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A Review On Recent Invivo And Invitro Screening Methods For Antiulcer Activity

[1]Tayum Yana, [2]Keserla Bhavani, and [3]Savi Biswakarma

[1, 2, 3] Department of Pharmacology
Krupanidhi College of Pharmacy, Bengaluru, India

Abstract A peptic ulcer is a gastrointestinal (GIT) disease that causes discomfort, morbidity, and mortality in human beings due to inappropriate diet, regular smoking, a sedentary lifestyle, alcohol intake, and extreme usage of drugs like non-steroidal anti-inflammatory drugs (NSAIDs), & numerous different models using animals, artificial stomach, in vivo and in vitro have been used to induce stomach ulcers to recognize the ulcer healing characteristics property of various novel and old drugs. This review describes animal screening models for anti-ulcer drugs and chemical constituents that are effective in the treatment of ulcers from several works of literature, search from various database sources like google scholar, PubMed, PMC, etc. using a mix of keywords: antiulcer models, in vivo models and in vitro methods of antiulcer action. In this paper, the digging of the literature for appropriate data was customized punctilious to get the best relevant articles to encounter the objectives of current in vivo animal and in vitro models principles, procedures, parameters and chemical constituents related to their uses deliberated in this review paper.

Keywords: Peptic ulcer; *Helicobacter pylori*; proton-pump blocker; Percentage Protection; Pharmacological Screening.

I. Introduction

Gastrointestinal (GIT) disease is a serious disorder in human beings which cause uneasiness, indigestion and morbidity in patients suffering from GIT disease even causing mortality in severe condition. One of GIT illnesses is peptic ulcers. (1). Characterized a break, wounds, haemorrhage, and holes in the segment of the gastrointestinal mucosa part is because of the coming contact of the stomach to enzyme endopeptidase & acidic gastric juice. Due to the nonequilibrium between aggressive forces like acid, pepsin, and *H.pylori* and defensive factors like gastric mucus, bicarbonate ions, and prostaglandins, as well as intrinsic resistance of mucosal cells exposed to gastric acid and pepsin, is disrupted. Other factors that induce peptic ulcers include inherited factors, particularly long-term alcohol usage, smoking cigarettes, or chronic NSAID use (2). The word "peptic" originates from the Greek term "peptikos" that's means "digestive"(3). “The occurrence of peptic ulcer illness in the overall population in lifespan was assessed to be around 6-101%, with an annual incidence of 1%–3%, with a global prevalence of roughly 40% in affluent nations and 80% in poor countries”(4). A loss of balance among the mucosal defensive and aggressive elements in the stomach is thought to be the main cause of peptic ulcers (5).

***Helicobacter-pylori*:** Etiologic of peptic-ulcer disorder is aggravated by *H. pylori* infection. around ≤ 90 % of patients suffer from duodenum ulcers and about 60 % to 85 % of patients who deal with gastric ulcers are found to be *H.pylori*-positive (6). *H. Pylori* is a bacteria which is known to be gram-negative bacillus, a single-cell organism, micro-aerophilic, flagellated, and spiral-shaped (7) It induces epithelial cell degeneration and damage by causing an inflammatory response via blood components including erythrocytes factor-like neutrophils, lymphocytes, plasma cells, and macrophages within the mucosal surface (8).

NSAIDs: these drugs are popular therapeutics uses, such as used to reduce inflammatory also as a pain-relievers and antipyretics. They are utilised to treat an extensive range of disease states, like arthritis and some other muscular-skeletal diseases. Due to its proclivity for causing stomach ulcers, their use has been restricted. Gastric ulcer disease affects nearly a quarter of long-term users of these medicines (9). Several studies on these medications show that they support the development of ulcers by suppressing the activity of the cyclooxygenase, whose role is to block the arachidonic acid to prostaglandins (PGs) conversion and degrade the stomach mucosal barrier, induce destructive action along with pepsin, and contribute to the progression of peptic ulceration (10) (11).

Signs and symptoms: Patients with peptic ulcer diseases may experience gnawing, searing discomfort or pain abdomen, loss of appetite, sickness, queasiness, bloating, and heartburn or abdominal pain, among other symptoms. Other people report no discomfort but black stools, which indicate that the ulcer is bleeding. Ulcer bleeding is a significant ulcer complication (12)(13).

