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Review Article

Experimental animal models for gastric ulcer / peptic ulcer: An overview

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Abstract

In the present study we have discussed around sixteen different animal models used worldwide for the scientific research and new drug discovery. The main aim of the using experimental animal models in drug discovery is to establish and provide evidence for non-clinical 'proof-of-concept' for the safety, efficacy, and target of interest for specific drug molecules. Experimental preparations developed in one species for the purpose of studying phenomena occurring in another species. The use of experimental animal models serves to better understand the origins, pathology, and the overall nature of comparable diseases of humans being. Similarly, animal models perform duties for in the development of safe and effective treatments and cures of such diseases and/or associated symptoms. Experimental animal models for drug discovery and development have played a major role in the characterization of the pathophysiology of diseases and associated mechanisms of injury, drug target identification, and evaluation of novel therapeutic agents for toxicity, pharmacokinetics and pharmacodynamics activity. Through animal model researchers can perform experiments that would be impractical or ethically prohibited with humans. There are various animal models used for screening of uncountable therapeutic activities, in this review our main focus is animal models used for peptic ulcer. Peptic ulcer is one of the worldwide diseases where 10% of adults are affected by peptic ulcer once in their lifetime. The antiulcer models for drug development against gastric and duodenal ulcer studies are limited in number that has hindered the progress of targeted therapy in this field. Therefore, it is necessary to review the literature on experimental animal models that are used to screen agents with potential anti-gastric ulcer activity and describe their biochemical basis in order to facilitate their use in the development of new preventive and curative antiulcer drugs. There are many models used to induce ulcer such as pylorus ligation or it can be chemically induced by ethanol, NSAIDs (e.g. indomethacin) or many more. In this review paper, current *in-vivo* animal models of ulcers and the pathophysiological mechanisms underlying their induction, their drawbacks, as well as the challenges associated with their use have been discussed.

Keywords: animal models, peptic ulcer, ethanol, NSAIDs, indomethacin, pylorus ligation

INTRODUCTION

Animal experimentation is a complex and controversial area of research that is subject to strict ethical and legal guidelines in many countries. The requirements for animal experimentation vary, but they generally include considerations related to ethical, scientific, and legal aspects. The common requirements include are, researchers must adhere to ethical principles, including the principles outlined in the three Rs: Replacement (use of alternatives), Reduction (minimizing the number of animals used), and Refinement (improving the welfare of animals). Ethical considerations also include minimizing pain and distress for the animals. Researchers are also required to justify why animals are necessary for the research and why alternative methods cannot be used. The scientific merit of the research and the potential benefits must outweigh the potential harm to the animals. Another important factor that needs to be taken into consideration is that, compliance with national and international animal welfare regulations is mandatory. These regulations often specify housing conditions, veterinary care, and monitoring of animal welfare. Researchers are required to provide a rationale for the choice of animal species and strains used in the study. The selection should be based on the scientific objectives and relevance to the research question. If procedures involve pain or distress to the animals, researchers must use anesthesia and analgesia to minimize suffering. The choice of anesthetic and analgesic agents should be based on the specific needs of the study and the welfare of the animals. Peptic ulcers are the areas of degeneration and necrosis of gastrointestinal mucosa exposed to acid-pepsin secretions. The term peptic ulcer (PU) defines a characteristic lesion consisting of a solution of continuity of the proximal gastric and/or duodenal mucosa, although it can also develop in the esophagus, small intestine, or in the ectopic gastric mucosa of Meckel's diverticulum. It may be a single lesion or multiple lesions and may extend beyond the *muscularis mucosae* into the sub mucosa. The size of the ulcer varies from 5 mm to several cm[1].It is generally recognized that peptic ulcer is caused by lack equilibrium between the gastric aggressive factors (*H. pylori*, HCl, pepsin) and mucosal defensive factors (prostaglandin, bicarbonate, mucus) [2]. Other factors that cause ulcer are hyper secretion of acid-pepsin, association with alcoholic cirrhosis, tobacco,

hyperparathyroidism, chronic pancreatitis, blood group O, genetic factor, bile reflux and some drugs. Based on site of attack, peptic ulcer may be classified as esophageal, duodenal, or gastric ulcer [2].

Pathophysiology of peptic ulcer:

HCl is secreted by the parietal cell whereas pepsin (as pepsinogen) by the peptic cell. Both the parietal and peptic cell is normally present in gastric mucosa. The pepsin can digest protein only when the pH is sufficiently low, between pH 2-3. At higher pH (>5), pepsin become inactive and cannot digest protein. Therefore, presence of strong acid like HCl is necessary. Acid-Pepsin Mixture (APM) which is formed in low pH initiates digesting intestinal mucosa which results into peptic ulcer.

Disadvantages of alternate models for peptic ulcer:

Unlike other screening model, screening of drug using animal models needs permission from regulatory authorities such as Institutional Animal Ethics Committee (IAEC), Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), etc. Apart from these, selection of proper species, strain, gender, appropriate body weight and sanitation & maintenance is also a strong concern. Animal models are needed for screening of anti-ulcer activity before the drug can be administered to human beings, it must be tested in animals to determine its adverse effects and toxicity profile. These tests are done *in-vivo* because the effect of pharmacokinetic processes, the interaction affecting them, effects on vital organs and the neuroendocrine system cannot be duplicated *in-vitro*. While testing new drug in animals, it is also necessary to test them in more than one species of animals, because extrapolation of the results of testing in animals to humans is not perfect. The most common animal model used for anti-ulcer activity are rodents, mainly mice and rats. The foundation for biomedical research states that 95% of animal models for research are mice and rats. Most human disease gene exists in mice and rats, making them suitable for research.

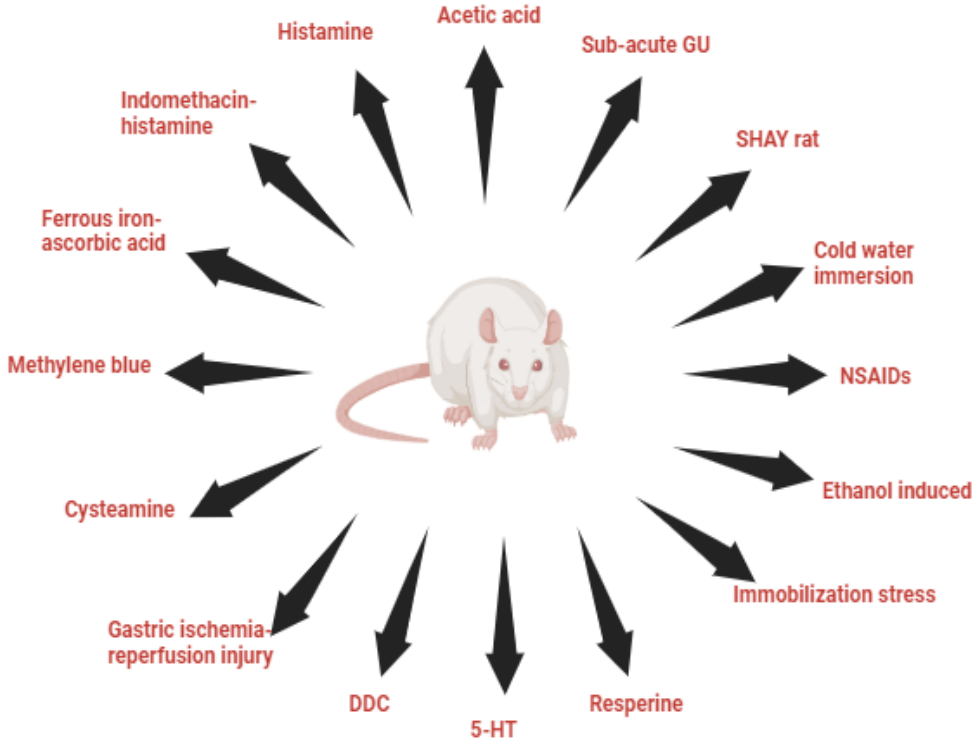

Figure 1: Diagrammatic representation of details of animal's model used for the evaluation of anti-ulcer activity

The following animal models used for the evaluation of anti-ulcer activity are:-

1. Pylorus ligation in rats (SHAY rat)
2. Stress ulcer through immobilization stress
3. Stress ulcers by cold water immersion
4. Indomethacin induced ulcers in rats (NSAID induced ulcers)
5. Ethanol induced mucosal damage in rats
6. Subacute gastric ulcer in rats
7. Gastric ischemia-reperfusion injury in rats
8. Acetic acid-induced gastric ulcers
9. Histamine-induced gastric ulcers
10. Reserpine-induced gastric ulcers
11. Serotonin-induced gastric ulcers
12. Diethyldithiocarbamate- (DDC)-induced peptic ulcers
13. Methylene blue-induced ulcers
14. Cysteamine-induced duodenal ulcers
15. Indomethacin-histamine-induced duodenal ulcers
16. Ferrous iron-ascorbic acid-induced gastric ulcers

Pylorus ligation in rats (SHAY rat) [3, 4, 5]

Principle:

Pylorus ligation model is widely used method for induction of experimental ulcer using rat model. This simple and reliable method for production of gastric ulceration in the rat based on ligature of the pylorus has been published by Shay et al. (1945).This model is also found suitable for screening of drugs for anti-GERD (Gastro Esophageal reflux disease) activity.

