Review Article

A Review of Screening Method of Anti-Ulcer Activity

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Received: 09-12-2024; **Revised:** 25-03-2025; **Accepted:** 04-04-2025; **Published online:** 20-04-2025.

ABSTRACT

Ulcers, including peptic, gastric, and duodenal ulcers, are common gastrointestinal disorders that can lead to significant morbidity and, in severe cases, mortality. Early diagnosis and effective management are essential in preventing complications such as perforation, bleeding, and malignancy. Various screening methods have been developed to identify individuals at risk and detect ulcers at an early stage. This abstract aim is to review the current screening methods for ulcers, encompassing non-invasive approaches, endoscopic evaluations, and laboratory tests. Non-invasive techniques, such as the urea breath test, stool antigen tests, and serological tests, are primarily used to detect Helicobacter pylori infection, a major etiological factor in ulcer formation. Endoscopy remains the gold standard for diagnosing ulcers, enabling direct visualization, biopsy, and assessment of severity. Imaging techniques, such as barium swallow and abdominal ultrasound, are used in specific cases, though they have limited sensitivity. Additionally, the role of biomarkers and advances in molecular diagnostics in ulcer detection are discussed.

Keywords: Ulcer, Gastric acid secretion, Helicobacter pylori infection, Non-steroidal anti-inflammatory drugs.

INTRODUCTION

The diverse conditions that make up peptic ulcer illnesses show up as a rupture in the gastrointestinal mucosa's lining that is surrounded by pepsin and acid. Among the gastrointestinal disorders, it is the most common. [1-2]Peptic ulceration is a common disease affecting millions of people. It is now reviewed to be one of the modern-age epidemics affecting nearly 10% of the world population [3]

The pathogenesis of peptic ulcers is widely acknowledged to be an imbalance between the mucosal defensive forces and the stomach aggressive elements[4]. Peptic ulcers can be categorized as gastric, duodenal, or oesophageal depending on where they occur. Acid-pepsin secretion, parietal cells, the mucosal barrier, mucus production, blood flow, cellular regeneration, and endogenous protective agents (prostaglandins and epidermal growth factors) are some of the aggressive and defensive elements that affect the ethology of gastro duodenal ulcers[5]. Peckenpaugh and Pole man say so. Peptic ulcer disorders are caused by several additional variables, including poor eating habits, excessive use of non-steroidal anti-inflammatory drugs, stress, genetic susceptibility, and Helicobacter pylori infection, which is thought to be the cause of over 70% of cases[6].

Physiology of Gastric Acid Secretion

Gastric acid secretion is a complex physiological process primarily regulated by neural, hormonal, and paracrine factors. This process occurs in the stomach and is essential for digestion, particularly for protein breakdown, as well as for activating digestive enzymes like pepsin. Here's an overview of how gastric acid is secreted.

Cellular Mechanism of Acid Secretion

The stomach lining contains specialized cells called **parietal cells**, which are responsible for producing hydrochloric acid (HCl). These cells are present in the gastric glands of the stomach mucosa, particularly in the fundus and stomach body.

The process of acid secretion occurs in the following steps:

Activation of Proton Pumps (H+/K+ ATPase): The parietal cells secrete H+ ions (protons) into the lumen of the stomach through a membrane-bound enzyme, **H+/K+ ATPase (proton pump)**. This pump exchanges intracellular protons for extracellular potassium ions (K+), which forms hydrochloric acid (HCl) in the lumen of the stomach.

Bicarbonate Buffering: As protons are pumped out, bicarbonate ions (HCO3−) are transported into the parietal cell, where they help maintain a balance in pH levels within the cell and the surrounding blood.

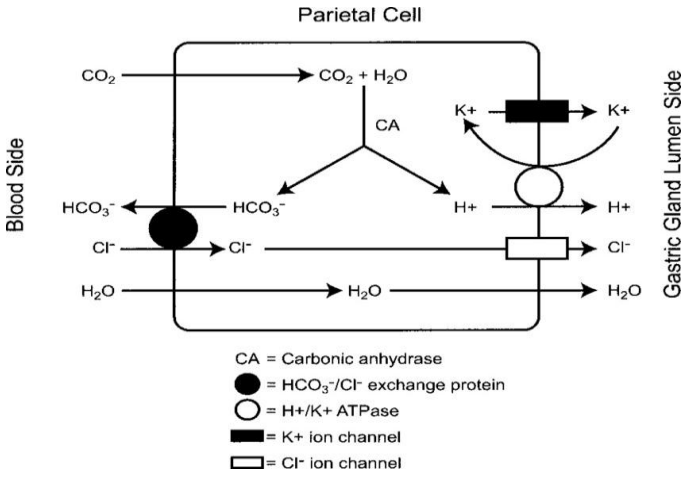

Figure 1: Process of HCl secretion

Regulation of Gastric Acid Secretion

Gastric acid secretion is tightly regulated by several factors:

Neural Regulation

- **Parasympathetic Nervous System:** The vagus nerve plays an important role in stimulating gastric acid secretion. When food enters in the stomach, the vagus nerve releases **acetylcholine (ACh)**, which binds to muscarinic receptors present in the parietal cells, activating the proton pumps. This leads to increased HCl production.
- **Gastrin Releasing Peptide (GRP):** The vagus nerve also stimulates the release of **gastrin-releasing peptide**, which in turn triggers the secretion of **gastrin**, a powerful gastric acid stimulant[7].

Hormonal Regulation

- **Gastrin:** This is the primary hormone required for stimulating acid secretion. Gastrin is produced by **G cells** in antrum of the stomach, and its secretion is stimulated by the presence of food, particularly protein, in the stomach. Gastrin acts on the **cholecystokinin-2 (CCK2) receptors** on parietal cells, enhancing the activity of proton pump. Additionally, gastrin indirectly increases acid secretion by stimulating histamine release from **enterochromaffin-like (ECL) cells**.
- **Histamine:** Histamine is released from ECL cells in response to gastrin. Histamine binds to **H2 receptors** on parietal cells, further stimulating proton pumps and increasing HCl secretion.
- **Somatostatin:** This hormone inhibits acid secretion. released by **D cells which present** in the stomach when the pH of the stomach becomes too low (acidic), acting as a negative feedback mechanism to prevent excessive acid production[8].

Paracrine Regulation

- **Prostaglandins:** Prostaglandins (especially PGE2) act as a modulator in gastric acid secretion. While they reduce acid secretion in the stomach, they also protect the gastric mucosa by stimulating the secretion of mucus and bicarbonate, which serves as a protective barrier against the corrosive effects of gastric acid.

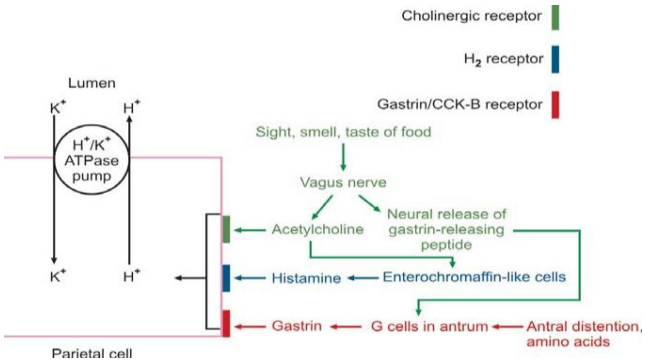

Figure 2: Regulation of Gastric Acid Secretion

Phases of Gastric Acid Secretion

Gastric acid secretion occurs in three distinct phases:

- **Cephalic Phase:** This phase is triggered by the sight, smell, taste, or thought of food. The vagus nerve stimulates parietal cells to begin secreting gastric acid before food even enters the stomach.
- **Gastric Phase:** The stomach wall is stretched when food is enters the stomach, and the presence of proteins and peptides further stimulates acid secretion. Gastrin release is at its highest during this phase, enhancing acid production.
- **Intestinal Phase:** This phase begins when the partially digested food (chyme) enters the duodenum. Initially, gastric acid secretion increases slightly due to the release of intestinal hormones, but as the pH of the chyme becomes too acidic, feedback mechanisms (including somatostatin) inhibit further gastric acid secretion.

Inhibition of gastric acid secretion

- Negative Feedback: When the stomach's pH becomes too acidic (typically a pH below 3), somatostatin is released, which inhibits the secretion of gastrin and, subsequently, gastric acid.
- Antacids and Medications: Pharmacologically, drugs like proton pump inhibitors (PPIs), H2 receptor antagonists, and antacids can reduce gastric acid secretion, often used in conditions such as gastroesophageal reflux disease (GERD), peptic ulcers, and gastritis[9].