Treatment: In order to treat peptic-ulcers there are a few existing classes of medications: proton-pump blockers, anticholinergics, histamine (H2) blockers, & antacids are commonly prescribed (14) (15) these drugs show unwanted serious side effects in prolonged use like low platelet levels, kidney problem, liver problem, and infertility (16) because of a severe adverse effect of above classes of medicines there is need for a safe and more effective therapy for ulcers.

II. Requirements of an ideal model (2)

It should be easy, reusable, and easy quantification of outcomes.
Ulcers in specific places should be differentiated.
Different mechanisms that cause ulceration should be considered.
During the monitoring period, ulcers should not heal immediately.

II. Ideal animal for screening anti-ulcer agents (17)
Rats are mostly used as they are omnivorous and nutritionally resemble humans, because of the continual release of acids; the glandular region of the rat stomach is physically and physiologically equivalent to the body of the stomach in humans. When histamine is used to cause ulcers, Guinea pigs are employed alongside rats.

IV. Preclinical anti-ulcer efficacy evaluation in vivo models

1. Nonsteroidal anti-inflammatory drug-induced gastric ulcer model (NSAIDs).
2. Swimming stress-cause gastric ulcer model.
3. Gastric ulcer model caused by Ethanol.
4. Pylorus ligated induced, ulcer model.
5. Gastric ulcer model caused by acetic acid.
6. Model of histamine-induced stomach ulcer.
7. Reserpine-cause gastric ulcer model.
8. Model of gastric ulceration produced by immersion in water or cold restraint:
9. Ulcer model caused by diethyl dithiocarbonate (DDC).
10. Cysteamine induces ulcers.

1. Nonsteroidal anti-inflammatory drug-induced gastric ulcer model (NSAIDs):

NSAIDs are a class of medications that are commonly used to treat pain, but they have also been demonstrated to be useful in disorders like rheumatoid-arthritis & inflammation. The extremely long period used, which may raise the risk of stomach injury NSAIDs such as the second most prevalent reason of stomach ulcers is aspirin & diclofenac (16).

Principle: Due to NSAID use, PG production is prevented in the stomach because of inhibition of the cyclooxygenase enzyme in the eicosanoid cyclooxygenase pathway (COX-1 AND COX-2). Blood flow, mucous secretion, and bicarbonate substance secretion, as well as mucous cell repair, are all disrupted (17) (18).

Procedure: Wister rats of any sex weighing 150 to 250 g are used for the procedure after proper measurement of the weight of the animals kept fast for 24 to 36 hours then with suitable vehicle (water, normal saline or carboxymethylcellulose) NSAIDs (aspirin, indomethacin, or others NSAIDs) ingested orally or intraperitoneal next one hour animals administered with a test product, 3 hours later rats are killed gently with a high dose of chloroform the stomach is removed, gastric juice collected, ulcer severity is estimated (16).

2. Swimming stress-cause gastric ulcer model:

This method is one of the finest and cheapest methods for the formation of ulcers in animals contrasted to other models, here cold water was used for depression for quite a long because there is a strong link between depression and ulcer severity scores (46).

Principle: stress caused by gastrointestinal motility increases when it reaches close contact with gastric acid, and the likelihood of destruction rises. Stressing over a longer period of time can affect the number as well as the quality of mucus secretion, triggering destruction to the layers of mucosa. The swimming stress-cause gastric ulcer model is useful for antiulcer evaluation since it reduces the mucosal component's synthesis (1).

Procedure:

Wister rats, both sexes, weighing 150 to 250 g, are used in the experiment. Prior to the experimentation, the Wister rats fasted for 24 to 36hours. 30 minutes later, rats are kept in a cylinder chamber and enforced to swim into the water at which levels are deep enough. Each rat is positioned carefully into a cylindrical shape water chamber which is filled with cold water that is sealed on both edges and dept. measured 30cm where water is filled maintaining 23-28ºC temperature such that the animals' feet do not come into contact with the cylinder's surface. Wister rats are euthanized 2–5hours later, the stomach is dissected, and ulcer parameters are assessed using the ulcer index and percentage of ulcer protection (47) (48).