Procedure:

In this method, female Wistar rats weighing 150–170 g are starved for 24-48 h having access to drinking water *ad libitum* are kept in polypropylene cages with elevated grated/perforated mesh floor to avoid coprophagy and cannibalism. Before the surgery, the animals are anaesthetized with sodium thiopental (50 mg/kg, I.P.) then the mid-line incision is made on abdominal portion, in the epigastric region nearly about 1.5 cm will be opened to expose the stomach and pyloric portion is identified after lifting the stomach. Grasping the stomach with instruments is to be meticulously avoided; otherwise ulceration may invariably develop at such points. The pyloric portion is tied with suture by avoiding the tissue damage. After tying the pyloric portion, the tissue is then replaced in its original position and outer layer of abdominal wall is closed by sutures. The animal will be returned to home cage again. The test compounds are given either orally by gavage or it can be injected subcutaneously. During this process the acid and pepsin accumulated in the stomach to develop ulcers. After 4 hours of pylorus ligation, the animals are sacrificed in CO_2 anesthesia. The abdomen is opened and a ligature is placed around the esophagus close to the diaphragm and stomach is cut at both ends, removed. The gastric content is collected to determine the free acidity and total acidity. After centrifugation, acidity is determined by titration with 0.1 n NaOH. The stomach is again cut-opened with greater curvature as shown in the fig-2 and cleaned with tap water. The stomach is then fixed on an appropriate flooring space and the ulcerated area is calculated with the following scores:

- 0 = no ulcer
- 1 = superficial ulcers
- 2 = deep ulcers
- 3 = perforation

Evaluation

An ulcer index UI is calculated:

UI = UN + US+ UP × 10–1

- UN= average of number of ulcers per animal
- US = average of severity score
- UP=percentage of animals with ulcers

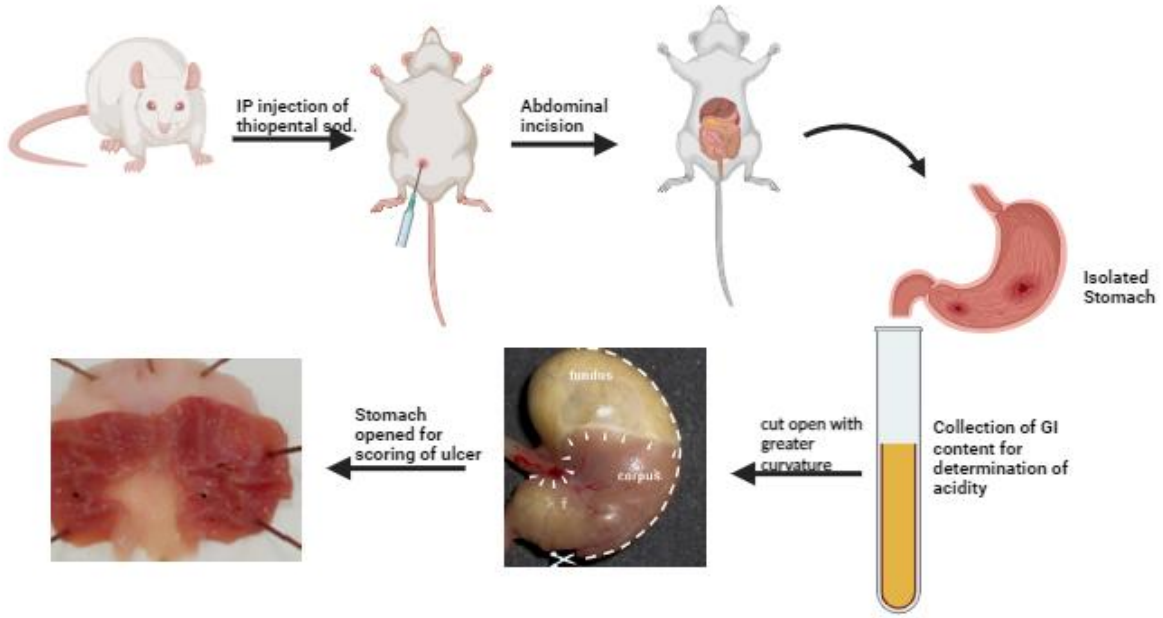

Figure 2: Diagrammatic representation of Pylorus ligation in rats (SHAY rat) model

The mucosa is subjected for examination under stereomicroscope. In the rat, the upper two fifths of the stomach form the rumen with squamous epithelium and possess little protective mechanisms against the corrosive action of gastric juice. Below a limiting ridge, in the glandular portion of the stomach, the protective mechanisms are better in the mucosa of the medium two fifths of the stomach than in the lowest part, forming the antrum. Therefore, lesions occur mainly in the rumen and in the antrum. The number of ulcers is noted and the severity recorded with the following scores:

- 0 = no ulcer
- 1 = superficial ulcers
- 2 = deep ulcers
- 3 = perforation.

The volume of the gastric content is measured. After centrifugation, acidity is determined by titration with 0.1 n NaOH. Ulcer index and acidity of the gastric content of treated animals are compared with controls. Using various doses, dose-response curves can be established for ulcer formation and gastric acid secretion. *ID*50 values can be calculated by probit analysis, whereby 0% corresponds to no and 100% to maximal stimulated gastric acid output.

Stress ulcer through immobilization stress[6, 7]

Principle:

A stress ulcer refers to a type of ulcer that develops in the gastrointestinal (GI) tract due to severe physiological stress. Immobilization stress is one of the stressors that can contribute to the development of stress ulcers. Immobilization stress occurs when an individual is subjected to prolonged periods of physical inactivity or confinement. This type of stress can activate the body's stress response, leading to various physiological changes. Stress ulcers are particularly associated with increased secretion of gastric acid, reduced blood flow to the stomach lining, and damage to the mucosal barrier of the GI tract. The mechanisms by which immobilization stress contributes to stress ulcers are complex and involve interactions between the nervous, endocrine, and immune systems. Some key factors for the development of stress induced ulcers are as follows.

Increased gastric acid secretion:

Stress, including immobilization stress, can stimulate the release of certain hormones like cortisol, which, in turn, can lead to an increase in gastric acid production. Elevated levels of gastric acid can contribute to the erosion of the stomach lining.

Reduced blood flow:

Stress can cause vasoconstriction (narrowing of blood vessels), including those that supply blood to the stomach. Reduced blood flow can compromise the delivery of oxygen and nutrients to the stomach lining, making it more susceptible to damage.

Inflammation and immune response:

Stress can trigger an inflammatory response in the body, and inflammation in the GI tract may contribute to the development of ulcers. Additionally, stress may affect the immune system, influencing the balance between protective and damaging factors in the stomach lining.

Impaired mucosal barrier:

Chronic stress, such as that induced by prolonged immobilization, may compromise the integrity of the mucosal barrier in the stomach. The mucosal barrier acts as a protective layer, and when it is weakened, the stomach is more susceptible to damage from gastric acid.

