

NOVEL PRECLINICAL PHARMACOLOGICAL SCREENING MODELS FOR ANTI-ULCER ACTIVITY WITH EMERGING THERAPEUTIC TARGETS

Laxmi*, Sanjita Das, Pranav Gupta, and Abhay Gahirwar

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Abstract

A peptic ulcer is a gastrointestinal disorder that causes discomfort, morbidity, and mortality in humans as a result of unhealthy food, smoking, alcohol intake, frequent use of Nonsteroidal Anti-inflammatory Drugs (NSAIDs), *Helicobacter pylori* (*H. pylori*), and a sedentary lifestyle. Ulceration forms in the stomach, or duodenum, when there is an imbalance between the protective factors of the gastroduodenal mucosa, such as bicarbonate and mucus, and the offensive factors, such as stomach acid and pepsin synthesis. This results in the development of tiny lesions that cause pain. So, it requires effective therapeutic strategies. There are several approaches for assessing the anti-ulcer activity of natural or synthetic drugs. These strategies are known as experimental animal models. The primary objective of this review is to explain the different types of models, such as *in vivo* and *in vitro*, that have been utilized to produce gastric and duodenal ulcers to identify the healing features and properties of various innovative and current medications. This review also discusses the principles, procedures, applications, and parameters of different types of models. In addition, it also delves into the advantages, disadvantages, and challenges related to the use of these screening models.

Keywords: *H. pylori*; Histamine; *in vitro*; Peptic ulcer *in vivo*; Protective; Stomach

Department of Pharmacy, School of Medical and Allied Sciences (SMAS), Galgotias University, Plot No. 2, Sector 17-A, Yamuna Expressway, Greater Noida 201310, Uttar Pradesh, India. E-mail: laxmigaur455@gmail.com*; sanjita8@yahoo.co.in; guptapranav002@gmail.com; abhaythakur7135@gmail.com

* Corresponding author

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Introduction

A peptic ulcer is a serious gastrointestinal disease, affecting 10% of the global population (Abbasi-Kangevari *et al.*, 2022; Salari *et al.*, 2022). It is commonly characterized as a mucosal rupture larger than 3-5 mm in the stomach or proximal duodenum with apparent depth caused by pepsin or gastric acid release. It is less common in the distal duodenum, lower esophagus, and jejunum (Pandey *et al.*, 2019). A gastric ulcer appears in the stomach, predominantly along the lesser curvature, while duodenal ulcers arise in the duodenal bulb, the initial segment of the small intestine. The clinical differential between these two ulcer types is notable: gastric ulcer pain generally worsens within 15-20 min after meals due to enhanced gastric acid secretion, whereas duodenal ulcer pain frequently alleviates with meal consumption but recurs 2-3 h later or nocturnally as gastric contents are depleted (Sung *et al.*, 2009; Malik *et al.*, 2022). The pain may be linked to dyspepsia, bloating, nausea, and/or early satiety (Ramakrishnan and Salinas, 2007; Sarfraz, 2018). Both ulcer types are significantly correlated with *H. pylori* and NSAIDs usage. Still, duodenal ulcers are more commonly associated with increased acid production, while gastric ulcers may additionally be connected to atrophic or, rarely, malignant (Lanas and Chan, 2017).

The main causes of peptic ulcer are *H. pylori* infection and the use of NSAIDs (Kavitt *et al.*, 2019). *H. pylori* contributes to ulcer formation mainly by disrupting the normal regulation of stomach acid production and compromising mucosal defences. The infection causes persistent inflammation in the stomach antrum, resulting in elevated gastrin secretion, which stimulates parietal cells to increase acid production. The excessive release of acid surpasses the protective functions of the duodenal and gastric mucosa, especially when bicarbonate is compromised by the infection (Olbe *et al.*, 2000). Moreover, *H. pylori* synthesizes virulence proteins such as CagA and VacA, which destroy epithelial cells and stimulate inflammation by attracting neutrophils and producing pro-inflammatory cytokines, resulting in mucosal damage and ulceration (Lenka and Bhuyan, 2022). Additionally, *H. pylori* can directly impact the α -subunit of the $H^+/K^+ATPase$, promote either somatostatin-related sensory neurons linked to calcitonin gene-related peptide (CGRP), or prevent the formation of gastrin (Zaki *et al.*, 2013). The bacteria can additionally build biofilms, augmenting their persistence and resistance to host defences (Fauzia *et al.*, 2024).

The utilization of NSAIDs is another contributing factor to peptic ulcers. NSAIDs induce ulcers mainly by disturbing the defensive systems of the stomach. NSAIDs inhibit cyclooxygenase (COX) enzymes, particularly COX-1, which are essential for producing prostaglandins that sustain mucosal blood flow, increase mucus and bicarbonate secretion, and facilitate epithelial cell repair. The decrease in prostaglandin levels compromises these cytoprotective elements, thereby increasing the vulnerability of the stomach lining to acid-induced damage. Furthermore, stomach epithelial cells may be directly damaged by NSAIDs, which would further undermine the integrity of the mucosa and the development of ulcers (Abdullah, 2011; Drini, 2017). In addition, occupational activities, psychological stress, smoking cigarettes, alcohol consumption, regular coffee intake, immunosuppressive drugs, and an age-related drop in prostaglandin levels have all been proven to contribute to peptic ulcer (Yuan *et al.*, 2006; Fazalda *et al.*, 2018). The complication of peptic ulcer disease is highly perilous and common, necessitating prompt intervention, either surgical or pharmacological, to maintain the life of the patient (Altweijry *et al.*, 2020).

The overall occurrence rate of peptic ulcer disease (PUD) in the general population is approximately 5-10%, with a 0.1-0.3% yearly incidence (Akash *et al.*, 2024). The prevalence of duodenal ulcers is four times higher than that of gastric ulcers. Moreover, there is a higher prevalence of duodenal ulcers in males compared to females (Malik *et al.*, 2022). It affects children less frequently than adults. In the United States, another study found that 17.4% of insured children diagnosed with any upper gastrointestinal ulcer experienced peptic ulcer hemorrhage (Ciubotaru and Leferman, 2021). Recent estimates indicate that approximately 8.09 million individuals were impacted by PUD globally in 2019 (Zhang *et al.*, 2023). However, the global prevalence of PUD has decreased due to improved hygienic and sanitary protocols, the use of effective therapeutic alternatives, and the prudent use of NSAIDs (Prayoga *et al.*, 2024). The greatest prevalence and mortality rates are found in countries with low and low-middle socioeconomic development index (SDI), especially in regions of the Pacific (Kiribati and Vanuatu), Southeast Asia, and Sub-Saharan Africa (Abbasi-Kangevari *et al.*, 2022; Xie *et al.*, 2022). In these high-burden regions, multiple variables exacerbate the impact of PUD. Restricted

access to healthcare and diagnostic services frequently results in underdiagnosis and postponed treatment, leading to more severe complications such as bleeding or perforation, which are associated with elevated perioperative mortality rates, particularly in Africa, where emergency surgeries for complicated ulcers can exhibit mortality rates as high as 30% (Peiffer *et al.*, 2020). Socioeconomic inequalities significantly influence healthcare quality, as low SDI countries exhibit inferior care and increased disability-adjusted life years (DALYs) lost to PUD relative to high SDI countries (Abbasi-Kangevari *et al.*, 2022; Zhang *et al.*, 2023). Conversely, developed countries have seen significant declines in PUD rates, primarily and effective management (Wang *et al.*, 2025).