3. Gastric ulcer model caused by Ethanol:

Principle: Ethanol consumption is linked to the development of stomach ulcers because ethanol can solubilize the protective mucus of the stomach and it put risk to the mucosa by exposing proteolytic & hydrolytic activities of HCl and pepsinogen, it effortlessly penetrates the gastric mucosa, damaging the mucus membrane (20).

Procedure: Wister rats are used for the experiment. Wistar rats of both sexes weigh animals between 150 to 250 grams and are placed into 6 different sets, with each set group comprising 6 rats.

Animals fast for 24 hours. Drugs to be tested will be given orally to animals on the 7^{th} the day of the 7^{th} after 1 hour, 1ml/per body weight of 100% ethanol is given through oral administration by using the gavage technique. An hour later each group of animals are sacrificed by overdosing on chloroform and the stomach parts are carefully dissected out, gastric juice collected for measured gastric juice. Along the greater curve of the stomach, it is opened. Cleaned with fresh water, and ulcer severity is assessed. Each gastric lesion's size is measured in millimetres (21) (40).

4. Pylorus ligated induced, ulcer model:

Shay et al. published this approach in 1945, and it has been used for ulceration production in rats ever since it is regarded as one of the most effective and simple procedures based on pylorus ligation.

Principle: The ligature of the stomach's pyloric ending, as a result, acid accumulates resulting in ulcer formation (22).

Procedure (5)(12): Wistar rats, both sexes, weighing 150-250g are used to carry out the procedure. Prior to pyloric ligation, animals fast for 48 hours before the procedure begins animals are anaesthetized by using general anaesthesia and below the xiphoid process, a one-inch middle of a front abdominal cut. The pyloric sphincter is cautiously pulled outside and tied without cause harmful to its bloodstream. The test substance is given orally or subcutaneously after the abdominal wall is sutured again after the stomach is restored. 10 to 19 hours after the Wistar rats are sacrificed, their stomachs portion are cut up. The whole stomach's contents are squished into a 2ml plastic tube and centrifuged for 10 minutes at revolving 2000 times in one minute. To determine the total gastric capacity, the 0.1 normality of NaOH titration is used to determine

total acidity. The ulcer index and percentage of ulcer prevention are computed after the stomach is sliced open.

5. Gastric ulcer model caused by acetic acid:

Principle: This approach is effective in treating chronically peptic ulcers and has anti-secretory and cytoprotective properties (23). In mice and rats, this model quickly causes circular, severe ulcers in the stomach and duodenum. (24).

Procedure: Adult Wistar rats of either sexes weight around 100 to 250g are employed for a procedure this method rats which randomly selected according to age and weight and fasted for 24 to 36 hours prior to experiments. Fasted animals are anaesthetized using general anaesthesia. A catheter which is made up of flexible plastic with a 2micrometres outer diameter is used to insert up to 8centimetres into the colon via the anus, and 2ml of 4% dilute acid is injected into the colon of rats. To prevent the acetic-acid solution from being leaky, rats are kept in a position where their heads are down for at least 2 minutes. Later 24 hours, animals are euthanasia, stomachs were dissected and slices are opened with greater curvature to determine the severity of an ulcer, an ulcer index is calculated (41) (19) (5).

6. Model of histamine-induced stomach ulcer:

Principle: Histamine is a substance released in the body by mast cells during infection, and it binds to the receptor that is present on the parietal cell surface. Once binding, they activate the enzyme adenyl-cyclase (AC), which transforms adenosine-triphosphate (ATP) to cyclic adenosine-monophosphate (cAMP), causing an increase in HCL secretion from the parietal cell of the stomach. Other factors such as the mucosa of the stomach, microcirculation, motility of the stomach, and mucus production are disrupted. (25).
Procedure: On male guinea pigs weighing 700g to 1.2 kg the experiment is carried out. Animals fast for 24-36 hours prior to the procedure. Histamine-acid sulphate in a dose of 50microgram is given intraperitoneally (*i.p*) to avoid histamine poisoning; promethazine hydrochloride 5microgram is administered intraperitoneal 15minutes before and after the histamine shot. 30minutes -1hour after the histamine injection, the drug to be tested is given orally. 4hours later guinea pigs are sacrificed, stomachs opened, and dissected. The ulcer index is used to assess the ulcers-severity and parameters are evaluated (26).