Procedure for induction of Stress ulcer through immobilization using rat model:

Randomly selected 10 female Wistar rats per dose of test drug and for controls weighing 150–170 g are used. Animals are deprived of food and water, 24h prior to the initiation of experiment. After oral or subcutaneous administration of the test compound/placebo, the animals are slightly anesthetized with suitable anesthesia such as ether. Both lower and upper extremities are fixed together and the animals are wrapped in wire gaze. They are horizontally suspended in the dark at 20 °C for 24 h and finally sacrificed in CO_2 anesthesia. The stomach is removed, fixed on a cork plate and the number and severity of ulcers is registered with a stereo-microscope using the following scores:

- 0 = no ulcer
- 1 = superficial ulcers
- 2 = deep ulcers
- 3 = perforation

Evaluation:

An ulcer index *U*I is calculated:

*U*I = *U*N + *U*S+ *U*P × 10–1

- *U*N = average of number of ulcers per animal
- *U*S = average of severity score
- *U*P = percentage of animals with ulcers

Assessment of the method:

The experimental model resembles the psychogenic factors in the pathogenesis of gastric ulcers in patients. Therefore, it is not surprising that not only antacids, anti-cholinergics, H_2-antagonists, proton-pump-inhibitors, but also psychotropic drugs, like neuroleptics, have been found to be effective in this test. The test is being used in final drug evaluation only.

Stress ulcers by cold water immersion[8, 9, 10]

Principle:

Cold water immersion can lead to various physiological responses in the body. While it's not accurate to directly attribute ulcers to cold water immersion, there are certain conditions and responses that may occur as a result of prolonged exposure to cold water, and these could potentially contribute to the development of ulcers indirectly. Major Key factors contribute to ulcers by cold water immersion are vasoconstriction, hypothermia and peripheral tissue damage. Prolonged vasoconstriction may reduce blood flow to certain areas of the body, potentially impacting tissue health leading to ulcer. Extended exposure to cold water can lead to hypothermia, which is a condition where the body loses heat faster than it can produce it. Severe hypothermia can affect various bodily functions, including digestion and blood circulation. Cold water immersion can lead to damage to peripheral tissues due to reduced blood flow. This may result in conditions such as frostbite, where the affected tissues may suffer damage or necrosis.

All these factors are responsible to accelerate the occurrence of gastric ulcers and shorten the time of necessary immobilization.

Procedure:

Wistar rats weighing between 150–200 g are used. Animals are randomly allocated into various groups depending upon protocol. Each group comprising of 6-8 animals. Animals are fasted properly as per CPCSEA guidelines. The test compound and standard drug are administered via PO route and the rats are placed vertically in individual restraint cages in water at 22 °C for one hour. Then animals are removed, dried and injected intravenously via the tail vein with 30 mg/kg Evans blue. Ten min. later, the animals are sacrificed using CO_2 anesthesia and their stomachs removed. Formal saline (2% v/v) is then injected into the totally ligated stomachs for storage overnight. The next day, the stomachs are opened along the greatest curvature, washed in warm water, and examined under a 3-fold magnifier. The lengths of the longest diameters of the lesions are measured and summated to give a total lesion score (in mm) for each animal, the mean count for each group being calculated.

Evaluation: The mean score in control rats is about 25 (reference range 20–28). Inhibition of the lesion production is expressed as percentage value.

Assessment of The method:

Like other stress models, the test resembling the psychogenic factor for ulcer disease in human beings, is used for final drug evaluation only.

Indomethacin induced ulcers in rats[11, 12, 13]

Principle:

Indomethacin is belongs to NSAID that works by inhibiting the activity of cyclooxygenase (COX) enzymes, which are involved in the synthesis of prostaglandins. The reduction in prostaglandin production can contribute to inflammation and compromise the protective mucosal barrier in the stomach, potentially leading to ulcer formation.

Procedure:

Wistar rats weighing between 150–200 g are used. Animals are randomly grouped into various groups test and standard drug treated groups etc. The test drugs are administered orally in 0.1% Tween 80 solution 10 min prior to oral Indomethacin in a dose of 20 mg/kg (4 mg/ml dissolved in 0.1% Tween 80 solution). Six hours later, the rats are sacrificed in CO2 anesthesia and their stomachs removed. Formolsaline (2% v/v) is then injected into the totally ligated stomachs for storage overnight. In the next day, the stomachs are opened along the greater curvature, then washed in warm water, and examined under a 3- fold magnifier. The lengths of

the longest diameters of the lesions are measured and summated to give a total lesion score (in mm) for each animal, the mean count for each group being calculated.

Evaluation:

The mean score in control rats is about 25 (reference range 20–28). Inhibition of the lesion production is expressed as percentage value.

Assessment of the method:

According to West (1982) the cold stress induced ulcer formation, but not the Indomethacin- or aspirin-induced ulcers are inhibited by H2-receptor antagonists, whereas other authors reported protective effects of H2-receptor antagonists under these conditions (Tarutani et al. 1985).

Ethanol induced mucosal damage in rats[14, 15, 16, 17]

Principle:

Ethanol, the type of alcohol found in alcoholic beverages, is known to cause damage to the mucosal lining of the stomach and intestines, potentially leading to the formation of ulcers. The exact mechanism is ethanol consumption can stimulate the production of gastric acid in the stomach. Elevated levels of stomach acid can help conversion of inactive pepsinogen to active pepsin. This acid-pepsin mixture can disrupt the mucosal barrier that protects the stomach and makes the stomach more susceptible to damage and ulcer formation. Intra-gastric application of absolute ethanol is a reproducible method to produce gastric lesions in experimental animals as per the data available by Robert et al. 1979 as well as by Szabo et al. 1981. These lesions can be at least partially inhibited by various drugs, such as some prostaglandins. The protective effect against various irritants has been called cytoprotective activity.

Procedure:

Male Wistar rats weighing 250–300 g are deprived of food 18 h prior to the experiment but are allowed free access to water. During this time they are kept in restraining cages to prevent coprophagy. The rats are administered either the appropriate vehicle or the cytoprotective drug, e.g. a prostanoid, intragastrally 30 min prior to administration of 1 ml absolute ethanol. Untreated animals are included as controls. One hour after administration of ethanol, the animals are euthanized with CO_2; the stomachs are excised, cut along the greater curvature, and gently rinsed under tap water. The stomachs are stretched on a piece of foam core mat, mucosal site up.

The subjective scores of the treated tissues are recorded; the graded response is reflecting the least (0) to most (3) damage. A circular full thickness area, about 13 mm in diameter, is cut with a cork borer from each lobe of the fundus just below the ridge dividing the glandular from the non-glandular portion of the stomach. A Plexiglas template (19 × 14 × 0.3 cm), burnished on one side with emery cloth, and with four rows with six holes 13 mm in diameter is placed on a sheet of clear glass, burnished side up, and bound to the glass with photographic tape along the periphery. The excised pairs of tissue from each stomach are placed into the holes of the template. Pairs of tissue from each stomach are examined to minimize sampling errors. The template is positioned on a rectangular central open area of an Aristo Model T-16 cold cathode trans-illuminator (38 × 38 cm) containing a W-45 blue-white lamp. A camera is mounted on a copy stand directly above the template. Photographs are taken, the film processed in a standard manner and a contact sheet is made from the negatives. A light transmission densitometer (e.g. Mac Beth model TD-501) is used to evaluate the negatives. The optical density of the test tissues is determined by placing each area of the negative in sequence over the aperture through which the light is transmitted. The optical density is displayed on a digital read out and recorded. Hemorrhagic or damaged areas appear bright on the negative, whereas undamaged tissue appears dark. Hence, lower optical density values are indicative of damage while higher optical densities are associated with little, or, as in the case of control, no damage.

Evaluation:

The significance of differences in optical density between control and ethanol-treated tissue is evaluated by non-paired single-tail Student's *t*-test.

Assessment of the method:

Several prostaglandins provide cytoprotection, particularly in rats, in a dose-range which has no anti-secretory activity. However, clinical experience with prostaglandins showed that ulcer healing is only achieved at anti-secretory doses (Lindberg et al. 1990). Therefore, it seems very likely that the cytoprotective property of a compound in rats has very limited relevance to prediction of its ulcer healing potential in humans if cytoprotection is really separated from its anti-secretory potential (Herling and Weidmann 1994).