Before commencing treatment on human subjects, it is imperative to evaluate the impact of the pharmaceuticals through a diverse range of methodologies. Multiple screening models are used to assess anti-ulcer drugs. However, choosing an appropriate model has been challenging due to each model's significant benefits and drawbacks. Local resources and study aims can occasionally impact model selection. Given these needs, this review aims to offer a complete summary of various *in vivo* and *in vitro* models for assessing the anti-ulcer characteristics of natural and synthetic drugs. These models could be used to better understand the pathophysiological mechanisms underpinning anti-ulcer drugs, such as antisecretory, gastroprotective, gastro-healing, and antioxidant pathways. This review also talks about their use and measuring parameters for peptic ulcers.

The Requirement of an Ideal Screening Model for Peptic Ulcer

In the pharmaceutical industry, discovering and developing a medicine is always a time-consuming and costly procedure. A probable molecular or cellular therapeutic target is identified and validated, followed by several thorough preclinical and clinical studies, and regulatory approvals mark the conclusion of the process. Using experimental animal models helps us better grasp the etiology, pathophysiology, and overall character of analogous human diseases. Animal models are essential for understanding the pathophysiology of diseases, identifying possible targets for drugs, and assessing the pharmacokinetics, safety, and efficacy of novel therapeutic agents. The purpose of the study determine the exact animal model used to test and

develop a certain medicine (Singh and Seed, 2021). The ideal model should have these properties:

- The model must reliably yield consistent outcomes under the same settings, facilitating reliable comparison of treatments (Singh *et al.*, 2022).
- It must be capable of identifying even minor variations in ulcer development or recovery, rendering it valuable for assessing potential therapeutics (Adinortey *et al.*, 2013a).
- The model must precisely replicate the pathophysiology of human peptic ulcers, encompassing elements such as acid secretion, mucosal damage, and healing mechanisms (Adinortey *et al.*, 2013a).
- It should be straightforward, using common laboratory equipment, not excessively labour-intensive (Ommurugan and Rao, 2019).
- The model must reduce animal suffering and adhere to ethical standards for animal research (Singh *et al.*, 2022).
- The model should not necessitate excessive resources or costly reagents, rendering it suitable for everyday application (Singh *et al.*, 2022).

Practical examples of current peptic ulcer models that fulfil optimal screening requirements include the pylorus ligation model, the ethanol-induced ulcer model, and the acetic acid-induced ulcer model. The pylorus ligation model in rats is extensively utilized due to its consistent induction of ulcer by increased stomach acid secretion, effectively stimulating human ulcer pathology and facilitating precise assessment of ulcer index and gastric secretions (Al-Gabri *et al.*, 2022). The ethanol-induced model is recognized for its simplicity and reproducibility, as ethanol rapidly damages the gastric mucosal barrier, resulting in ulcers similar to those observed in humans (Toktay and Selli, 2022). The acetic acid ulcer model notable recent advancement utilizing filter paper infused with acetic acid, yields ulcers of consistent size and shape, closely replicates the morphology and healing characteristics of original gastric ulcers, and is particularly advantageous for assessing both liquid and solid drug formulations (Toktay and Selli, 2022; Dong *et al.*, 2025). These screening models are cost-effective, replicable, and provide quantitative evaluation of ulcer severity and drug efficacy, rendering them practical options for preclinical research (Satapathy *et al.*, 2024).

The Ideal Animal for Evaluating Anti-Ulcer Drugs

Rodents serve as preferred models for investigating PUD owing to several significant advantages. Their genetic, physiological, and immunological similarities to humans enable them appropriate for mimic human stomach pathology, encompassing the inflammatory and healing responses observed in peptic ulcers. Rodents, including mice, rats, guinea pigs, and Mongolian gerbils, can develop stomach lesions and an inflammatory profile that closely resembles those in humans, facilitating pertinent translational research. They are also exceptionally cost-efficient, rodents are affordable to acquire and sustain, reproduce rapidly, and necessitate less room and resources than larger animals (Zhang and Moss, 2012; Ansari and Yamaoka, 2022). Additionally, the presence of many inbred and genetically modified strains allows researchers to investigate particular genetic factors and pathways involved in ulcer

formation and healing (Zhang and Moss, 2012). Their small size and simplicity of handling enhance experimental procedures and high-throughput drug screening, rendering them essential for both fundamental and preclinical investigations of peptic ulcer (Ansari and Yamaoka, 2022; Satapathy *et al.*, 2024).

Types of Screening Models for Anti-ulcer Activity

Certain animal models are more suitable for testing specific drugs for peptic ulcer because each model replicates a unique mechanism of ulcer development and repair, closely resembling the human situation they are intended to duplicate. Based on the approach, anti-ulcer models are divided into two types: *in vivo* and *in vitro* (Shivam and Chandra, 2020) *In vivo* and *in vitro* models are both crucial for the investigation of peptic ulcers, however, they

Table 1. Various anti-ulcer models and their applications (Adinortey *et al.*, 2013b; Beiranvand, 2022; Toktay and Selli, 2022)