7. Reserpine-cause gastric ulcer model:

Reserpine is an adrenergic blocking agent derived from the plant *Rauwolfia serpentine* roots and is considered a drug that is utilized in the treatment of high blood pressure but it can cause gastric mucosal ulcers when used over longer periods (27).

Principle: Reserpine-drug works by influencing the cholinergic system. It induces histamine release, causing stomach mast cells to degranulation. It causes ulcers in the stomach by depressing sympathetic nerves response & liberating cholinergic signalling, which is responsible for causing extreme stomach acidity output (28).

Procedure (29) (19): Adult albino rats weighing 100 to 200g are employed in the study. For 48 hours, animals fast, the drug to be tested is injected intraperitoneally afterwards 30 minutes later, reserpine in a dosage of 15mg/kg is given *i.p* rats are euthanized four hours advanced, the rats' stomachs are gently dissected, portions are cut open, and an ulcer score is assigned. Ulcer-index and % of ulcer defence are calculated for test drugs.

8. Model of gastric ulceration produced by immersion in water or cold restraint:

Principle: Histamine release will accumulate as a result of the constant stress of being restrained in cold water, which will cause ulceration because histamine causes a significant increase in gastric-acid production, lower mucus supply, pancreatic liquid repeatedly reflux into bile, rising gastrointestinal motility, and harmed gastric blood flow (18)(19).

Procedure (30) (31) (32): In this method, the experiment is carried out on male Wistar rats. Animals being fast for 24 to 36 hours before doing the investigation

Animals are individually put down in a restricted cage & then submerged in a water chamber where the temperature of the water was maintained at 15-20ºc for induced ulcers. in the water-immersed model, 17 hours long experiment was carried out or in the case of the cold water restraint model 2 to 4 hours at a temperature of 2-3ºc cold water then after sufficient hours according to the requirement the animals were sacrificed by euthanasia method of a blow to the head, the stomach is dissected out carefully, Fill a sample collection cup with 1 per cent formalin and set it aside for 10 minutes. The test drug was given 30minutes before the procedure. the ulcer index is determined by the overall length of the lesions (30) (31) (32).

9. Ulcer model caused by diethyl dithiocarbonate (DDC):

Principle: Diethyldithiocarbamate-induced gastric ulcer (DDC) causes ulcers by releasing superoxide and hydroxyl radicals into the body. In the formation of ulcers, superoxide and hydroxyl radicals play a role. This approach is used to evaluate the antioxidant properties of medications intended to prevent stomach damage (33).

Procedure: Both males & females Wistar rats 120-150g are used for the experiment. Animals fast for a period of 24 hours the injection of 1ml of diethyl dithiocarbonate in normal saline under the dermis (s.c), afterwards by an orally intake of 1microlitre of 0.1normality hydrochloric acid, causes acute glandular lesions. In this experimental model, consumption of food is withheld for 24 hours & H2O is withheld for 2 hours right before the experiment begins (34) (35).

10. Cysteamine induces ulcers:

Selye and Szabo were the first to describe a cysteamine hydrochloric acid-induced duodenal lesion in rats. Duodenal-ulcer caused by ketamine has long been utilized as an animal investigational model for peptic-ulcers disease. In the terms of location, histology, and pathogenesis, cysteamine hydrochloric acid chemically generated ulcers similar to duodenal ulcers in humans (36).

Principle: Brunner's gland, which is located in the submucosa duodenum, stimulates gastric acid secretion while preventing the flow of alkali mucus; cysteamine promotes the formation of duodenal ulcers (37).

Procedure: Adult Wistar albino rats weighing 100-200g are employed in the experiment. Duodenal ulcers are divided into two categories. i.e. acute duodenal ulcer and chronic duodenal ulcers.

Acute duodenal ulcers can be created by giving 400mg per kg total body weight of cystamine hydrochloric acid once, and severe duodenal-ulcers can be induced by giving 400mg per kg of bodyweight of cystamine hydrochloric acid two times at a 4-hour break and a long period of time when cysteamine hydrochloric acid is added to drinking water. 24 hours after the ulcers are produced; the rats are killed by euthanasia. The ulcer regions are measured after the duodena are gently removed and sliced open along the antimesenteric side (38) (39).

