A Comprehensive Review on the Screening Models for the Pharmacological Assessment of Antiulcer Drugs

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Abstract: Background: Due to inappropriate diet, smoking, alcohol consumption, regular use of drugs like NSAIDs and sedentary lifestyle, one may feel upper abdominal pain which may be the predictor of the gastrointestinal disorder called Peptic Ulcer. When an imbalance occurs between the defensive factor and aggressive factor of the stomach, ulcer formation in the esophageal lining, stomach, or duodenum takes place. This leads to the formation of small sores that cause pain. Another condition that synergizes the abdominal pain is vomiting materials which look like coffee grounds, blood in the stool, black or tarry stools. This pain may increase after lunch or dinner. This problem persists, that often leads to the gastroenterologist's consultation.

Objective: There are many antiulcer screening models present for the determination of antiulcer activity of the drug molecule. The main objective of this study is to find which model is best for the determination of antiulcer activity.

Methods: A literature search was conducted on the databases namely Science direct and PubMed with the help of different keywords such as "Anti-ulcer", "In-vitro models" and "In-vivo models". The search was customized by applying the appropriate filters so as to get the most relevant articles to meet the objective of this review article.

Result: There are different research and review papers based on the antiulcer screening models for the determination of antiulcer activity of new drug molecules.

Conclusion: On the basis of our study, we found some useful models for the antiulcer activity of drugs and suggested that, if we use in-vitro and in-vivo methods together, then we may obtain the most relevant result in our research area.

Keywords: Helicobacter pylori, proton pump inhibitor, cysteamine, histamine, NSAIDs, cushing ulcer.

1. INTRODUCTION

Gastrointestinal diseases are very serious and common problems, which are causing maximum discomfort, morbidity, and mobility in human beings. It occurs in 10-15% of the population at a time. A peptic ulcer is a group of disorders which is responsible for the ulcer formation or mucosal lesions formation in the esophageal lining (swallowing pipe), stomach or duodenum (the first part of the small intestine). The small sores are formed due to the imbalance between mucosal defensive factors (bicarbonate, mucin, prostaglandin, nitric oxide, and other peptides and growth factors) and injurious factors (pepsin, Helicobacter pylori, NSAIDs gastric acid). Ulcer in the stomach is known as gastric ulcer while ulcer in the first part of the intestine is also known as a duodenal ulcer [1]. Sometimes, people feel that upper abdominal pain may increase after lunch or dinner, and sometimes people vomit materials which looks like coffee grounds, blood comes with stool, have black or tarry stools, all these symptoms cause severe abdominal pain. The gastric ulcer pain may increase with eating and we feel burning-like sensation in our stomach. While the duodenal ulcer increases with improper sleep or waking up late at night and eating. These symptoms indicate the severe presence of a peptic ulcer in your body. When these types of symptoms are not controlled by the counter drug, then the patient may be referred to a specialist called a gastroenterologist [2].

The microbe Helicobacter pylori (H. pylori) plays a critical role in peptic ulcer disease, and eradication of this microbe can minimize the complication of this disease. Many studies reveal that more than half of the world's population is affected by chronic H. pylori infection which directly affects gastroduodenal mucosa. By using triple chemotherapy i.e. histamine receptor antagonist, proton pump inhibitors and
sequential regimen, management of this disease can be done. If the disease condition is severe, we proceed with the surgical approach for the treatment. In the absence of *H. pylori* infection and NSAIDs drugs, a different category of ulcers may occur which are Zollinger-Ellison syndrome, truly idiopathic ulcers, cushingulcer and high dose upper abdominal radiotherapy that can also lead to a type of ulcer [3]. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are valuable agents in the treatment of various diseases like arthritis musculoskeletal disorders and inflammation, in a wide variety of clinical scenarios but these agents also cause peptic ulcer [4]. If NSAIDs are given in the presence of *Helicobacter pylori* (infection) significantly increases the risk of peptic ulcer bleeding [5]. Mammalian stomach has an ability to secrete concentrated hydrochloric acid in a very large quantity as we know that proteolytic enzyme pepsin and gastric acid are required to initiate digestion. Gastric acid does play a very significant and important role in protein hydrolysis and other digestive processes [6].

This secreted acid plays an etiologic role in producing different forms of discomfort like esophageal and duodenal injury under different conditions. In response of physiologic stimuli, human stomach produces/contains approximately 1 billion parietal cells which secrete hydrogen ions into the gastric lumen and the generation of these hydrogen ions is mediated by 3 pathways namely endocrine, paracrine, and neurocrine. By vagal postganglionic neurons, a neurotransmitter, acetylcholine is released which stimulates hydrogen ion generation directly via a parietal cell M3 muscarinic receptor. On parietal cells, Paracrine transmitter name Histamine binds with H2-specific receptors. In response to this, Adenylate cyclase is activated which increases adenosine 38, 58-Cyclic Monophosphate (cAMP) levels, and subsequently stimulates the generation of hydrogen ions. Gastrin secretion from antral G-cell which follows the endocrine pathway and stimulates the hydrogen ion secretion both directly or indirectly, in corpus and fundus, increases the stimulation of histamine secretion from enterochromaffin-like cells. A short pathogenesis of peptic ulcer is described in Fig. (1) [7, 8].

