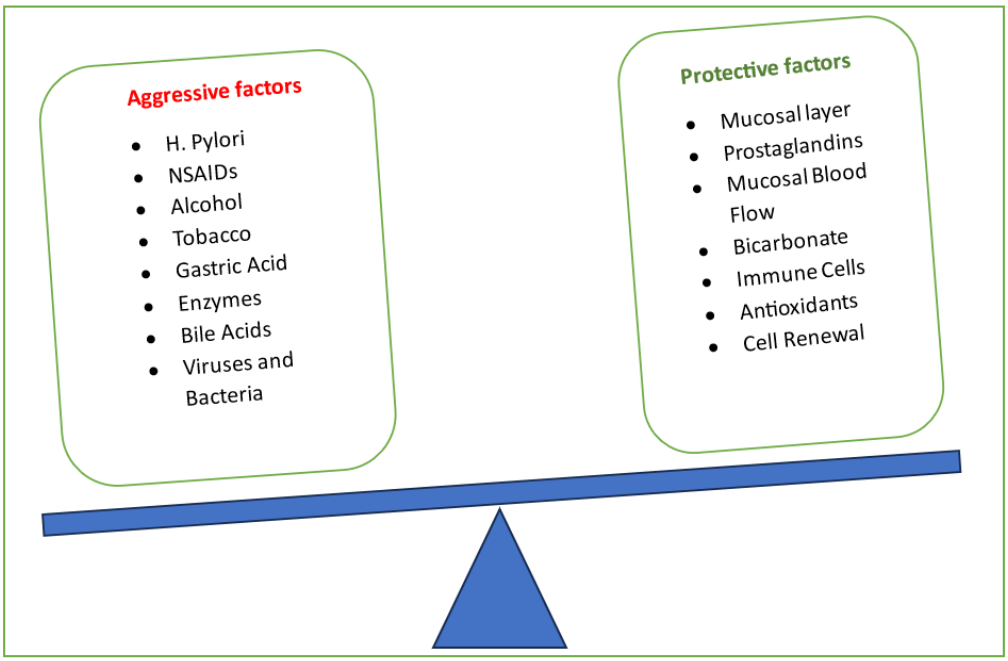

Fig. 1 Imbalance between aggressive and defensive factors leading to peptic ulcer

Peptic Ulcer Overview:

Peptic ulcer represents a persistent inflammatory condition encompassing a cluster of disorders marked by ulceration within the upper gastrointestinal tract regions, where parietal cells secrete pepsin and hydrochloric acid. [1]

Clinical Manifestations:

In individuals with peptic ulcer diseases, patients may either exhibit no symptoms or present with manifestations such as anorexia, nausea, vomiting, belching, bloating, heartburn, or epigastric pain. [1,2]

Epidemiological Insights:

The lifetime prevalence of peptic ulcer diseases is estimated to be between 5 to 10% in the general population. Within the United States, approximately 3.9 million individuals are affected by peptic ulcer diseases, with 200,000 to 400,000 new cases reported annually. The peak incidence is observed between the ages of 50 to 70 years. [2]

Gastric Acid and Pepsin versus Mucosal Resistance in Peptic Ulceration:

The primary instigator of peptic ulceration is the digestion of the mucosa facilitated by the acid and pepsin components within the gastric juice. However, the precise sequence of events culminating in this pathology remains elusive. Digestion by acid and pepsin alone cannot be the exclusive determinant, given the inherent capability of the normal stomach to resist self-digestion by its secretions. The conceptual framework for ulcer etiology can thus be articulated as “acid plus pepsin versus mucosal resistance”. [1]