V. List of in-vitro preclinical models for evaluating anti-ulcer efficacy:

1. Effects on artificial gastric acid neutralisation
2. The neutralisation duration capacity of a prepared preparation on artificial stomach acid.
3. A titration method was used to assess neutralising capacity in vitro.
4. Valuation of hydrogen potassium ATPase action.

1. Effects on artificial gastric acid neutralisation:

The artificial acid is created in the lab in this experimental model and its pH is set to be similar to that of genuine stomach acid, ranging from 1.2 to 3.2.

Procedure: 2 grammes of sodium chloride and 3milligrammes of pepsin-powder are dissolved in 500millilitres of distilled H_2O to make artificial stomach acid juice. Prepare an HCl solution as well as enough water. The pH of the freshly prepared solution was modified to 1.2, which is a very acidic state.

The formation of gastric ulcers is caused by a very high acidic condition, which damages the mucosal layer. If this pH is not managed, it will develop into more severe ulcers in the stomach. For assessing the test drugs and reference medication for their neutralizing action on gastric acidity, both drugs were added

individually to prepare gastric juice with a low pH value of 1 to 2.4, and the neutralization impact was assessed using artificial gastric juice titration (61).

2. The neutralisation duration capacity of prepared artificial stomach acid:

Principle: 'Vatiers artificial stomach device' explained this approach for determining the neutralization time of gastric acid. The pH record portion, the stomach, and the roller pump are all included in this instrumental model. In this apparatus model, the stomach is divided into three sections: S-1 (reservoir tank), S-2 (secretory chamber flux), & S-3 (gastric draining) (62).

Procedure: Each fresh plant extract, distilled water, or standard medication is mixed with artificial stomach juice at a pH of 1.2. At 37°C, and with 2.5 cm magnetic stirring equipment, the artificial stomach's reservoir was constantly stirred (30 rpm). Artificial gastric juice with a pH of 1.2 is pumped straight to the reservoir chamber where the artificial stomach fluids content is stored at the same rate as it is pumped out. To help assess pH variations, a pH metre is attached to the reservoir of an artificial stomach (62).

3. A titration method was used to assess neutralising capacity in vitro:

The titration approach, which is one of the most extensively, used in vitro methods for evaluating antiulcer activity, can be used to assess neutralising capacity.

Procedure: For titration, the test drug from the plant is freshly extracted, and the reference drugs are put into the beaker & heat up in a water bath to 37ºC with a magnetic mixer running continuously at 30revolutions per minute to mimic stomach activities. Individually plant extract and reference medicine is titrated using laboratory prepared stomach juice till the endpoint pH value reaches 3. Finally, following titration, the total volume of laboratory stomach juice consumed is evaluated, the total hydrogen ion consumption is determined, and the neutralized capacity is assessed (63).

4. Valuation of hydrogen potassium ATPase action:

The ion channel hydrogen-potassium ATPase is found in the oxyntic cell and is important for acid secretion control. It's commonly referred to as a proton pump. When defence factors are impaired due to harmful elements including hydrochloric acid & stomach acid discharge toward the gastric cavity, the canaliculi of parietal cells exchange intracellular H+ for external K+, resulting in gastric mucosal damage. Proton pump inhibitor-containing antiulcer medications are becoming more extensively used (64) (65).

VI. Estimation of parameters:

1. **Macroscopic Evaluation of Stomach/ Histopathological Examination**: Stomach tissue will be fixed in paraffin for one day after being immersed in 10 percentage formalin thin sections (3–5 m) are taken. The dyes hematoxylin and eosin are used to colour the samples, which are then observed by an electron microscope. To measure the development of stomach ulcers, use a magnifying lens with a magnification factor of ten (42) (43).

Ulcer severity is used to calculate a score for ulcers:

Scoring	Ulcer based severity
0	Usual colour stomach
0.5	Red colouration
1	Inflames & spot ulceration
1.5	Haemorrhagic stripe
2	Severe ulcer
3	Perforation of stomach mucosal layer

Table1: Ulcer severity scoring.