Subacute gastric ulcer in rats[18, 19]

Principle:

Subacute gastric ulcers in rats are often induced in laboratory research settings to study the pathophysiology of ulcer formation and to evaluate the effects of various substances, interventions, or treatments on gastric mucosal damage. Subacute gastric ulcers to simulate conditions where ulcers develop over a more extended period compared to acute models. Here are some key points typically associated with the induction and study of sub-acute gastric ulcers in rats. Ezer is a scientist in 1988, described a method for producing standard sub acute gastric ulcers in rats and for the quantitative evaluation of the healing process.

procedure

Female Wistar rats weighing 120–150 g are fasted for 24 h having access to water at libitum in cages with wire sieves at the bottom. The rats are anesthetized with ether and a polyethylene catheter including a fine steel wire with a needle tip (1.2 mm diameter) at the lower end is orally inserted into the stomach. After the cannula reaches the gastric wall, the upper end of the steel wire is pressed in a definitive manner, so as to puncture the gastric wall. Each rat is kept in the same position during the intervention in order to localize the puncture at nearly the same region of the glandular part of the stomach. The test substances are administered orally, 30 min or 24 h after puncture. Free access to food and water is provided from 2 h up to the end of the experiment. Each group consists of 8–15 rats. The animals are sacrificed by overdose of ether at definitive time intervals after puncture. The stomach is dissected and opened along the lesser curvature, extensively rinsed in tap water and fixed to the end of a polyethylene tube of 10 mm diameter (plastic tip of an automatic pipette) in a position with the punched ulcer in the center.

The end of the tube with the gastric wall is suspended in a beaker containing physiological saline, and the pressure in the tube is gradually increased with a valved rubber ball connected to the other end of the tube. The third part of the system is a tonometer calibrated up to 1 bar. The value of tension at which bubbles appear at the ulcerous gastric wall is

noted. This value is termed as tensile strength and can be expressed in mm Hg.

Evaluation:

The extent of the healing of gastric ulcers can be characterized by the healing rate (*HR*) according the following equation:

HR = (*A* – *B*) / *C* (mmHg/h) with

• *A* = tensile strength (mm Hg) at *C* time-point after puncture

• *B* = tensile strength 30 min after puncture (the average value is 143 mm Hg)

• *C* = time course (h) of the experiment.

Anti-ulcer drugs, such as H2 antagonists, significantly increase the healing rate, which is decreased by non-steroidal anti-inflammatory drugs.

Assessment of the method:

Similarly, to the method of Takagi et al. (1969) who injected 50 µl of acetic acid into the stomach wall (Szelenyi et al. 1982), the method of Ezer (1988) allows to judge the time course of healing of the ulcers.

Gastric ischemia-reperfusion injury in rats[20, 21, 22]

Principle:

Hassan et al. (1997) described the effect of an endothelin converting enzyme inhibitor on local gastric ischemia-reperfusion injury in rats. Endothelin-1 has potent ulcerogenic effects in the stomach (Wallace et al. 1988). Endogenous endothelin-1 has been implicated for ethanol, indomethacin and hemorrhagic shock induced gastric ischemia-reperfusion injuries (Masuda et al. 1993; Kitajima et al. 1993; Michida et al. 1994; Kitajima et al. 1995).

Procedure:

Male Wistar rats weighing 200–250 g are fasted for 24 h with free access to water. The rats are anesthetized with 1.5 g/kg urethane i.p. The stomach is exposed by a medial laparatomy and instilled with 0.15 M HCl (1 ml/100 g) via the fore-stomach. The left gastric artery is clamped by a small vascular clamp for 5 min to induce ischemia and 30 min of reperfusion is done by releasing the clamp. Pretreatment with test drug or standard is given to groups of 5 rats immediately before the induction of ischemia. At the end of the experiment, the rats are sacrificed by cervical dislocation. The stomach is fixed with 10% buffered formalin and photographed for macroscopic evaluation of injuries. For the assessment of microscopic injuries, a sample of corpus 0.5 cm below the limiting ridge containing the entire width of the anterior wall is taken from each stomach and processed for subsequent histological evaluation. A planimeter attached to a computer is used to trace the macroscopic mucosal injury from color photographs. The results are expressed as a percentage of the total glandular mucosal area. Each histological section is stained with hematoxylin/eosin and examined under light microscope. An one cm length of each histological section is assessed for epithelial damage (score = 1), glandular disruption, vasocongestion or edema in the upper mucosa (score = 2), hemorrhagic damage in the mid to lower mucosa (score = 3) and deep necrosis and ulceration (score = 4). Each section is evaluated on a cumulative basis to give the histological index, the maximum score thus being 10.

Evaluation:

Data are expressed as mean ±SEM. Comparisons between different groups are made by one way analysis of variance followed by Fisher's least significant difference test. P-values of <0.05 are considered as statistically significant.

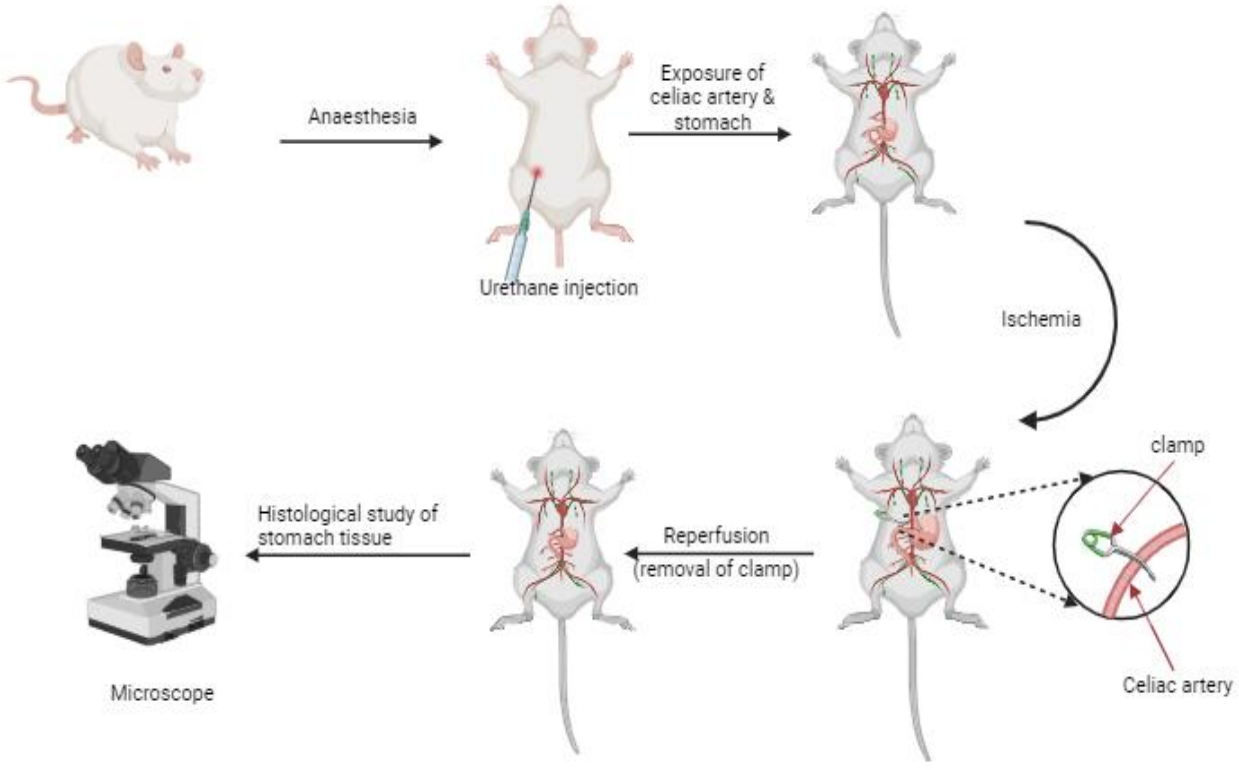

Figure 3: Gastric ischemia-reperfusion injury in rats

Acetic Acid-Induced Gastric Ulcers[23, 24, 25]