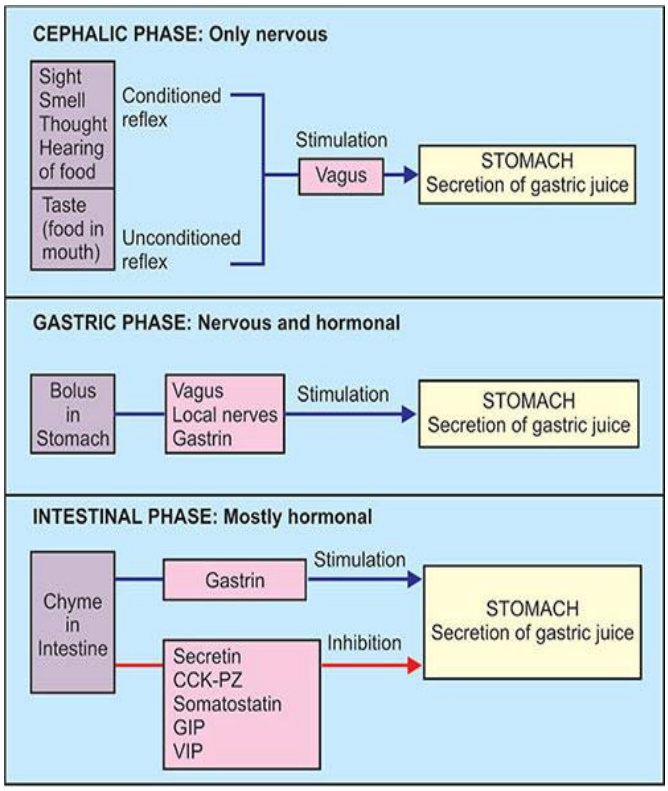

Figure 2: Phases of Gastric Acid Secretion

Conditions for the Ideal Model

- While the observation period is in effect, the ulcers should not heal on their own.
- It should include a variety of mechanisms that cause ulceration.
- Should cause distinctive ulceration in specific areas.
- Allow for easy quantification of results by being simple, reproducible, and AMP [10]

Peptic Ulcer Models

In many animal species, peptic ulcers can be brought on by physiological, pharmacological, or surgical procedures. However, rats are used in the majority of peptic ulcer research investigations. The following are some of the models that are used in experiments to evaluate or assess the anti-peptic ulcer action of medications or agents:

Water-immersion stress or cold-water-restraint or cold-restraint stress model

NSAIDs- (indomethacin, aspirin, and ibuprofen) induced gastric ulcers model

Ethanol-induced gastric ulcers model

Acetic acid-induced gastric ulcers model

Histamine-induced gastric ulcers model

Reserpine-induced gastric ulcers model

Serotonin-induced gastric ulcers model

Pylorus-ligated-induced peptic ulcers model

Diethyldithiocarbamate- (DDC)-induced peptic ulcers model

Methylene blue-induced ulcers model

Ischemia-reperfusion- (I-R-) induced gastric ulcers model

Cysteamine-induced duodenal ulcers model

Indomethacin-histamine-induced duodenal ulcers model

Ferrous iron-ascorbic acid-induced gastric ulcers model

Acetic acid-H. pylori-induced ulcers model

I. Water-immersion stress or cold-water-restraint Ulcer model [11-12.]

In order to produce ulcers using the water immersion-stress-induced ulcer model, animals must fast for 24 to 36 hours before the experiment. In order to induce ulcers, each animal is placed in the restricted cage and submerged vertically in a water tank (15–20°C) gradually until it reaches the level of the xiphoid for 17 hours in the case of the "water-immersed model," in cold water for 2–4 hours in the case of the "cold water immersed model," or in a cold ventilated refrigerator under restraint at a temperature of 2-3°C for 2–4 hours in the case of the "cold restraint stress model."

II. NSAIDs Induced Mucosal Damage. [13-14]

The two most common ulcerogens used to induce peptic ulcers are aspirin and indomethacin. After the chosen animals have fasted for 24 to 36 hours, the ulcerogen is typically given via the proper route in a suitable vehicle. In rats, the oral aspirin dosage is typically between 125 and 150 mg/kg of body weight, and the animals are killed four hours later. When using indomethacin, the dosage ranges from 40 to 100 mg/kg body weight, and the ulcers is scored four to eight hours later. In the ibuprofen-induced ulcer model, animals are typically given a 400 mg/kg of body weight p.o. dose and are killed five hours following the dose. A pilot study is typically advised in order to ascertain the effective dosage required to cause the stomach ulcers.