2. **Determination of pH & Volume of Gastric Secretions:** First of all the stomach was dissected, the ending section of the animal's stomach cut opened & the components contained by the stomach were collected in a small plastic conical tube. The total volume of digestive juice was estimated by centrifuging at 3000 revolutions per minute for 15minutes, an aliquot of the supernatant obtained was

mixed with water; shake properly to make them dissolve and the pH value acquired was determined via a pH digital metre (42).

3. **Total Acidity Measurement:** In a 50ml conical flask, the supernatant aliquot of gastric contents was taken. Topfer's reagent was also added in a few drops. In a burette, 0.01normality sodium chloride was added and titrated until the liquid turned yellow. Then some few droplets of phenolphthalein pH indicator were added on and titrated till a bright orange shade appeared. The quantity of sodium chloride consumed was recorded, and the reading was calculated (44).

4. **Ulcer index:** the ulcer index can be calculated from the glandular portions of the stomach by the given formula below:

   The ulcer will calculate as:

   Ulcer Index (UI) = $\frac{10}{X}$

   Where X denotes the total mucosal surface divided by the total ulcerated surface (19).

5. **Percentage of ulcer protection** (45) :

$$\% \text{ of ulcer Protective} = 100 - \left(\frac{\text{UI pretreated}}{\text{UI control}}\right) \times 100$$

6. **Estimation of the production of free radicals**: To estimate free radical production, the fundus is homogenised in an ice saline solution for thirty seconds. Following that, the homogenate result is centrifuged in the centrifugal machine for another 10minutes, the separate liquids are centrifuged again for 15 minutes, and the fraction obtained is used to calculate free radicals. (2).

VII. Medicinal plants & their chemical constituent that proved antiulcer activity:

Allium sativum: Allicin is one of the major active chemical components of *Allium sativum* extracts that have been revealed in antiulcer research to prevent the growth of H. pylori (60).

Aloe vera Barbalin, isobarbolin, and saponins, active elements of *aloe vera* powder are estimated to have antiulcer effectiveness comparable to that of the control group (49).

Mimosa pudica leaf extract contains active components, including the alkaloid mimosine, which has been demonstrated to have antiulcer activity and in the management of ulcers, it's beneficial as a naturally occurring antioxidant (50).

Ficus religiosa bioactive substances such as flavonoids, saponins, and tannins are considered to be active compounds of *Ficus religiosa*, and they are believed to dramatically reduce the ulcer-index when matched to the control group (51).

Carica papaya Linn. Extract of *c. papaya* contains the active compounds chymopapain and papain, which protect the gastrointestinal mucosa while also lowering gastric juice volume and acidity (52).

Mangifera indica the flower decoction was administered in rats with gastric lesions, the active component mangiferin considerably lowered the volume of stomach juice & gastric acidity (53).

Momordica charantia The active components flavonoids, sterols, and saponins found in an extract of *M. charantia* fruit are known to be beneficial against causing ulcers in rats when matched to the control group, the ulcer-index of these extracts showed much lower (54).

Zingiber officinalis extracts of *Zingiber officinalis* containing phenol components: gingerol & zingerone have a substantial inhibitory effect on parietal cell hydrogen potassium -ATPase. As a response, stomach acid output is reduced and the proton pump is inhibited. It also works as a preventative against *H. pylori* ulcers (55).

Solanum nigrum L. This herbal plant, which contains active flavonoids, is considered to have protected rats from induced gastric ulcers. (56).

Murraya koenigii *M. koenigii* extracts containing the active alkaloids compounds such as girinimbine reduce glutathione, nonprotein sulphydryls level and NO concentration in the plasma and elevated PGE2 together with a decreased level of IL-6 which help in heal peptic ulcers (57).

Aegle marmelos constituents that are active luvangetin, a pyranocoumarin, were found to have a remarkable lowering ulcer in rats when compared to a control (58).

Azadirachta indica the neem tree contains numerous active compounds one of the main active constituents is nimbidin which has antiulcer, properties (59).

Conclusion:

This peer-reviewed paper discussed several models that are effectively useful for testing drugs for antiulcer activity, as well as some important medicinal plants with active chemical constituents that have proven antiulcer activity, and it will assist investigators in selecting the best appropriate model and active constituents for experimentation in gastric ulcer studies.

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