Principle:

Acetic acid-induced gastric ulcers are a commonly used experimental model to study the pathophysiology of gastric ulcers and to evaluate the efficacy of potential therapeutic agents. This model involves the induction of gastric ulcers in laboratory animals, usually rodents, by the application of acetic acid to the gastric mucosa. Typically, rats or mice are used for this model. Acetic acid induces localized damage to the gastric mucosa by causing necrosis and inflammation. The application of acetic acid triggers an inflammatory response, leading to the formation of ulcers. Acetic acid causes an increase in vascular permeability, leading to edema and infiltration of inflammatory cells. The inflammatory response results in the release of various mediators, including cytokines, prostaglandins, and reactive oxygen species. The severity of gastric ulcers is often assessed by histological examination of the stomach tissue. This includes the measurement of ulcer area, depth, and the extent of inflammatory cell infiltration. Various biochemical markers, such as myeloperoxidase activity and pro-inflammatory cytokine levels, may be measured to quantify the extent of inflammation. The model is commonly used to evaluate the effectiveness of potential therapeutic agents in preventing or treating gastric ulcers. Compounds with anti-inflammatory, antioxidant, or cytoprotective properties are often studied for their ability to attenuate acetic acid-induced ulcer formation. While the model is valuable for studying certain aspects of ulcer formation, it has limitations and may not fully replicate the complexity of human gastric ulcers. Despite its limitations, the acetic acid-induced ulcer model provides valuable insights into the basic mechanisms of ulcer formation and the potential efficacy of therapeutic intervention. Takagi et al. developed a model for inducing chronic gastric ulcer in rats by means of sub-mucosal injection of acetic acid and reported on the healing process of lesions for extended intervals after the ulcer preparation. The experimental gastric ulcer was considered as chronic due to its persistence for a long time and resemblance to human chronic ulcer both grossly and histologically. The model easily and reliably produces round, deep ulcers in the stomach and duodenum of mice, rats, Mongolian gerbils, guinea pigs, cats, dogs, miniature pigs, and monkeys.

Procedure:

Healthy adult laboratory rats (typically Sprague-Dawley or Wistar rats) are randomly allocated for different groups. Animals are kept fasted for a specified period before the experiment (usually overnight with access to water *ad libitum*. Rats are anesthetized using an appropriate method (e.g., isoflurane inhalation). The investigator should ensure that the adequate anesthesia depth throughout the procedure. Animal (rat) is to be placed in a supine position on a surgical table. The abdomen is exposed through a midline or lateral incision, following aseptic surgical procedures. Stomach is identified to expose carefully. After exteriorizing, the stomach is injected with 0.01, 0.05 or 0.14 ml of 20% acetic acid into the sub-mucosal layer of the anterior or posterior wall of the glandular stomach with microsyringe (0.05 ml). While injecting, a finger or thumb must be placed on the injecting needle to avoid solution leakage. Even after the needle is withdrawn, the thumb is maintained at the injection site for 45 s to prevent acetic acid leakage upon removal of the needle. The gastric mucosal surface starts damaging within 30 min after injection. After a week of acid injection, round/oval, deep ulcers (approximately 50-60 mm^2) are observed in the stomach surface corresponding to the injected area. In the pre-treatment groups, rats were injected intraperitoneally with either saline (n=8) for 10 days prior to ulcer induction and the treatments were continued for the following 3 days. In the treatment groups, saline (n=16) was administered at the same dose for either 5 or 10 days starting on the day of ulcer induction. Control group (n=8) underwent the surgical procedure of ulcer induction without the application of acetic acid. Upon the completion of the treatments on the 3rd, 5th or 10th days of ulcer induction, the rats were decapitated. Immediately after decapitation, freshly excised stomachs were dissected out, cut along the greater curvature and the mucosa were rinsed with normal saline for the macroscopical analysis of haemorrhagic lesions in the glandular mucosa. The length (mm) of each lesion was measured (three petechia were counted as 1 mm), summed per stomach and expressed as ulcer index.

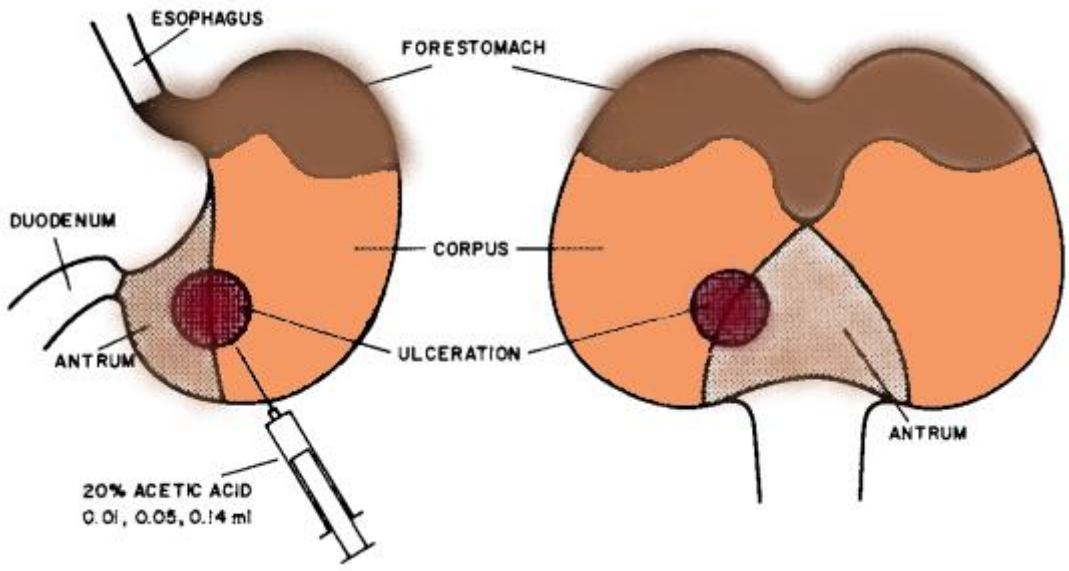

Figure 4: Schematic drawing for ulcer induction in stomach of rat.

Histamine-Induced Gastric Ulcer [26, 27]

Principle:

Histamine has long been a candidate for inclusion in this exclusive club of ulcer-promoting factors (Babkin, 1950). Thus chronic or continuous application of histamine produces ulcers in animals (Biichner et al., 1928; Walpote et at., 1940). Gastric ulceration is mediated by several factors including the release of histamine, and this is the basis for the histamine model for producing ulcers. There are histamine receptors, particularly H_2 receptors which are located on parietal cells of the stomach. Histamine does not only enhance gastric acid secretion, but it also causes disturbances of the gastric mucosa, microcirculation, abnormal motility, and reduction in mucus production. The mechanism by which histamine induces gastric ulcers is through its potent acid stimulating and vasodilating capability, which leads to increased vascular permeability. These pharmacological effects of histamine underlie the histamine-induced ulcer model and hence its usefulness in evaluating anti-secretory effects of potential drugs against ulcers and agents that function as H_2-receptor antagonists.

Procedure:

The animal selected for this method are male guinea pigs weighing 300-400 g are fasted for 36 hours prior the experiment with water *ad libitum*. Ulcers are induced by administering histamine phosphate (40–100 mg/kg body weight) subcutaneously or intraperitoneally. To protect the animals against systemic histamine toxicity, a drug: promethazine hydrochloride (5 mg) is injected i.p. 15 min before and 15 min after the histamine administration. The test drugs are administered p.o. or s.c. 30-45 min before histamine injection. After four hours of histamine administration, the animals are sacrificed. The stomach is dissected out of the animal's body. The gastric contents are subjected to analysis for free and total acidity and the stomach is cut open. Then the degree of ulceration is graded according to methods described under pylorus ligated rats. Pilot studies to determine the effective dose for ulcer induction are usually needed.