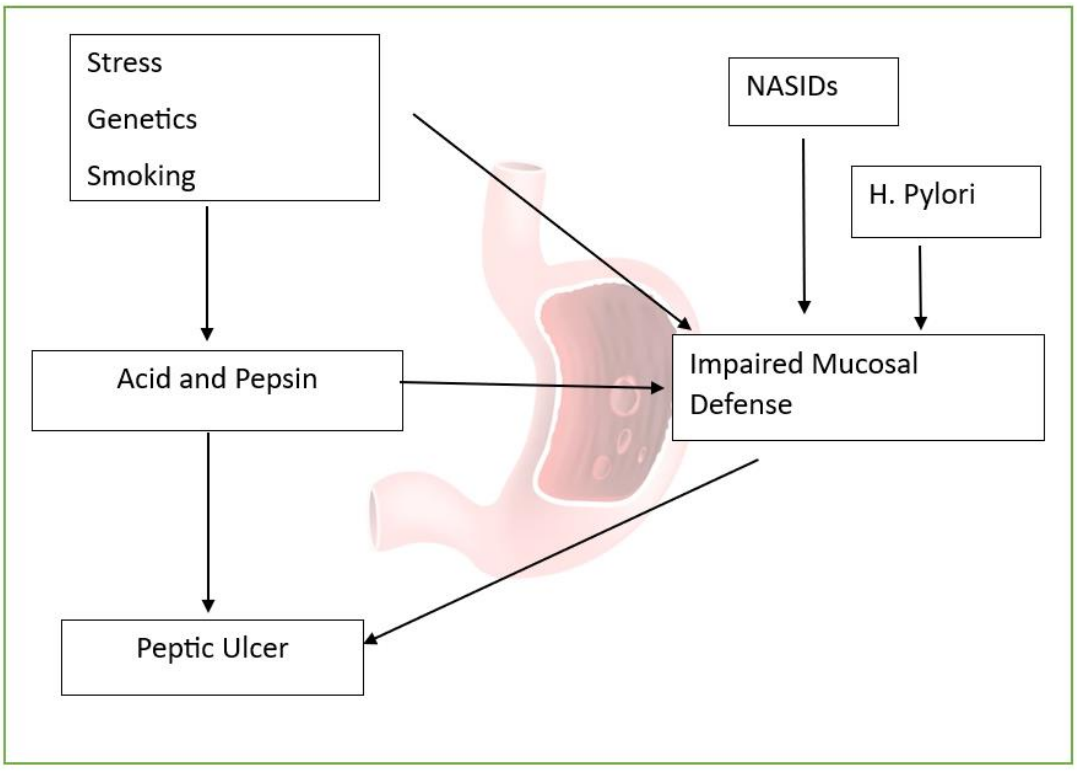

Fig.2 Factors causing peptic ulcers

Using Animal Models to Study Peptic Ulcers [24]:

- Ethanol-induced ulcers
- Cold restraint stress-induced ulcers
- Stress-induced gastric ulceration
- Pylorus ligated (PL)-induced ulcers
- Acetic acid-induced ulcers
- Histamine-induced ulcers
- Indomethacin-induced ulcers
- Serotonin-induced ulcer
- Aspirin-induced ulcers
- Reserpine-induced ulcers

1. **ALCOHOL-INDUCED GASTRIC ULCER:**

Principle:

Alcohol triggers the secretion of gastric juice and reduces mucosal resistance, leading to a substantial increase in the protein content of gastric juice due to ethanol. This may result in the leakage of plasma proteins into the gastric juice, weakening the mucosal resistance barrier of the gastric mucosa and consequently contributing to the development of peptic ulcers. [3,4]

Procedure:

Albino rats of either sex weighing between (150-200 gm.) are divided into six groups of animals in each group. The animals are fasted for 24 hours with free access to water. Animals are given test drugs or standard drugs. 1 hour later 1ml/200gm of 99.80% alcohol is administered p.o to each animal. Animals are sacrificed 1 hour after alcohol administration stomach is isolated and cut open along the greater curvature and pinned on a soft board. The length of each gastric lesion is measured in mm. The % inhibition is expressed as the sum of the length of the control-mean lesion index of text / mean lesion index of control × 10. [3,4]

2. H. pylori-induced gastric ulcer:

Principle:

This gram-negative bacterium is commonly present in the gastric and duodenal mucosa, especially in older individuals. Within the mucosa, it undergoes a process of splitting into ammonia, leading to increased local alkalinity. This mechanism significantly contributes to the development of peptic ulcers. [5]

Procedure:

Albino Wistar rats, irrespective of gender, with weights ranging from 150 to 200 grams, were divided into five groups, each comprising six animals. The rats underwent a 24-hour fasting period in individual cages, with careful attention to prevent coprophagy. The test drug, standard drug, or control vehicle was administered 30 minutes before pyloric ligation. Following light ether anesthesia, a laparotomy was performed, and pylorus ligation ensued. The abdominal cavity was subsequently sutured. Four hours post-ligation, animals were humanely sacrificed using excess anesthetic ether. Gastric juice was collected, and centrifuged, and its volume was noted. The pH of the gastric juice was measured using a pH meter. The contents underwent analysis for free and total acidity. Stomachs were rinsed with running water to assess ulcers in the glandular portion. [5]

The number of ulcers per stomach was recorded, and ulcer severity was microscopically scored using a hand lens (10x), following Kulkarni's criteria (1987):

- 0 = Normal stomach
- 0.5 = Red coloration
- 1 = Spot ulcers
- 1.5 = Haemorrhagic streaks
- 2 = Ulcer > 3 mm but < 5 mm
- 3 = Ulcers > 5 mm

Percentage protection = 100- ut / uc × 100

The mean ulcer score for each group was expressed as the ulcer index, and percentage protection was determined based on the given formula, where it represents the ulcer index of the treated group and uc represents the ulcer index of the control group. [5]