Model	Type	Mechanism of induction	Application
Pylorus ligation (SHAY rat)	<i>In vivo</i>	Surgical ligation of the pylorus causes gastric acid accumulation and mucosal self-digestion	Screening anti-secretory, cytoprotective, and acid-neutralizing agents
Stress-induced ulcer (Water/CWI/Swimming)	<i>In vivo</i>	Physical restraint, water immersion, or cold exposure induces stress, acid secretion, and ischemia	Studying stress ulcer pathogenesis, testing anti-stress/anti-ulcer drugs
Acetic acid-induced chronic ulcer	<i>In vivo</i>	Local application of acetic acid to the gastric mucosa creates chronic ulcers	Evaluating chronic ulcer healing, long-term drug efficacy
Histamine-induced gastric ulcer	<i>In vivo</i>	Administration of histamine increases acid secretion, leading to ulceration	Testing antihistaminic and anti-secretory drugs
Ethanol-induced gastric ulcer	<i>In vivo</i>	Oral ethanol damages the gastric mucosa and disrupts the mucus	Assessing cytoprotective and mucosal defense agents
Reserpine-induced duodenal ulcer	<i>In vivo</i>	Reserpine increases gastric acid and depletes protective amines	Screening drugs for duodenal ulcer prevention
NSAID-induced gastric ulcer	<i>In vivo</i>	NSAIDs inhibit prostaglandin synthesis, reducing mucosal defense	Evaluating the gastroprotective effects of drugs against NSAID injury
Cysteamine-induced duodenal ulcer	<i>In vivo</i>	Cysteamine administration causes duodenal ulceration via cytotoxicity	Used for duodenal ulcer pathogenesis and therapy studies
Serotonin-induced gastric ulcer	<i>In vivo</i>	Serotonin increases acid secretion and motility, leading to ulcers	Testing anti-serotonergic and anti-ulcer agents
Diethyl dithiocarbonate (DDC) induced an ulcer	<i>In vivo</i>	DDC induces oxidative stress and mucosal damage	Studying oxidative stress-related ulcer mechanisms
H ⁺ /K ⁺ ATPase Inhibition Assay	<i>In vitro</i>	Measures the inhibition of the gastric proton pump enzyme	Screening for proton pump inhibitors (PPIs)
Neutralizing effect on artificial stomach acid	<i>In vitro</i>	Assesses the duration and extent of acid neutralization by substances	Testing antacid potency and duration
Fordtran's Model (Titration for neutralization)	<i>In vitro</i>	Titration method to quantify acid neutralizing capacity	Comparative assessment of antacid formulations
3D Gastric Organoids	<i>In vitro</i> (3D)	Stem cell-derived mini-stomachs mimic gastric tissue structure and function	Disease modeling, drug screening, personalized medicine
2D Gastric Epithelial Cell Cultures	<i>In vitro</i> (2D)	Monolayer cultures of gastric epithelial cells	Mechanistic studies, cytotoxicity testing, infection models
Tissue Engineering (Air-Liquid Interface, etc.)	<i>In vitro</i>	Engineered gastric tissue using scaffolds and cell cultures	Advanced drug testing, regenerative medicine, barrier function studies

fundamentally differ in their methodologies and implications. *In vivo* models comprise the induction of an ulcer in living animals (such as mice or rats), enabling researchers to examine the intricate physiological, biochemical, and healing processes within a complete organism, thereby closely resembling human disease (Adinortey *et al.*, 2013a; Khan *et al.*, 2022). These models can assess the efficacy and safety of anti-ulcer drugs within conditions that mimic the human disease state; however, they are constrained by interspecies variations and ethical concerns (Beiranvand, 2022). Conversely, *in vitro* models utilize isolated tissues, cells, or biochemical assays external to living organisms, facilitating comprehensive mechanistic investigations and high-throughput drug screening. It provides simplicity, control, and cost-effectiveness, nevertheless, they are devoid of the systemic interactions found *in vivo* and may not accurately predict clinical consequences. The choice of a particular model is dependent upon the mechanism of drugs and the clinical context it aims to address, ensuring the predictive validity of preclinical data for human treatment outcomes (Khan *et al.*, 2022; Khatoon *et al.*, 2024). These anti-ulcer (*in vivo* and *in vitro*) models are as follows in Table 1.

***In-vivo* Models**

In vivo approaches are commonly used to simulate human disease using animal models. Rodents, such as mice and rats, are popular animal models due to their morphological, physiological, and genetic parallels with humans. *In vivo* models require animals to acquire gastric lesions. Without employing an *in vivo* model, the evaluation of anti-ulcer medications is insufficient because Ulcers are a condition that develops within the body and causes lesions in individuals. Typically, most trials are conducted on gnawers, including mice, rats, and guinea pigs. The key benefit of these models is that they allow us to determine the agent's true anti-ulcer activity; nevertheless, this is a time-consuming approach, which is also a downside (Mishra *et al.*, 2019a; Khan *et al.*, 2022). Anti-ulcer medications are evaluated using *in vivo* models, which are as follows:

Pylorus Ligation in Rat (SHAY Rat)

Principle

Ulcers created by this procedure are also known as Shay ulcers, as Shay originally represented in 1945 (Shay, 1945). Pylorus ligation

is a surgical procedure used to induce stomach ulcers by surgically ligating the stomach pylorus (Sairam *et al.*, 2002). The closure of the stomach's pyloric end results in an accumulation of gastric acid, which causes ulcers (Satapathy *et al.*, 2024).

Procedure

Albino Wistar rats (150-200 g) are segregated into five groups (n=6). After 24-48 h of fasting, rats are anesthetized with sodium thiopental (50 mg/kg, I.P.), and a midline abdominal incision is made. The stomach is opened, and the pylorus is meticulously ligated to prevent tissue injury. The abdomen is sutured. Test substances are administered orally or subcutaneously. After 4 h, rats are euthanized using ether, and gastric ulcers are assessed due to pepsin and acid accumulation. After placing a ligature around the esophagus at the diaphragm, the abdominal cavity is opened, and the stomach is removed, cut at both ends, and rinsed. Stomach contents are collected and centrifuged (10 min, 1,000 rpm). Acidity is measured via titration with 0.1 N NaOH, and pH is determined using a pH meter. Ulcer presence is examined, and gastric volume, acidity, pH, and ulcer index are compared across test, standard, and control groups (Kulkarni, 1987; Monteiro *et al.*, 2013; Andargie *et al.*, 2022). This model is beneficial for evaluating anti-ulcer drugs with different mechanisms and doses. However, its drawback is that ulcers are confined to the stomach's antrum, which may not fully represent broader ulcer conditions or produce desired outcomes. (Mishra *et al.*, 2019a).

Application

This model has been frequently utilized and has proven suitable for evaluating medications for anti-GERD (gastroesophageal reflux disease) action (Satapathy *et al.*, 2024).

Stress-induced Ulcer

Selye (1936) was the first to report on constraint as a stress factor, followed by Brodie and Hanson (1960) and Bonfils and Lambling (1963), who provided procedures for studying the influence of anti-ulcer agents on rats' stress during immobility. Levine created simple water immersion or cold water procedures (Brodie and Hanson, 1960; Levine, 1971).

Stress may originate from continuous anxiety, powerful emotions, significant physical discomfort, hemorrhage, surgical shock, burns, and trauma, all of which can lead to the formation of serious stomach ulcers. Patients in the intensive care unit who are critically ill may develop stress ulcers. This may result in major bleeding in the upper

gastrointestinal system. This bleeding is linked to a considerably greater rate of illness and mortality, even with thorough treatment to reduce stomach acid (Jia *et al.*, 2007). Stress generates extremely reactive hydroxyl radicals, which induce oxidative damage to the gastric mucosa (Bandyopadhyay *et al.*, 1999). One benefit of this model is that the animals react to drugs that act centrally, including imipramine and diazepam, indicating that central neurotransmission plays a part in the formation of gastric ulcers. This approach is time-consuming and requires constant observation of the animals housed in the exercise cages, which are its drawbacks (Mishra *et al.*, 2019a).