Evaluation:

An ulcer index UI is calculated:

UI = UN + US+ UP × 10–1

- UN= average of number of ulcers per animal
- US = average of severity score
- UP = percentage of animals with ulcers

Reserpine-Induced Peptic Ulcer [28, 29, 30]

Principle:

The ulcerogenic properties of reserpine in humans and animals are well established, and appear to be associated with enhanced gastric acid secretion (Kim and Shore, 1963), central liberation of 5-hydroxytryptamine (Blackman et al., 1959), reduced peripheral tissue levels of monoamines (Doteuchi, 1971) and release of histamine through degranulation of gastric mast cells (Rasanen, 1971 and Ogle and Cho, 1978 a, b). Scientists have also used reserpine to induce ulcers. Reserpine-induced gastric ulceration has been attributed to the de-granulation of gastric mast cells consequent to liberation of histamine, believed to be mediated by the cholinergic system.

Procedure:

The female Sprague-Dawley (albino) rats weighing 130-180g are taken and fasted for 48 hours prior the experiment. During fasting, they have free access to liquid diet made of 0.8% sucrose in 0.2% NaCl w/v which is withdrawn one hour before the experiment. Normally, drugs or plant extracts to be evaluated are administered to the test animals, at least, 30 minutes before the administration of the reserpine. After 1-2 hour(s) later, reserpine 5mg/kg dissolved in 10% Tween 80 is injected intraperitoneally. Although the model is acid dependent, hyper motility seems to be more important than hyper secretion for the induction of gastric mucosal lesion. The test animals are then sacrificed 24 hours later and stomach is removed. The stomach is cut through greater curvature and mucosal lesions are examined.

Serotonin-Induced Gastric Ulcer [31, 32]

Principle:

Serotonin, which has also been used to induce ulcers, is known to cause vasoconstriction, thereby reducing gastric mucosal blood flow (GMBF) resulting in acute mucosal injury. Abnormal regulation of 5-HT in the gut has been implicated in some GI disorders, including inflammatory bowel disease (Magro et al. 2002; Coates et al. 2004). Similarly, TNF is a pro-inflammatory cytokine that is involved in the pathogenesis of gastric ulcers; it can cause apoptosis of gastric parietal cells (Neu et al. 2003) and act as an inhibitor of constitutive nitric oxide synthase (cNOS) (Bauer et al. 1997). This is important in that this could lead to reductions in local levels of NO, a vasodilator that maintains adequate blood flow to gastric mucosa (Pique et al. 1989).

Procedure:

In this model, rats are weighed and fasted for 24–36 hours prior the experiment with water *ad libitum*. The fasted animals are denied of water 2 hours just before commencement of the experiments. Glandular lesions are established following the administration of a single dose of serotonin creatinine sulfate (0.5 mL of 50 mg/kg subcutaneous injection). Serotonin is administered by intra-gastric intubation with the aid of an oro-gastric cannula. The animals are sacrificed by cervical dislocation 6 hours later. The stomach is dissected out for the further studies of ulcer index.

Diethyldithiocarbamate-Induced Gastric Ulcer [33, 34, 35]

Principle:

The diethyldithiocarbamate model is used to assess the antioxidant activities of drugs in the prevention of gastric damage. This model is also used to assess the cytoprotective actions of potential drugs. Diethyldithiocarbamate has been reported to induce antral lesions through the mobilization of superoxide and hydroxyl radicals. Superoxide radical and hydroxyl radicals play a pathogenic role in the induction of this ulcer.

Procedure:

The groups of rats are taken, each weighing 200-230 g which are previously fasted for 24 hours before the experiment with free access of water *ad libitum*. The water is withdrawn two hours just before the commencement of the experiment. Acute glandular lesions are induced by subcutaneous administration of 1 mL of diethyldithiocarbamate (DDC) in saline (800 mg/kg body weight) followed by 1 mL oral dose of 0.1N HCl. After the administration of DDC, rats are sacrificed at 1 h, 3 h, and 7 h, and the stomachs were removed immediately and placed in ice-cold buffer (0.1 M Tris-HC1 at pH 7.4). The stomach is cut opened to observe the area of ulceration and due to DDC, a large ulcer can be observed in the antrum along the lesser curvature, penetrating into the muscular layer.

Evaluation:

The ulcer index was measured under a dissecting microscope with a square-grid eyepiece and expressed as the area of the antral ulcer (mm^2).

Methylene Blue-Induced Ulcer[36, 37, 38]

Principle:

Methylene blue (MB), a synthetic drug is known to uncouple ATPases and generate superoxide radical ions. MB has been used as an ulcerogenic agent to study antiulcer agents and their mechanisms of action. It can be used as a pharmacological tool to screen various antiulcer agents, which modulate the H+/K+ ATPase system. When administered to animals, MB induces gastric and duodenal lesions. The compound also inhibits nitric oxide synthase activity and hence reduces the bioavailability of nitric oxide. Additionally, MB has affinity for acetylcholine or muscarinic receptors and has been reported to inhibit cholinesterase activity. This implies that the model could be used for assessing antiulcer agents with anticholinergic effects and proton-pump inhibitory activity. Methylene blue decreases blood supply to gastric mucosa, which causes oxidative stress and subsequently produces erosion and ulceration of gastric mucosa.

Procedure:

For induction of ulcers with MB, animals are fasted 24 hours before the experiment with water *ad libitum.* MB administered at a dose of 125 mg/kg body weight p.o.(oral route) followed by the administration of the drug(s) or test substances. Animals are sacrificed after 4 hours of MB administration. The stomach of the experimental animals are dissected out and cut opened by the greater curvature to determine ulcer index.

Evaluation:

Cysteamine-Induced Duodenal Ulcers[39, 40, 41]

Principle:

A duodenal ulcer in rats induced by cysteamine hydrochloride was first described by Selye and Szabo. Cysteamine induced duodenal ulcer in rat has been widely used as a model of peptic ulcer disease. Cysteamine stimulates gastric acid secretion rate and inhibits the alkaline mucus secretion from Brunner's glands in the proximal duodenum resulting in the formation of duodenal ulcer. Cysteamine also affects processes that increase gastric acid and pepsin secretion in the gastric mucosa, with a decrease in defensive processes that lower levels of bicarbonate, mucus, and epidermal growth factor. This model is widely used to evaluate the anti-ulcer activity of anticholinergics, antacids, prostaglandins and H_2 receptor antagonists.

Procedure:

Female Sprague-Dawley rats are used. Cysteamine (10% in normal saline) is administered in dose of 28 mg/100 g body weight, 3 times at intervals of 3.5 hours orally or 20 mg/100 g body weight, twice at an interval of 4 hours subcutaneously. The animals are sacrificed 28 hours after the first dose in case of orally administered cysteamine and 40 hours after subcutaneous administration of cysteamine. Perforating duodenal ulcers are produced that are located 2-4 mm from the pylorus, mainly on the anterior wall of the duodenum. Presence of necrotic material and acute inflammatory response on the luminal layers of the crater are characteristic of active ulcers. The ulcer and its features in test group are compared to those in control group.

Indomethacin, Plus Histamine-Induced Duodenal Ulcer[42, 43]

Principle:

Another method for inducing duodenal ulcers described by Takeuchi et al. involves administering Indomethacin and histamine to rats. This combined treatment has been reported to induce one or two round lesions in the proximal duodenum at an incidence of 100%, and a few lesions in the corpus and antrum of the stomach as well. The development of duodenal lesions induced by indomethacin and histamine in rats is due to both an increase in gastric acid secretion and an impairment of acid-induced duodenal HCO_3 secretion. This model for duodenal ulcers is useful for studying the pathogenesis of duodenal ulcers and for screening anti-duodenal ulcer drugs or agents.

Procedure:

Wistar or Sprague Dawley rats weighing 150-200 g are fasted for 24 hours with water ad libitum and randomly distributed in groups comprising of 6-8 rats. Indomethacin 5 mg/kg s.c. is first administered to these animals and subsequently histamine dihydrochloride (40mg/kg) is given three times at 2.5 hours intervals, beginning 30 min after the injection of indomethacin. The development of duodenal lesions induced by indomethacin plus histamine in rats is due to both an increase in gastric secretion and an impairment of acid-induced duodenal HCO_3-secretion. This model is expected to be useful for studying the pathogenesis of duodenal ulcers and for the screening of anti-ulcer agents. After 3 hours, duodena are excised carefully, cut opened along the anti-mesentric side, and the ulcer area(s) is measured.