3. Stress-induced gastric ulcers:

Prolonged stress resulting from persistent anxiety, tension, emotional strain, severe physical discomfort, hemorrhage, surgical shock, burns, and trauma can lead to the development of severe gastric ulcers. The mechanism behind gastric ulceration in response to stress is not fully understood. Recent research indicates that resistant cold stress induces severe hemorrhagic ulcers by disrupting mucosal antioxidant enzymes, such as superoxide dismutase and peroxides. This stress condition, primarily arising from physiological discomfort, likely involves a different mechanism of ulceration compared to ulcers caused by other factors. Stress generates highly reactive OH* radicals, causing oxidative damage to the gastric mucosa. These radicals are formed through a metal-catalyzed Herbert Weiss reaction between O2 and H2O2 following the induction of superoxide dismutase, leading to oxidative damage of gastric peroxides. [3,4,5]

4. Restraint ulcers:

The Brodie and Hanson method is employed for generating restraint ulcers. Albino rats, weighing 150–200 g of either sex, are individually housed and organized into groups. The animals undergo a 36-hour food deprivation before the experiment. Each rat is placed within a galvanized steel window screen, molded around the animal, and secured in place with staples. Limbs are conjoined and secured with adhesive tape to restrict movement. Drugs under investigation are administered 30 minutes before subjecting the animal to restraint. After 24 hours, animals are removed from the screen, euthanized using an ether overdose, and their stomachs are opened along the greater curvature. The stomachs are rinsed with tap water, and ulcers are examined and scored using a suitable method. [5,7]

One method involves noting the total areas of stomach mucosa and ulcerated regions to determine the ulcer index. The stomach is removed, opened along the greater curvature, cleaned, and spread on cardboard with the mucous surface facing up. Tracing paper is placed over the stomach, and the outlines of the stomach and erosion areas are traced. This approach is not only useful for studying ulcer healing but has been modified by various researchers to make it less time-consuming, less cumbersome, and more reproducible. While Brodie and coworkers found the restraint technique useful in North America, they highlighted disadvantages, including the lesions not penetrating the muscularis mucosa, not being true ulcers, and the method appearing somewhat species-specific. Several modifications have been introduced to address these concerns, as excellently reviewed by Glavin. [5,6]

I. Water Immersion-Induced Restraint Ulcers:

Exposing rats to restraint stress has been demonstrated to significantly reduce gastric acid secretion. However, there is an observed increase in gastric acid secretion toward restress levels for a few hours when restrained animals undergo additional water immersion. Exposure to water immersion has been found to significantly enhance the development of gastric lesions during stress. The surge in acid secretion may play a crucial role in exacerbating lesions during water immersion. [7,8]

In this method, male Wistar rats, fasting for 24 hours, are immobilized in a stress cage and then immersed up to the level of the xiphoid process in a water bath (23°C) for 16 hours. The animals are euthanized by a blow to the head, each stomach is removed, filled with 1% formalin, and left in it for 10 minutes. The ulcer index can be estimated by measuring the total length of the lesions, and the test drugs are administered 30 minutes before the stress is induced. [8]

II. Hypothermic Restraint Ulcer Model:

In 1977, Vincent et al. introduced a method for restraining animals that eliminated the need for an extensive starvation period beforehand. This approach avoided prolonged restraint, limited nearly all animal movements without causing respiratory or circulatory trauma, and rapidly induced highly reliable gastric glandular restraint ulcers in rodents. Termed the hypothermic restraint ulcer model, it has proven to be a valuable experimental model and research tool. It serves psychologists and pharmacologists in investigating the causes, progression, consequences, and treatment of peptic ulcer disease. [10]

In this method, Wistar rats are fasted for 12 hours, then immobilized in a stress cage and compelled to stay in a cold room (4° - 6°C) for 3 hours. The animals are euthanized by a blow to the head, and the ulcer index is calculated following the procedure described for restraint ulcers. Test drugs are administered 30 minutes before immobilizing the animals. This model is particularly useful for studying gastric erosion resulting from short-term stress and concurrent administration of nonsteroidal anti-inflammatory drugs. [7,10]

Estimation of Parameters:

Estimation of Free Radical Generation:

The funding portion of the stomach undergoes homogenization (5%) in ice-cold 0.9% saline using a Potter-Elvehzem glass homogenizer for 30 seconds. The resulting homogenate is then centrifuged at 800× g for 10 minutes, followed by centrifugation of the supernatant at 12,000× g for 15 minutes. The obtained mitochondrial fraction is utilized for subsequent estimations. Statistical analysis is conducted using the student's t-test. [10,11,12]

Lipid Peroxides (LPO):

The LPO product, malondialdehyde (MDA), is estimated employing 1, 1, 3, 3-tetraethoxypropane as the standard and is expressed as nmol/mg protein[2].