There are three stress ulcer approaches that can be used for *in vivo* research:

Water Immersion-Induced Ulcer

Principle

Water immersion induces stomach ulcers by increasing acid production, reducing mucus secretion, and disrupting blood flow due to gastric fold contraction (Kitagawa *et al.*, 1979).

Procedure

Wistar rats (150-200 g, both sexes) were divided into 10 groups and fasted for 24 h. After receiving the test drug or vehicle orally, each rat was immersed in a temperature-controlled water bath (22°C-23°C) up to its xiphoid for 10-16 h. Extract the animal from the water immersion and let it air dry. Administer Evans Blue intravenously into the tail vein at a dosage of 30 mg/kg. After 10 min, they were euthanized, the stomach was collected, injected with 1% formaldehyde, and stored overnight. The stomach is cut the next day along its larger curve, cleaned with warm water, and inspected using a magnifying glass. To ascertain the total lesion score in mm for each animal, measure the longest diameters of the lesions and sum them. The average count of each group is calculated. Lesion inhibition was assessed by comparing it with the control, and statistical analysis was performed (Arige and Rao, 2017; Lu *et al.*, 2019).

Application

This model is particularly effective for testing various classes of anti-ulcer agents, such as Proton pump inhibitors (PPIs), histamine-2 receptor (H₂ receptor) antagonists, cytoprotective agents, antioxidants, and anti-inflammatory drugs (Ahmad *et al.*, 2015).

Cold Water Immersion-Induced Gastric Ulcers

Principle

Immersing the rats in water to cool them during the restraint phase enhances the formation of stomach mucosal lesions and reduces the duration of immobility (Itoh and Noguchi, 2000).

Procedure

Albino Wistar rats (150-200 g, both sexes) were separated into 5 groups (n=6) (Jain *et al.*, 2010), and fasted for 24 h. The rats received a single oral administration of the test, standard, and solvent. Cold resistance stress (CRS) was used to cause stomach ulcers, one hour after treatment, in a closed cylindrical steel cage that was stored in a refrigerator set to 2-4°C. All rats were euthanized after 3 h (Sisay and Jemere, 2020). The stomach was incised along its longitudinal curvature and meticulously cleansed and documented by photography. Ulcer index was calculated by measuring thrombi length, and the ulcer ratio was analyzed, which is the ratio of bleeding stomach ulcers to the overall stomach area. Finally, the removed stomach was kept at -80°C until it was measured for heat-shock protein (HSP 70) concentration (Bhajoni *et al.*, 2016; Itoh and Noguchi, 2000).

Application

The cold water immersion ulcer model is employed to assess the efficacy of certain anti-ulcer medications, such as H₂ receptor antagonists, cytoprotective agents, antioxidants, and anti-inflammatory drugs (Khoshnoud *et al.*, 2022).

Swimming stress-induced gastric ulcer model

Principle

The swimming stress ulcer method causes animals to develop ulcers more severely than other stress methods, and there is a direct link between the severity of ulcers and sadness. This technique entails compelling animals to navigate across deep water by using external pressure (Pare and Redei, 1993).

Procedure

Male Wistar rats were fasted for 24-36 h and placed in a cylindrical chamber with two closed sides. Rats are compelled to engage in swimming

activities within cylindrical structures that are 30 cm in height and 15 cm in diameter. The cylinders are kept between 23-28°C in temperature and are loaded with water to a depth of 15 cm. This arrangement makes sure that the rats' feet stay off the cylinder's surface. At least two swimming sessions were conducted, with the medication administered in between. Stress from swimming increases histamine release and gastric acid, enhances gastrointestinal motility, reduces mucus quantity and quality, and promotes damage, leading to ulcer formation (Mishra *et al.*, 2019a).

Application

The stress ulcer model is useful for evaluating ulcer treatments, including anticholinergics, H₂ antagonists, and PPI medications, and psychotropic drugs such as neuroleptics, which stimulate psychogenic stress-induced ulcers (Mishra *et al.*, 2019a).

Acetic Acid-Induced Chronic Ulcer Model

Principle

The acetic acid ulcer model, developed by Takagi and his coworkers in 1969, reliably induces chronic deep gastric and duodenal ulcers in various rodents, including rats, cats, dogs, guinea pigs, miniature pigs, and Mongolian gerbils. The resulting ulcers closely mimic human chronic ulcers in both appearance and tissue structure (Takagi *et al.*, 1969; Okabe and Amagase, 2005).

Procedure

Albino rats (150 and 200 g) were divided into groups and sedated with phenobarbitone (35 mg/kg intraperitoneally). The abdomen is surgically opened to allow for visualization of the stomach. A solution of 50% acetic acid (0.06 mL/rat) is administered by dropping it into a 6mm diameter tube to induce ulcers. The test and standard drugs are given orally starting four hours after the acetic acid application, and continue for three or seven days. The animals were euthanized 18 h after the final dose to assess ulcer size, ulcer index, ulcer scores, and inhibition percentage (Bhattamisra *et al.*, 2019). The primary benefit of this approach is that it is a simple procedure that consistently generates ulcers of the same size and intensity with a success rate of 100 %. The drawbacks of this model include the formation of ulcers that penetrate the entire gastric wall, and may lead to adhesion to nearby organs like the liver, posing a potential risk (Mishra *et al.*, 2019a).

Application

This strategy is equally applicable to their antisecretory and cytoprotective action (Okabe and Amagase, 2005).

Histamine-Induced Gastric Ulcer Model

Principle

This model was discovered in 1947, using guinea pigs, which showed 100% ulceration with a substantial elevation in stomach volume, free acidity, and total acidity (Ommurugan and Rao, 2019). Histamine is stored in mast cells, attaches to receptors on parietal cells, activating adenylyl cyclase, which stimulates the proton pump (H⁺/K⁺ATPase) to secrete HCl. It also disrupts gastrointestinal motility, reducing mucus formation, mucosal integrity, and microcirculation in the stomach (Tripathi, 2013).

Procedure

Male guinea pigs (300-400g) were fasted for 36 h (water was given) and divided into 3 groups (n=6). Control, standard, and test drugs are given for two days, with histamine (50 mg/kg, intraperitoneal) being given 30 min before each dose, to cause gastric ulcers. Promethazine hydrochloride (5 mg/kg, intraperitoneal) was administered 15 min following the administration of histamine. Following a duration of 4 h, the animals were euthanized, their stomachs were dissected, and the ulcer scores and gastric contents were compared to those of the control group (Arige and Rao, 2017; Sirmagul *et al.*, 2004). The main benefit of this model is its high suitability since it consistently generates complete stomach ulcers. The drawback is that it does not provide a comprehensive representation of chronic ulcers observed in humans.

Application

This best model to assess the H₂ receptor-blocking activity of the proposed product (Mishra *et al.*, 2019a).