III. Ethanol-Induced Peptic Ulcer Model [15-16]

In order to use ethanol to cause ulcers, animals are fasted for 24 to 36 hours, after which they are given a dose of 1 mL/200 g of body weight of absolute ethanol (95%–99%), and they are then slaughtered after an hour. It is advised that an initial evaluation be conducted for each study in order to ascertain the optimal dosage needed to induce ulcers.

IV. Acetic Acid-Induced Gastric Ulcers. [17-18]

Rats are fasted for 24 to 36 hours while having unlimited access to water in order to induce ulcers via acetic acid. Before ulcers are induced, the animals are monitored to make sure they are healthy. The animals are first anesthetized with mild ether. In order to deliver diluted acetic acid (4%) (2 mL) into the colon, a flexible plastic catheter with an outer diameter of 2 mm is placed 8 cm into the colon through the anus. To stop the acetic acid solution from leaking, the rats are then kept head down for two minutes. The animals are slaughtered 24 hours after being administered acetic acid. To establish the ideal dose for ulcer induction, it is important to carry out a first dose-finding investigation.

V. Histamine-Induced Gastric Ulcer [19]

The chosen animals are fasted for eighteen hours before the experiment starts in order to use histamine to cause ulcers. Histamine phosphate (40–100 mg/kg body weight) is administered subcutaneously to cause ulcers. The animals are sacrificed two hours later. Usually, pilot trials are required to identify the ideal dosage for ulcer induction.

VI. Reserpine-Induced Peptic Ulcer [20]

Reserpine diluted in 10% Tween 80 (5–8 mg/kg, i.p.) is given to rats who have fasted for 36 hours . Hypermotility appears to be more crucial than hypersecretion for the production of gastric mucosal lesion, despite the fact that the model is acid dependent. Typically, test animals are given medications or plant extracts to be assessed at least half an hour prior to reserpine delivery. Twenty-four hours later, the test animals are killed.

VII. Serotonin-Induced Gastric Ulcer [21-22]

Rats are starved for 24 to 36 hours in this model. Two hours before to the start of the studies, the fasting animals are not given any water. After receiving a single dosage of serotonin creatinine sulphate (0.5 mL of 50 mg/kg subcutaneous injection), glandular lesions are formed. Using an orogastric cannula, intragastric intubation is used to deliver serotonin. Six hours later, the animals are sacrificed via cervical dislocation.

VIII. Pylorus-Ligated-Induced Peptic Ulcer [23]

A 36–72-hour fast is given to the animals before pylorus ligation. In this model, ether anesthesia is used to ligate the pylorus using the "Shay" approach[22]. One hour prior to the pylorus being tied, the medication or test substance is taken orally. After 18 to 20 hours, the animals are slaughtered, and the ulcers are evaluated.

IX. Diethyldithiocarbamate-Induced Gastric Ulcer [24]

One millilitre of diethyldithiocarbamate in saline (800 mg/kg body weight) is administered subcutaneously, followed by a one millilitre Loral dosage of 0.1 N HCl, to cause acute glandular lesions. In this model, water is taken out two hours before the experiment starts, and food is taken out 24 hours beforehand.

X. Methylene Blue-Induced Ulcer [25]

Animals are fasted for 24 hours prior to receiving MB at a dosage of 125 mg/kg body weight p.o., and then the drug or drugs under inquiry are administered in order to cause ulcers. After administering MB for four hours, the animals are slaughtered, and the ulcer index is calculated.

XI. Ischemia-Reperfusion (I-R) Gastric Ulcer Model. [26]

Rats in this paradigm undergo a 24-hour fast before being anesthetized with xylazine (16 mg/kg, i.m.), ketamine (100 mg/kg), and an intramuscular injection (i.m.). Bulldog clips are used to clamp the stomach's pyloric and oesophageal ends during laparotomy. After that, the celiac artery is occluded for 30 minutes at a location 0.5 cm distal to the aorta. After that, the GI receives 20 minutes of reperfusion[35]. After the rats are killed, the ulcer index is determined. Antiulcer medications can be assessed using this model in a preclinical context.

XII. Cysteamine-Induced Duodenal Ulcer [27]

Duodenal ulcers can be classified as either acute or chronic. Rats are experimentally given 400 mg/kg p.o. of cysteamine hydrochloride, which causes acute duodenal ulcers. Cysteine is given twice to treat persistent duodenal ulcers: 400 mg/kg (p.o.) at 4-hour intervals and by incorporating cysteamine-HCl into drinking water for a while. The animals are sacrificed 24 hours after the ulcers are induced, and the duodena is carefully removed, the anti-mesenteric side is cut open, and the ulcer regions are measured.