Superoxide Dismutase (SOD) Activity:

The inhibition of the reduction of nitro blue tetrazolium (NBT) to blue-colored Formosan in the presence of phenazine methosulfate (PMS) and NADH is measured at 560 nm, using n-butanol as a blank. One unit of enzyme activity is defined as the amount of enzyme that inhibits the rate of reaction by 50% in one minute under the defined assay conditions. Results are expressed as units (U) of SOD activity/mg protein. [13,14,15]

Estimation of Mucosal Glycoproteins:

Gastric mucosal scraping samples are homogenized in distilled water, treated with 90% ethanol, and subjected to carbohydrate and protein estimation using the methods previously described for gastric juice contents. Statistical analysis is carried out using the student's t-test.

Estimation of Free Acidity and Total Acidity:

A 1 ml sample of gastric juice is carefully pipetted into a 100 ml conical flask. To this, 2 to 3 drops of Topfer's reagent are added, followed by treatment with 0.01 N sodium hydroxide until the disappearance of all traces of red color, resulting in a yellowish-orange solution. The volume of alkali added at this point corresponds to free acidity.

Next, 2 to 3 drops of phenolphthalein solution are introduced, and titration continues until a definite red tinge reappears. The total volume of alkali added during this titration is noted. Acidity is then calculated using the formula:

Total Acidity = Volume of NaOH × Normality of NaOH × 100 / 0.1 × meq/L/100 gm [15,16,17].

Histological studies:

Gastric tissue samples from each group were fixed in 10% formalin for 24 h. The formalin-fixed specimens are embedded in paraffin and section (3-5µm) and stained with hematoxylin and eosin dye. The histochemical sections are evaluated by light microscopy.

Studies based on animal models to evaluate the antiulcer potential of test substance:

- Researchers examined AETP's antiulcer effects in rats using various ulcer-inducing agents. AETP, given orally (1-20 mg/kg) 30 minutes before induction, showed antiulcer activity by comparing ulcer indices with a control group. Gastric acid output and pepsin activity were measured in pylorus-ligated rats, with omeprazole as a reference drug.[03]

- The study aimed to compare the antiulcer potential of aqueous and ethanolic extracts of *Citrullus colocynthis*. A preliminary toxicity study determined doses of 200 mg/kg and 400 mg/kg for further investigation. Using a pylorus ligation ulcer model, oral administration of both extracts at these doses showed significant reductions in gastric volume, total acidity, and free acidity, while increasing gastric juice pH. The extracts also demonstrated a substantial decrease in the number of ulcers and ulcer score index. Ranitidine at 50 mg/kg served as the standard drug. [04]

- The study compared the antiulcer effects of water and ethanol extracts from *Citrullus colocynthis*. After a preliminary toxicity study, doses of 200 mg/kg and 400 mg/kg were chosen for further investigation. In a pylorus ligation ulcer model, both extracts, especially at 400 mg/kg, significantly reduced gastric volume, total acidity, and free acidity, while increasing gastric juice pH. The number of ulcers and ulcer score index also significantly decreased. Ranitidine at 50 mg/kg was used as a standard drug.[05]

- The study looked at how a methanolic extract from *Asparagus racemosus* Willd. affects stomach ulcers induced by a combination of indomethacin (an anti-inflammatory drug) and pyloric ligation in rats. The goal was to understand its antisecretory and antiulcer properties. [06]

- The study aimed to explore the potential of *Centella asiatica* ethanol extract in protecting against ethanol-induced gastric mucosal injury in rats. Rats were pre-treated with different

substances, and those treated with C. asiatica extract showed significant protection from mucosal damage, both visually and histologically. This indicates that C. asiatica leaf extract may have a positive impact on preventing gastric ulcers in rats without causing any toxic effects.[09]

- The study results indicate that the *S. mahagoni* leaf extract effectively hinders the formation of gastric lesions induced by ethanol. The noteworthy rise in mucus production suggests a reinforcement of the gastric mucosa, contributing to the anti-irritant properties of *S. mahagoni*. The preventive impact of the extract is associated with a substantial increase in mucus production, emphasizing the crucial role of gastric wall mucus as a protective factor against gastrointestinal damage.[10]

Conclusion:

Various methodologies, such as the Aspirin-induced ulcer model, Stress-induced ulcer model, Pylorus-ligated-induced ulcer model, Ethanol-induced ulcer model, Acetic acid-induced ulcer model, Cold-resistant stress-induced ulcer model, and Histamine-induced ulcer model, offer diverse avenues for evaluating green pharmaceuticals as potential anti-ulcer agents. Through scientific assessments using these models, both natural and synthetic products can be rigorously evaluated to establish their therapeutic efficacy. The wide array of techniques mentioned provides a comprehensive framework for the systematic evaluation of compounds with potential anti-ulcer properties.

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