Ethanol-Induced Gastric Ulcer Model

Principle

The ethanol-induced ulcer model induces gastric ulcer by mucus depletion, oxidative stress, and mucus necrosis. Ethanol elevates free radical generation, impairs cell permeability, and enhances malondialdehyde concentrations, resulting in lipid

peroxidation and cellular apoptosis (Okokon and Nwafor, 2009; Beiranvand, 2022).

Procedure

Wistar rats (250 to 300 g, both sexes) were fasted for 18 h and divided into six groups (n=6). After receiving the test or standard drugs, rats were given 99.80% ethanol (1 mL/ 200 g) orally. Control received no treatment. One hour later, animals were euthanized by administering an excessive amount of chloroform. The stomach is then excised, opened, and the lesions are measured. Ulcer inhibition is calculated by comparing the lesion index to the control group (Bae *et al.*, 2011; Raza *et al.*, 2017; Zhou *et al.*, 2023). The benefit of this model is that it increases the release of acid and decreases blood flow, causing microvascular injury through the disruption of vascular endothelium and the promotion of vascular permeability. There are certain drawbacks to this concept. Ethanol directly damages the mucosa and does not involve acid secretion, it is not suitable for testing antisecretory or acid-suppressing drugs (Mishra *et al.*, 2019a).

Application

This model is important in evaluating the efficacy of pharmaceuticals possessing antioxidant or cytoprotective characteristics (Mishra *et al.*, 2019a).

Reserpine Induced Duodenal Ulcer Model

Principle

Reserpine is an adrenergic-blocking agent derived from the plant *Rauvolfia serpentina* roots, which treats high blood pressure but induces hemorrhagic glandular ulceration through mast cell degranulation and histamine release. This leads to free radical production and inhibits prostaglandin synthesis, mimicking the change seen in human gastric ulcers (Yusuf *et al.*, 2008; Dilpreet *et al.*, 2012).

Procedure

Female Sprague-Dawley rats (130-180 g) were fasted for 24 h, then separated into four groups (n=6). During the fasting period, they had access to a liquid diet consisting of 0.8% sucrose in 0.2% NaCl, discontinued one hour before the experiment. Test drugs or plant extracts were given at least 30 min before reserpine (5 mg/kg, i.p.), which is dissolved in a solution containing 10% Tween 80. After 18 h, all rats were euthanized, extracted their stomachs, and carefully opened them along the larger curve, and ulcers were measured to

calculate the ulcer index. Gastric contents were analyzed for acidity or pH levels (Li *et al.*, 2014; Zhou *et al.*, 2014). The benefit of this model is to evaluate both acute and chronic ulcers, and it is ideal for studying how calcitonin inhibits ulcers. However, it has a drawback, it is dose and acidity dependent, with hypermotility seeming to have a greater impact than hypersecretion (Mishra *et al.*, 2019a).

Application

This model is employed to assess medicines exhibiting cytoprotective, antioxidant, and anti-inflammatory properties (Fulga *et al.*, 2020).

Nonsteroidal Anti-Inflammatory Drug-Induced Gastric Ulcer

Principle

NSAIDs used to induce gastric ulcers in animals include aspirin, phenylbutazone, and indomethacin. It is a widely accepted model for testing anti-ulcer activity (Hatware *et al.*, 2018). NSAIDs induce gastric ulcers by blocking the release of prostaglandins, decreasing the production of bicarbonate and mucus. This impairs mucosal defence and promotes oxidative damage through increased peroxide and free radical generation (Suleyman *et al.*, 2010; Cheng *et al.*, 2017). NSAIDs inhibit COX-1 and COX-2 enzymes, reducing mucosal blood flow, mucus, bicarbonate, and platelet aggregation (Wallace *et al.*, 2000).

Procedure

Male Wistar rats (150-200 g) were fasted for 24-36 h and separated into five groups. Test drugs were given once daily for two days, 30 min before ulcer-inducing drugs (200 mg/kg of aspirin, 100 mg/kg of phenylbutazone, and 10 mg/kg of indomethacin, intraperitoneal). After 6 h, the rats were euthanized, and their stomachs were extracted, filled with formalin solution (2% v/v), and stored for a night. The next day, the stomachs were opened along the bigger curvature, cleaned with warm water, and examined under a three-fold magnification. Lesion lengths were measured to calculate total and average lesion scores per group (Feng *et al.*, 2023; Mahmoud *et al.*, 2023). The benefit of this model is that it evaluates anti-ulcer drug effects on gastric blood flow and leukocyte adherence using COX inhibitors, but it has a major drawback: it requires inhibition of both COX-1 and COX-2 to produce gastric injury, as blockage alone typically causes no significant stomach damage (Mishra *et al.*, 2019a).

Application

This model is employed to assess the effectiveness of antisecretory and cytoprotective agents in preventing stomach ulcer formation (Adinortey *et al.*, 2013a).

Cysteamine-Induced Ulcer (Duodenal)**Principle**

Cysteamine-induced duodenal ulcer, first reported by Selye and Szabo. It is widely used in rats to model peptic ulcer. Cysteamine stimulates the production of stomach acid while inhibiting alkaline mucus from Brunner's glands, leading to ulcer formation (Selye and Szabo, 1973; Warzecha *et al.*, 2012).

Procedure

Male Wistar albino rats (200-250 g) were fasted for 24 h and separated into groups. The first group receives saline solution, whereas the second group receives a standard (gastro-protecting) drug. Acute duodenal ulcers were caused by a single 400 mg/kg dose of cysteamine hydrochloric acid, while chronic duodenal ulcers can be caused by receiving 400 mg/kg of body weight of cysteamine hydrochloric acid twice, with a 4-hour break. Rats were euthanized 24 h later, the duodenum isolated and opened, and ulcers measured (mm²) to calculate ulcer score and index for comparison between groups (Saghaei *et al.*, 2012; Sahoo *et al.*, 2016). This model offers the benefits of being easily replicable and suitable for investigating the pathogenesis of duodenal ulcers. The drawbacks of this model include the ulcers often forming on the anterior wall, leading to perforation into the liver or pancreas, which can cause complications and limit its reliability (Mishra *et al.*, 2019a).

Application

This method is often utilized to assess the anti-ulcer properties of prostaglandins, antacids, anticholinergics, and H₂ receptor antagonists (Rethinavel *et al.*, 2022).

Serotonin-Induced Gastric Ulcer Model**Principle**

Serotonin-induced ulcer, as classified by Wilhelmi, Hedinger, and Veraguth. Serotonin facilitates vasoconstriction, reducing blood supply to the mucosa of the gastrointestinal tract and leading to immediate mucosal damage (Naik *et al.*, 2016).