XIII. Indomethacin, Plus Histamine-Induced Duodenal Ulcer. [28]

I Rats fasting for 24 hours are initially administered indomethacin (5 mg/kg) subcutaneously, then 30 minutes later, histamine dihydrochloride (40 mg/kg also subcutaneously) three times at 2.5-hour intervals. Three hours later, the duodena are carefully removed, the antimesentric side is cut open, and the ulcer area or areas are measured. According to reports, this combination medication has a 100% chance of causing one or two circular lesions in the proximal duodenum, along with a few lesions in the stomach's corpus and antrum.

IVX. Ferrous Iron-Plus Ascorbic Acid-Induced Gastric Ulcer Model [29].

This type of gastric ulcer model is induced by the local injection of ferrous iron with ascorbic acid(Fe/ASA) solution into the gastric wall. The ulcers produced resemble human gastric ulcers that penetrate the muscular is mucosa. Lipid peroxidation mediated by oxygen radicals plays a crucial role in the pathogenesis of the gastric ulceration induced by the Fe/AS A solution.

VX. Acetic Acid-Plus H. Pylori-Induced Ulcer Model[30].

Using a cannula suitable for gastric gavage, animals are intragastrically injected with 1 mL of a verified pathogenic strain of Helicobacter pylori, such as ATCC 43504 (9 × 108), suspended in Mueller Hinton broth or Brain-Heart Infusion Broth 24 hours after acetic acid induces ulcers.

Only Mueller-Hinton or Brain Heart Infusion Broth is given orally to the animals in the control and Acetic Acid-induced ulcer groups that are not infected with Helicobacter pylori. The Helicobacter pylori gastric injection is performed twice daily for seven days, while the test medications, control and standard pharmaceuticals, are given twice daily for fourteen days in a row, beginning on the third day following the acetic acid-induced ulcer.

Following therapy, the animals are dislocated cervically, their stomachs are removed to assess any gastrointestinal lesions, and blood is drawn from the inferior vena cava.

In experimental models, such as animal research, the ulcer index is a quantitative metric used to assess the severity of gastrointestinal ulcers. It offers a means of determining the degree of damage to the stomach mucosa brought on by different elements, such as medications, stress, or infections. In pharmacological and toxicological research, the ulcer index is frequently used to assess how well medications protect the stomach lining.

Parameters for Calculating Ulcer Index

Several parameters are used for calculating ulcer index, with the most common method involving the **scoring of gastric lesions** based on their size and severity. Below is an outline of these parameters [31].

Number of Ulcers (Lesions)

- The **total number of ulcers** (or lesions) in a particular area of the stomach (typically the glandular region of the stomach) is counted. A higher number of ulcers indicates more severe damage.

Ulcer Size (Length or Area)

- The **size of the ulcers** is also measured. It can either be based on the length of the ulcers (measured in millimetres) or the total surface area affected by the ulcers (measured in square millimetres). The area of the ulcers can be calculated by summing the areas of individual ulcers or using a grid overlay technique.

Ulcer Score/Severity

- **Scoring systems** are used to rate the severity of each ulcer, often using a scale (usually from 0 to 3, with increasing numbers indicating more severe damage). For example:
  - **Score 0**: No ulceration
  - **Score 1**: Superficial ulceration (minor damage)
  - **Score 2**: Moderate ulceration (more pronounced damage but not deep)
  - **Score 3**: Severe ulceration (deep, extensive ulceration)

Total Lesion Area

- This is the cumulative area of all ulcers observed in the stomach. It can be expressed as the **total ulcer area (in mm²)**[32].

Ulcer Index Formula

The commonly used formula for calculating ulcer index is:

$$\text{Ulcer Index (UI)} = \frac{\sum (\text{Ulcer Score}) \times (\text{Number of Ulcers})}{\text{Number of Animals}}$$

Modified Methods for Ulcer Index Calculation

Some studies may use modifications or more detailed methods to enhance accuracy. For example:

- **Quantitative Methods**: In addition to visual inspection, imaging techniques such as endoscopy or histopathological evaluation of tissue samples is used to measure ulcer severity more precisely.
- **Ulcer Protection**: Some experiments also calculate the percent protection offered by a test compound by comparing the ulcer index of treated versus untreated animals[33].

CONCLUSION

Ulcer formation is primarily induced by factors such as *H. pylori* infection, NSAID use, stress, Ethanol and lifestyle habits like smoking and alcohol consumption. Understanding these contributing factors is essential for prevention and effective treatment strategies.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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