Ferrous Iron-Plus Ascorbic Acid-Induced Gastric Ulcer Model[44, 45]

Principle:

This type of gastric ulcer model is induced by the local injection of ferrous iron with ascorbic acid (Fe/AS A) solution into the gastric wall. The ulcers produced resemble human gastric ulcers that penetrate the muscularis 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.

Procedure:

The experimental animals used are male Sprague-Dawley rats weighing 180-200 g were obtained from Keari Co. Ltd. (Osaka, Japan). They are housed at 22°C in a maintained environment with 12 h of artificial light per day and 12 h dark cycle. They fed rat chow *ad libitum*. They are fasted for 18 h before the experiments, but are allowed free access to water *ad libitum*. The drug in a dose of twenty-five micro liters dissolved in saline was injected into the sub mucosa of the anterior wall of the glandular stomach using a micro syringe. The animals are sacrificed and stomach is dissected out to measure ulcer index.

Evaluation:

The area of the ulcers accompanied by white plaques that developed in the gastric mucosa was calculated according to the formula: n/4 × major axis (a) x minor axis (b) (mm^2).

REFERENCES:

1. Laucirica I, Iglesias PG, Calvet X. Peptic ulcer. Medicina Clinica. 2023;169: 260-66. https://doi.org/10.1016/j.medcli.2023.05.008 PMid:37365037

2. Adinortey MB, Ansah C, Galyuon I, Nyarko A. In Vivo Models Used for Evaluation of Potential Antigastroduodenal Ulcer Agents.

Hindawi Publishing Corporation Ulcer. 2013; 2013:12. https://doi.org/10.1155/2013/796405

3. Monteiro KM, Spindola HM, Possenti A, Tinti SV , Ruiz ALTG, Longato GB et.al. Characterization of a refinement of the "pylorus ligation" model of rat gastric ulceration resulting in "no pain" and a more specific pharmacological response. Journal of Pharmacological and Toxicological methods. 2013; 67:121-128. https://doi.org/10.1016/j.vascn.2012.09.002 PMid:23000280

4. Mohd. T, SiddiquiHH, Mohd. K, Md. AAzizur R. Protective effect of hydro-alcoholic extract of Rutagraveolens Linn. leaves on indomethacin and pylorus ligation-induced gastric ulcer in rats. Journal of Ayurveda and Integrative Medicine. 2016;7:38-43. https://doi.org/10.1016/j.jaim.2015.09.002 PMid:27297508 PMCid:PMC4910575

5. SatapathyT, Panda PK, GoyalAK&Rath G. Evaluation of Anti-GERD Activity of Gastro Retentive Drug Delivery System of Itopride Hydrochloride. Artificial Cells, Blood Substitutes, and Biotechnology. 2010;38(4):200-207. https://doi.org/10.3109/10731191003776751 PMid:20515421

6. Yadin E, Thomas E. Stimulation of the lateral septum attenuates immobilization-induced stress ulcers. Physiology & Behavior. 1996;59:883-886. https://doi.org/10.1016/0031-9384(95)02184-1 PMid:8778882

7. Watanabe K, Matsuka N, Okazaki M, Hashimoto Y, Araki H, and Gomita Y. The Effect of Immobilization Stress on the Pharmacokinetics of Omeprazole in Rats.AcitaMedica Okayama. 2002;56(1):19-23.

8. Muthuraman A, Sood S. Antisecretory, antioxidative and antiapoptotic effects of montelukast on pyloric ligation and water immersion stress induced peptic ulcer in rat. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2010;83:55-60. https://doi.org/10.1016/j.plefa.2010.01.003 PMid:20153621

9. Dejban P, Eslami F, Rahimi N, TakzareN, Jahansouz M, DehpourAR. Involvement of nitric oxide pathway in the anti-inflammatory effect of modafinil on indomethacin-, stress-, and ethanol -induced gastric mucosal injury in rat. European Journal of Pharmacology. 2020;887. https://doi.org/10.1016/j.ejphar.2020.173579 PMid:32950497 PMCid:PMC7495189

10. Guo S, Gao Q, Jiao Q, Hao W, Gao X, and Cao J. Gastric mucosal damage in water immersion stress: Mechanism and prevention with GHRP-6. World J Gastroenterol. 2012;18(24):3145-3155. https://doi.org/10.3748/wjg.v18.i24.3145 PMid:22791951 PMCid:PMC3386329

11. Tamaddonfard E, Erfanparast A, Farshid AA,Imani M,Navideh M, SalighedarR et.al. Safranal, a constituent of saffron, exerts gastro-protective effects against indomethacin-induced gastric ulcer. Life Sciences. 2019;224:88-94. https://doi.org/10.1016/j.lfs.2019.03.054 PMid:30914317

12. Mahmoud MF, Abdo W, Mohamed N, Drissi B, Abdelfattah M AO, Sobeh M et.al. Apple (MalusdomesticaBorkh) leaves attenuate indomethacin-induced gastric ulcer in rats. Biomedicine & Pharmacotherapy. 2023;160. https://doi.org/10.1016/j.biopha.2023.114331 PMid:36736281

13. Feng L,BaoT,BaiL, Mu X, Ta Na, BaoM et.al. Mongolian medicine formulae Ruda-6 alleviates indomethacin-induced gastric ulcer by regulating gut microbiome and serum metabolomics in rats. Journal of Ethnopharmacology. 2023;314. https://doi.org/10.1016/j.jep.2023.116545 PMid:37196816

14. da Silva DT, Rodrigues RF, Machado NM, Maurer LH, Ferreira LF, SomacalS et.al. Natural deep eutectic solvent (NADES)-based blueberry extracts protect against ethanol-induced gastric ulcer in rats. Food Research International. 2020;138(A). https://doi.org/10.1016/j.foodres.2020.109718 PMid:33292963

15. Zhou C, Chen J, Liu K, MaharajanK, Zhang Y, HouL at.al.Isoalantolactone protects against ethanol-induced gastric ulcer via alleviating inflammation through regulation of PI3K-Akt signaling pathway and Th17 cell differentiation. Biomedicine & Pharmacotherapy. 2023;160. https://doi.org/10.1016/j.biopha.2023.114315 PMid:36716661

16. Arab HH,SalamaSA, Omar HA, ArafaEl-ShaimaaA,andMaghrabi IA.Diosmin Protects against Ethanol-Induced Gastric Injury in Rats: Novel Anti-Ulcer Actions. PLoS One.2015;10(3). https://doi.org/10.1371/journal.pone.0122417 PMid:25821971 PMCid:PMC4378914

17. Hashim H, MughrabiFF, Mahmood A, KhalediH, Ali HM.Cytoprotective effect of benzyl N'-(5-chloro-indol-3-yl-methylidene)hydrazinecarbodithioate against ethanol-induced gastric mucosal injury in rats. Molecules 2012;17(8):9306-9320. https://doi.org/10.3390/molecules17089306 PMid:22864239 PMCid:PMC6268369

18. Ezer E. Effect of cytoprotective and antiulcer drugs on the healing process of subacute gastric ulcer in rat (a new model). ActaPhysiol Hung. 1989;73(2-3):233-240.