Procedure

Albino rats were subjected to a 24-hour fasting period and thereafter categorized into groups, with the control group receiving saline. The rats get a solitary subcutaneous administration of serotonin (0.5 mL at a concentration of 50 mg/kg). Water is withheld for 2 h post-induction. Rats were euthanized within 6 h of administering the test medicine. The stomachs were dissected, cleaned, and examined for ulcers. Ulcer index and gastric acidity or pH are measured (Salem Sokar *et al.*, 2016; Satapathy *et al.*, 2024). The main benefit of this model is to facilitates vasoconstriction, reducing blood supply to the mucosa and leading to immediate mucosal damage. However, its main drawback is limited clinical relevance, as it does not fully mimic the multifactorial causes of human ulcers (Meena and Jayanthi, 2018).

Application

This method is utilized to assess the effectiveness of anti-ulcer drugs, that hinder serotonin, decrease acid secretion, or enhance mucosal defenses (Satapathy *et al.*, 2024)

Diethyl Dithiocarbonate (DDC) Induced An Ulcer Model**Principle**

DDC causes ulcers by generating free radicals, specifically superoxide and hydroxyl radicals. These radicals inhibit the activity of gastric mucosal copper-zinc superoxide dismutase and reduce mucosal blood flow, leading to gastric ulcers (Ogino *et al.*, 1990).

Procedure

Wistar rats (120-150 g, both sexes) were categorized into groups. After 24 h of fasting, all animals were injected with 1 mL of DDC in normal saline (800 mg/kg body weight) under the dermis (s.c.), subsequently ingested 1 mL of 0.1N HCl orally, resulting in acute glandular lesions. The test drug was supplied to the rats after two h. The rats were euthanized four h post-administration of the test drug. The stomachs were promptly extracted and transferred to a buffer solution at a cold temperature (0.1 M Tris-HCl with a pH of 7.4). The stomach is incised to inspect the precise location of ulceration, and as a result of DDC, a substantial ulcer is visible in the antrum following the minor curvature, and it infiltrates the muscle layer (Shivam and Chandra, 2020; Satapathy *et al.*, 2024). The benefit of this model, is useful for

studying oxidative stress-related gastric injury and testing antioxidant therapies, but its drawback is that it does not mimic the complex causes of human ulcers (Ogino *et al.*, 1990).

Application

This model is utilized to evaluate the antioxidant properties of medications designed to prevent stomach damage (Meena and Jayanthi, 2018).

In-vitro Models

In vitro models have been established to help determine the drug's potential and effect on gastric ulcers. These models are user-friendly and save time. Nevertheless, a significant drawback of these models is their limited reliability due to the multitude of laboratory variables that can influence their outcomes. Failure to adhere to correct protocol during the manufacture of synthetic gastric fluid, for instance, can result in significant inaccuracies in the obtained data (Thabrew and Arawwawala, 2018).

2D Gastric Epithelial Cell Cultures

Principle

2D gastric cell culture offers a streamlined and accessible framework for investigating cellular reactions to ulcerogenic substances and drugs (Schlaermann *et al.*, 2014; Lourenço *et al.*, 2018).

Procedure

Primary gastric epithelial cells or existing lines are cultivated on coated plates in specific media. These cells may be subjected to ulcerogenic chemicals, pharmaceuticals, or pathogens to evaluate cellular response, including viability, barrier function (tight junction integrity), and inflammatory signalling (Schlaermann *et al.*, 2014; Lourenço *et al.*, 2018). The main benefit of this model is that it provides a simplified, accessible, and manipulable system to study cellular responses, infection mechanisms, and drug effects, making them suitable for high-throughput screening and genetic manipulation. Nonetheless, their primary drawback is the absence of intricate three-dimensional architecture and cellular heterogeneity characteristic of the human stomach, which constrains their capacity to accurately emulate *in vivo* physiology and disease mechanisms (Schlaermann *et al.*, 2014; Canadas-Ortega *et al.*, 2024).

Application

2D cultures are extensively utilized for preliminary drug screening, mechanistic investigations, and toxicity assessments. They have high throughput capabilities and are appropriate for experiments such as $H^+/K^+ATPase$, binding assays, and cryoprotection investigations (Schlaermann *et al.*, 2014; Lourenço *et al.*, 2018).

Tissue Engineering Model (Air-liquid Interface Cultures)

Principle

These models utilize primary epithelial cells cultured at the interface of air and nutritional medium, facilitating differentiation and the development of tissue-like structures.

Procedure

Isolated gastric epithelial cells are inoculated onto permeable substrates and cultivated with media provided from beneath, while the upper surface remains exposed to air. This configuration promotes the formation of differentiated, polarized epithelial layers that resemble the stomach mucosa *in vivo* (Schlaermann *et al.*, 2014). The benefit of this model is that it closely resembles the natural gastric lining and supports studies of epithelial barrier function, but they are technically demanding and cannot fully replicate the complexity of the human stomach (Choi *et al.*, 2020; Sabapaty *et al.*, 2023).

Application

ALI cultures are employed to investigate epithelial barrier function, wound healing, and drug absorption. They are essential for evaluating chemicals that influence mucosal integrity or facilitate ulcer healing (Schlaermann *et al.*, 2014).

Hydrogen Potassium ATPase ($H^+/K^+ATPase$) Inhibition Assay

Principle

The $H^+/K^+ATPase$ inhibition assay was used to detect anti-ulcer activity by measuring the inhibition of the proton pump, a key enzyme regulating gastric acid secretion (Vogel and Vogel, 2002; Rao *et al.*, 2014). $H^+/K^+ATPase$ inhibitors, especially those with sulfhydryl groups, that are activated in acidic environments, forming sulfenic acid or sulfenamide. These reactive compounds bind covalently to the sulfhydryl groups of the enzyme, inhibiting its function (Houshia *et al.*, 2012).

Procedure

Healthy rats were selected, and their stomach chambers were gently rinsed with water. A cold phosphate buffer with a pH of 7.4 was used to thoroughly remove and homogenize the mucosal layer from the fundus region. The homogenate is subsequently centrifuged at 18,000 rpm for 20 min to eliminate debris. The resulting supernatant was subjected to ultracentrifugation at 100,000 rpm for 60 minutes to isolate membrane fractions. The pellet was re-suspended in a homogenization buffer, and the $H^+/K^{+}ATPase$ enzyme was purified using ficoll-sucrose discontinuous density gradient centrifugation. Finally, the protein concentration is determined using the Bradford method, and enzyme activity assays are conducted at both acidic and neutral pH levels to evaluate inhibition by test compounds. The main benefit of this model is to measure the anti-ulcer potential of compounds by analyzing their ability to inhibit $H^+/K^{+}ATPase$. A limitation of the assay is that it relies on complex ultracentrifugation, may not accurately represent *in vivo* conditions, and only measures enzyme inhibition, excluding mucosal healing or other anti-ulcer effects (Yadav *et al.*, 2012; Umre *et al.*, 2018).

Application

The assay helps screen proton pump inhibitors, identify anti-ulcer compounds, and study acid secretion mechanisms (Yadav *et al.*, 2012; Umre *et al.*, 2018).

Assessment of the Period of Neutralizing Effects of Produced Substances on Artificial Stomach Acid

Principle

The model stimulates human gastric conditions to measure the duration and effectiveness of acid neutralization by observing pH change over time in a controlled artificial stomach environment.

Procedure

This model consists of three components: a pH recording system that monitors the duration of acid neutralization, a peristaltic pump that replicates the conditions of the human body, and an artificial stomach. The artificial stomach in the equipment consists of three components: the gastric emptying flux, the reservoir, and the secretory flux. A mixture of 100 mL of gastric juice (pH 1.2 at 37°C) and 90 mL of test sample, distilled water, or standard solution is added to the reservoir. A magnetic device

with a length of 2.5 cm is employed to stir in a continuous manner at a rate of 30 rpm. Simultaneously, 3 mL/min of synthetic gastric juice enters and exits the reservoir of an artificial stomach to assist in the evaluation of pH fluctuations (Vatier *et al.*, 1992). The benefit of this model is to evaluate acid neutralization, provide clear, continuous data on pH fluctuation, and drug performance. The drawback of this model is that it requires technical setup and careful calibration, and as *in vitro* systems, they may not fully replicate the physiological factors like gastric emptying and mucosal interactions (Vatier *et al.*, 1998; Sandhya *et al.*, 2015).

Assessment of Neutralizing Capacity Utilizing Fordtran's Model through a Titration Method

Principle

The method simulates gastric conditions by mixing a test preparation and a reference antacid with artificial gastric juice at 37°C, agitating them to mimic stomach movements, and titrating until a target pH (usually 3) is reached, allowing assessment of acid-neutralizing capacity (Lirazan *et al.*, 2018).

Procedure

Place 90 mL of the plant extract and reference drug are each placed in separate 250 mL beakers, maintain at 37°C, and stir using a magnetic stirrer at 30 rpm. This will simulate the movements and surroundings of the stomach. Artificial gastric juice is gradually added to each beaker while continuously monitoring the pH. The process continues until the pH drops to 3, and the volume of gastric juice required to reach this point is recorded. This volume reflects the neutralizing capacity of each sample, allowing for a direct comparison between the antacid potential of the plant extract and the reference standard (Fordtran *et al.*, 1973; Mugwena, 2020). At the end of the titration process, we did not acquire a pH of 3.5, indicating that our plant extract lacked neutralizing capacity (Fokunang *et al.*, 2019). The benefit of this method, it provides a reproducible, controlled, and direct comparison of acid-neutralizing capacity between test (natural and synthetic) and standard substances. The drawback of this method, the plant extract failed to reach the target pH, showing no neutralizing capacity. Moreover, the *in vitro* setup may not reflect *in vivo* conditions (Cua *et al.*, 2018; Shatri, 2023).

Parameters to be Estimated in Peptic Ulcer

Several parameters can be calculated using the various methodologies given by different scientists to assess the drug's anti-ulcer activity in *in vivo* models. These are as follows in Figure 1.

Macroscopic Assessment of the Stomach

The stomachs were excised, incised along the greater curvature, and irrigated with normal saline to remove gastric contents and blood clots. Subsequently, elongated stomach sections were captured, and the number of lesions was determined utilizing a magnification lens (10x) (Reddy *et al.*, 2012; Shams and Eissa, 2022). The ulcer will be scored as follows in Table 2.

The percentage of protection was calculated using the ulcer index, which shows the average ulcer score for each group. The formula for calculating % protection is based on the ulcer index of the treatment control group (Mekonnen *et al.*, 2020).

The ulcer index (UI) was quantified utilizing the following mathematical expression:

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

In this formula, UN represents the average number of ulcers per animal. The US represents the average severity score, and UP represents the percentage of animals with ulcers.

The calculation of the % inhibition of ulcer was performed as follows:

$$\% \text{ Inhibition of Ulceration} = [(Ulcer \text{ Index Control} - Ulcer \text{ Index Test}) / Ulcer \text{ Index Control}] \times 100$$

Estimation of Lipid Peroxidase (LPO)

The stomach tissue's glandular section will be combined with trichloroacetic acid (TCA) to create a homogeneous mixture. This mixture will then be used to determine the amount of malondialdehyde present. To summarize, lipid peroxidation will be triggered by the addition of 10 μ l of ferric chloride (400 mM) and 10 μ l of 1-ascorbic acid (4,000 mM) to a mixture that contains 0.3 mL of stomach homogenate in phosphate buffer (5 mL, pH 7.4, 0.2 M). After an hour of incubation at 37°C, the reaction will be terminated via the addition of HCL (2 mL, 0.25 N) containing thiobarbituric acid (0.5 mL, 0.375% w/v) and TCA (1 mL, 15% w/v), which will be heated for 15 min. The mixture will be chilled and then subjected to centrifugation. The percentage absorbance of the resulting supernatant will be measured at a wavelength of 532 nm (Duh *et al.*, 2001).

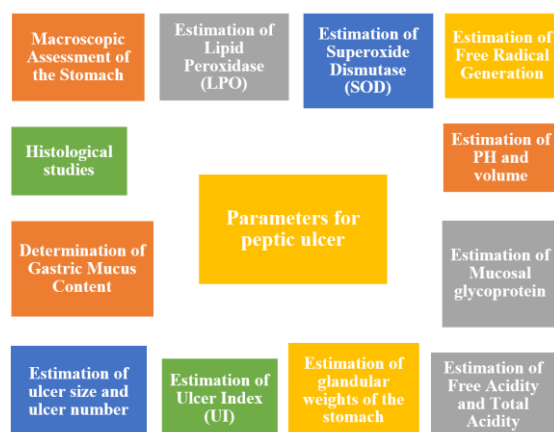


Figure 1. Evaluation parameters commonly used in the assessment of peptic ulcers

Table 2. Score of ulcer severity (Reddy *et al.*, 2012; Shams and Eissa, 2022)

Scoring	Severity
0	Healthy stomach
0.5	Red colouring
1	Inflames & spot ulceration
1.5	Hemorrhagic stripe
2	Severe ulcer >3mm but <5mm
3	Perforation of the stomach mucosal layer >5mm

Estimation of Superoxide Dismutase (SOD)

The assessment of the inhibition of nitro blue tetrazolium reduction to blue Formosan is conducted at 560 nm. This is done in the presence of phenazine methosulfate (PMS) and Nicotinamide Adenine Dinucleotide (NADH). The control used for this measurement is n-butanol. The amount of enzyme required to reduce the reaction rate by 50% in one minute under the given test circumstances is known as a single unit of enzyme activity. Units of SOD activity per mg of protein have been used to quantify the findings (Arige and Rao, 2017).