19. Ezer E.Novel method for producing standard subacute gastric ulcer in rats and for the quantitative evaluation of the healing process. Effect of several drugs on healing. J Pharmacol Methods. 1988;20(4):279-91. https://doi.org/10.1016/0160-5402(88)90050-2 PMid:3210680

20. Iwata F, JohT, Ueda F, Yokoyama Y, andItohM. Role of gap junctions in inhibiting ischemia-reperfusion injury of rat gastric mucosa. Am J Physiol. 1998;275(5):G883-G888. https://doi.org/10.1152/ajpgi.1998.275.5.G883 PMid:9815015

21. Kuyrukluyildiz U, DelenLA, OnkD, YaziciGN, GulabogluM, Suleyman H;The effect of dexmedetomidine on gastric ischemia reperfusion injury in rats. Biochemical and histopathological evaluation. Acta Cir Bras. 2021;36(1):e360104. https://doi.org/10.1590/acb360104 PMid:33533828 PMCid:PMC7853698

22. OharaR, Larissa LucenaPérico, ViniciusPeixoto Rodrigues, Bueno G, Ana Caroline Zanatta,Vilegas W et.al.Terminaliacatappa L. infusion accelerates the healing process of gastric ischemia-reperfusion injury in rats. Journal of Ethnopharmacology. 2020; 256:112793. https://doi.org/10.1016/j.jep.2020.112793 PMid:32240780

23. Shi Z, XuF, Xia T, Bian Z, Li S. Pharmacokinetics and anti-ulcerogenic effects of Zuojin gastric floating bio-adhesive pellets in rats with acetic acid-induced gastric ulcer. Phytomedicine Plus. 2023; 3:100442. https://doi.org/10.1016/j.phyplu.2023.100442

24. da Silva LM, PezziniBC, SomensiLB, Mariano LNB, Mariott M, Boeing Tet.al.Hesperidin, a citrus flavanone glycoside, accelerates the gastric healing process of acetic acid-induced ulcer in rats.Chemico-Biological Interactions. 2019;308: 45-50. https://doi.org/10.1016/j.cbi.2019.05.011 PMid:31095933

25. Han T, Tang Y, Li J, XueB, Gong L, Li J, Yu X, Liu C. Nitric oxide donor protects against acetic acid-induced gastric ulcer in rats via S-nitrosylation of TRPV1 on vagus nerve. Sci Rep. 2017;7(1):2063. https://doi.org/10.1038/s41598-017-02275-1 PMid:28522805 PMCid:PMC5437002

26. Sirmagul B, Kilic FS, Batu O, Erol K. The effects of verapamil on stress- and histamine-induced gastric lesions in rats. Methods Find ExpClin Pharmacol.2004;26(10):763-67. https://doi.org/10.1358/mf.2004.26.10.872557 PMid:15672118

27. Troidl H, Lorenz W, Rohde H, Häfner G, Ronzheimer M. Histamine and peptic ulcer: A prospective study of mucosal histamine concentration in duodenal ulcer patients and in control subjects suffering from various gastrointestinal diseases. KlinischeWochenschrift . 1976; 54: 947-56. https://doi.org/10.1007/BF01469008 PMid:979074

28. Zhou Y, Wang R, Feng X, Zhao X. Preventive effect of insect tea against reserpine-induced gastric ulcers in mice. Experimental and Therapeutic Medicine. 2014; 8:1318-324. https://doi.org/10.3892/etm.2014.1859 PMid:25187847 PMCid:PMC4151677

29. Pfeiffer CJ, Cho CH, Cheema A, Saltman D. Reserpine-induced gastric ulcers: protection by lysosomal stabilization due to zinc. European Journal of Pharmacology. 1980;61(4):347-53. https://doi.org/10.1016/0014-2999(80)90073-4 PMid:7371712

30. Salomone S, Caruso A, Cutuli VM, Mangano NG, Prato A, Bianchi A, et.al. Effects of adrenomedullin on the contraction of gastric

arteries during reserpine-induced gastric ulcer.2003;24(1):117-22. https://doi.org/10.1016/S0196-9781(02)00283-8 PMid:12576092

31. Hashizume T, Aibara S, Hirokawa K, Nishida A, Ogawa H, Kasahara A. Protective effect of cetraxate, a new antiulcer drug, against serotonin-induced ulcer. Arch IntPharmacodynTher. 1979 Aug; 240(2):314-27.

32. Abdel-SaterKA, Abdel-Daiem WM, BakheetMd S .The gender difference of selective serotonin reuptake inhibitor, fluoxetine in adult rats with stress-induced gastric ulcer. European Journal of Pharmacology. 2012; 688:42-48. https://doi.org/10.1016/j.ejphar.2012.04.019 PMid:22546225

33. Ogino K, Hobara T, Kawamoto T, Kobayashi H, Iwamoto S, Oka S et.al. Mechanism of diethyldithiocarbamate-induced gastric ulcer formation in the rat. Pharmacol Toxicol. 1990; 66(2):133-37. https://doi.org/10.1111/j.1600-0773.1990.tb00719.x PMid:2156249

34. Takeuchi K, Nishiwaki H, Niida H, Ueshima K, Okabe S.Duodenal ulcers induced by diethyldithiocarbamate, a superoxide dismutase inhibitor, in the rat: role of antioxidative system in the pathogenesis.Jpn J Pharmacol. 1991;57(3):299-310. https://doi.org/10.1016/S0021-5198(19)39814-2 PMid:1667532

35. OginoK, HobaraT, Kawamoto T, Kobayashi H, Iwamoto S, Oka S, et.al.;Mechanism of Diethyldithiocarbamate-Induced Gastric Ulcer Formation in the Rat.Pharmacology & Toxicology.1990;66: 133-37. https://doi.org/10.1111/j.1600-0773.1990.tb00719.x PMid:2156249

36. Shah DI, SantaniDD, GoswamiSS. A novel use of methylene blue as a pharmacological tool. Journal of Pharmacological and Toxicological Methods. 2006;54:273-277. https://doi.org/10.1016/j.vascn.2005.12.003 PMid:16488629

37. Shivam, SachanN, Chandra P. Pharmacological Screening Techniques for Evaluation of Gastric Ulcers: Principles, Mechanism and Procedures. Int. J. Pharm. Sci. Rev. Res., 2020;65(1):224-32. https://doi.org/10.47583/ijpsrr.2020.v65i01.032

38. AgbajeEO, FageyinboMS, AlabiOO. Gastro-duodenal protective effect of aqueous leaf extract of Daucuscarotasativus Linn. (Apiaceae) in rats and its possible mechanism of action. The Journal of Phytopharmacology. 2017; 6(3):156-63. https://doi.org/10.31254/phyto.2017.6301

39. Kanemasa K, Okamura H, Kodama T, Kashima K, Ibata Y. Time course of the induction of VGF mRNA in the dorsal vagal complex in rats with cysteamine-induced peptic ulcers.Molecular Brain Research. 1995;34:309-314. https://doi.org/10.1016/0169-328X(95)00183-S PMid:8750834

40. Rethinavel HS, Selvaraj DB, BalakrishnanSJ , Andrews JFV , Joseph JHM , Kandasamy M. Omeprazole treatment manifests anxiolytic effects in a cysteamine hydrochloride induced mouse model of gastrointestinal disorder. Heliyon. 2022; 8:01-06. https://doi.org/10.1016/j.heliyon.2022.e09787 PMid:35800723 PMCid:PMC9253648

41. Hernandez DE, Adcock JW, Nemeroff CB, Arthur JP range Jr. The effect of intra cisternally administered bombesin on cysteamine-induced duodenal ulcers in rats. European Journal of Pharmacology. 1982; 84:205-209. https://doi.org/10.1016/0014-2999(82)90203-5 PMid:7173320

42. Takeuchi K, Okada M, Niida H, Okabe S. Healing process of duodenal ulcers induced by indomethacin plus histamine in rats. Digestion. 1989; 42(4):202-211. https://doi.org/10.1159/000199847 PMid:2806753

43. Takeuchi K, Furukawa O, Okada M, Okabe S. Duodenal ulcers induced by indomethacin plus histamine in the dog: involvement of the impaired duodenal alkaline secretion in their pathogenesis. Digestion 1988; 39(4):230-240. https://doi.org/10.1159/000199631 PMid:3220176

44. Naito Y, Yoshikawa T, Yoneta T, Yagi N, Matsuyama K, Arai M et.al. A New Gastric Ulcer Model in Rats Produced by Ferrous Iron and Ascorbic Acid Injection. Digestion 1995; 56 (6): 472-478. https://doi.org/10.1159/000201278 PMid:8536816

45. Grisham MB, Ritter CV , Smith BF, Lamont JT, Granger DN. Interaction between oxygen radicals and gastric mucin. American Journal of Physiology-Gastrointestinal and Liver Physiology. 1987; 253(1): G93-G96. https://doi.org/10.1152/ajpgi.1987.253.1.G93 PMid:3605340