Estimation of Generation of Free Radical

Using a Potter-Elvehjem glass homogenizer, the fundus of the stomach is thoroughly homogenized in ice-cold 0.9% saline for 30 sec. After that, the mixture is centrifuged twice: once for 10 min at 800 $\times$ g and again for 15 min at 12,000 $\times$ g, which separates the supernatant. The Student's t-test is used in statistical analysis (Chattopadhyay *et al.*, 2006).

Estimation of Glycoprotein of Mucosa

After being combined with distilled water, the gastric mucosal scrape samples are treated with 90 percent ethanol. The techniques are used to assess the amounts of proteins and carbs that were previously outlined for analyzing the contents of gastric juice. The t-test for students is used in statistical analysis (Adinortey *et al.*, 2022).

Estimation of Volume and pH

Following the incision of the abdomen, the upper part of the stomach, known as the cardiac end, was carefully separated, and the contents were transferred into a glass tube. After centrifuging for 10 min at 2,000 revolutions per minute, the amount of digestive juice was measured. From the supernatant, a subsample (1 mL each) was taken and combined with 1 mL of water. A pH meter was used to measure the fluid's pH (Sisay and Jemere, 2020).

Estimation of Total Acidity and Free Acidity

The gastrointestinal contents were subjected to centrifugation and filtration. 1 mL of gastric juice was extracted using a pipette, and the resulting solution was subjected to titration using 0.1 N sodium hydroxide. Topfer's reagent was added as an indicator, and the titration continued until the solution changed color to a yellowish-orange hue. This measurement represents the quantity of NaOH needed to neutralize the unbound acid present in the gastric juice. Next, a small amount of phenolphthalein solution, approximately 2-3 drops, was introduced. The titration process was then repeated until a noticeable red coloration emerged. The disparity between the two figures unveiled the quantity of NaOH needed to neutralize the collective acid content in the digestive juice. The results of the two titrations were combined to determine the overall acidity of the gastric juice (Kaur *et al.*, 2012; Kalaichelvi *et al.*, 2018).

The formula for calculating the total acidity is:

$$\text{Total Acidity (mEq/L)} = (V \times N \times 100) / 0.1,$$

where

V represents the volume.

N refers to normality.

Estimation of Stomach Weight

The weight of the rumen is subtracted from the weight of the entire stomach to find the mass of the glandular segment of the stomach. This value is expressed in mg/100 g of the animal's body mass. The student's t-test is used for the statistical analysis. (Aman *et al.*, 2021).

Estimation of Ulcer Size, Ulcer Number, and Ulcer Index (UI)

The stomach is dissected along its larger curvature and secured onto a board. Tracing was then performed on translucent paper and transferred onto graph sheets using a magnifying hand lens. The ulcer index was determined by multiplying the size of each ulcer by the total number of ulcers (Mishra *et al.*, 2019b).

Determination of Gastric Mucus Content

This method was employed to ascertain the presence of stomach wall mucus with minor alterations. The gastric areas containing glands were excised and quantified by weight. Each segment was immersed in a 10 mL solution of 0.1% Alcian blue 8GX for 2 h. The residual color was eliminated using two rounds of washing with a 0.25M sucrose solution, each lasting 15 and 45 min, respectively. The dye that formed a complex with the mucus on the stomach wall was subsequently isolated by adding 20 mL of a 0.05M magnesium chloride solution and shaking intermittently. Subsequently, a 5 mL portion of blue extract was vigorously agitated for 2 h, with intervals of 30 min, using an equal volume of diethyl ether. Then, for fifteen minutes, the mixture is centrifuged at three thousand revolutions per minute using a UV-visible spectrophotometer set to a wavelength of 580 nanometers. The absorbance of the liquid layer was subsequently measured and recorded. The quantity of Alcian blue extracted per gram of glandular tissue was subsequently measured using the Alcian blue standard curve (Andargie *et al.*, 2022).

Histological Examinations

Samples of stomach tissue from every group are kept for a full day in 10% formalin. Following preservation, the tissues were sectioned into 3-5 millimeter pieces, fixed in paraffin, and stained with hematoxylin and eosin. The histological properties of the stained sections are analyzed using light microscopy examination (Jainu and Devi, 2006; Sistani Karampour *et al.*, 2019).

Recent Advancement

Organoids are complex three-dimensional structures that are created using either adult or pluripotent stem cells (Pleguezuelos-Manzano *et al.*, 2020). Initially, LGR5+ adult stomach stem cells were used to create gastric organoids; subsequently, pluripotent stem cells were used. These organoids are made up of every type of cell present in stomach tissue, such as chief cells, parietal cells, neck cells of the mucous gland, and surface mucous pits. To replicate

gastrointestinal ulcers and investigate the organoids' repair mechanism, microbial habitats must be added to the culture. This is achieved by using specific substances such as Wnt3a, R-Spondin (which interacts with Wnt), Noggin, A83-01 (inhibitors of TGF β), SB202190 (inhibitors of MAPK), growth factors (FGF2, FGF7, FGF10 and EGF), N-Acetyl Cysteine, Nicotinamide, gastrin and N2 supplement (containing human insulin, sodium selenite, human transferrin, and progesterone) (Ollivier *et al.*, 2021). Despite the stomach having a highly resistant epithelial lining, it is still susceptible to various infections. *H. pylori*, a widely recognized bacterium that causes infections in the stomach lining, has been connected to stomach cancer, gastric ulcers, and gastritis. Understanding the pathophysiology of human infection has improved with the use of stomach organoids in research on *H. pylori* infection. Organoid technology has been employed to discern bacterial proteins and cellular receptors, offering insights into the mechanisms underlying the link between cellular growth and *H. pylori*, which serves as a precursor to stomach cancer. An *H. pylori* chemoreceptor is stimulated, specifically detecting chemicals such as urea produced by stomach organoids. The identification of this factor is essential in understanding why organisms are attracted to the epithelium, offering initial insights into the mechanisms of colonization (Blutt and Estes, 2022; Idowu *et al.*, 2022).

Conclusions

In conclusion, evaluating anti-ulcer activity in a variety of animal models is essential to advancing our knowledge of the pathophysiology of PU and the effectiveness of potential therapeutic medications. These *in-vivo* and *in-vitro* models are useful in understanding the fundamental mechanisms of ulcer formation and healing processes. These models have made substantial contributions to preclinical research. Differences in anatomy and physiology between animal models and humans might prevent transferring results to clinical trials. Furthermore, ethical concerns and the difficulty of adequately simulating human ulcer situations in animals must be carefully addressed. Despite these obstacles, the ongoing refining of these models and the development of fresh methodologies are critical. These developments will improve the predictive value of preclinical investigations, allowing for the identification and development of more effective and safer anti-ulcer medicines. Finally, a thorough understanding of the pathophysiological mechanisms involved in ulcer formation, combined with the use of robust and

ethically sound experimental models, will drive progress in the treatment of peptic ulcers, lowering the global burden and improving patient outcomes.

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