Combination of these three pathways control and regulate hydrogen ion secretion. A negative feedback mechanism governs and controls both gastrin release and the return of acid secretion to the basal level. Enterochromaffin-like cells are also known as controller cells. Many studies indicate that under different physiological conditions, some of the other neurotransmitters, like galanin, pituitary adenylate cyclase–activating peptide, and Vasoactive Intestinal Peptide (VIP), may play a very important role in regulating gastric acid secretion both directly or indirectly [9]. When gastric acid increases in our stomach, it causes mucosal damage and leads to the formation of gastric lesions. Gastric lesions are one of the most important tools for the determination of an antiulcer property of drug molecule because the size changes constitute useful information like if a drug is effective in the case of ulcer or not, and if the size of the lesion is small and less in number, then we can say that the prepared preparation is an effective antiulcer agent [10]. The measurement of gastric ulcer or gastric lesions is done after dissection of the stomach along its greater curvature from the rat and fixed on a}

plane board or transparent glass. After fixing it into board or glass, the gastric lesions are examined by microscopes like light or scanning microscope. Another examination method is performed by hand lens, though it is an old method of examination and ulcer investigation, later the size of lesions are measured. Nowadays, the stomach is also scanned by using the camera in ulcer investigation and later investigated by suitable or appropriate softwate programs like Scion, Image J and others. After examination, the investigator can calculate the ulcer index by different methods as per their vantage [10, 11]. The choice of a particular model for the evaluation of the antiulcer drug is difficult because every model has significant pros and cons. The choice of screening model is also influenced by local resources, study objective, the hypothesis being tested or researcher’s questions. Preclinical experiments were carried out in *in-vivo* models.

Colours for all the figures in this review are prepared according to the guidelines of “Guidelines for preparing color figures for everyone including the colorblind” given by Robert Roskoski Jr. [12]. In our review work, we have discussed different types of *in-vivo* and *in-vitro* screening model for the assessment of the antiulcer property of prepared preparation. We have also discussed the measurement of gastric lesions.

2. MATERIALS AND METHODS

A literature search was conducted on various database sources (like science direct, PubMed) with the help of the combination of different keywords: Anti-ulcer model, *in-vitro* *in-vivo* models of ulcer activity. The search was customized by applying the appropriate filter so as to get the most relevant articles to meet the objective of this review. The various numbers of papers are present on the antiulcer model for the newly synthesized drugs to determine their antiulcer activity.

3. TYPES OF MODELS FOR ANTIULCER ACTIVITY

After the exhausting literature survey, we have observed that there is ‘n’ number of models present in which, some of them are not in use nowadays because of difficulties in their procedure or because of poor result, while there are some other models present which provide good results in a short span of time. So basically, these models are either used in the laboratory with the help of instruments and chemicals while in some models, animal requirement is necessary. On the basis of the methodology, we divide antiulcer models with respect to *in-vitro* or *in-vivo*. The animal is not required for *in-vitro* models, while *in-vivo* models, animals are required for the development of gastric lesions and by the help of *in-vivo* model, we determine the activity of newly synthesized drug or molecule. So, these models are divided into two groups which are as follows.

3.1. *In-vivo* Models

The term *in-vivo* directly indicates the evaluation of drugs in a living organism, and evaluation of the anti-ulcer drugs is incomplete without using *in-vivo* model because ulcer is a disease which occurs in the internal environment of the body and forms lesions in humans. Peptic ulcer induced
by the pharmacological, surgical and by physiological manipulation in the animal. Usually, most of the experiments are carried out in rodents [11]. The main advantage of these models is that we can determine the actual antiulcer activity of drug preparation, though it is a time-taking process which is also a disadvantage of these models. Antiulcer activity of drug preparation also depends upon the type of models we select for the determination of antiulcer property. *In-vivo* models are used for evaluation of antiulcer drugs and these are as follows.

**Fig. (1).** Secretion of gastric acid and pathophysiology of peptic ulcer.
3.1.1. Stress Ulcer Models

Psychogenic factors like stress, play a very important role in the pathogenesis of gastric ulcers in man. The first time restraint as stress factor was published by Selye (1936) and after that Hanson and Brodie (1960) and Bonfils et al. (1966) described methods to study the effect of anti-ulcer drugs on immobilization stress in rats [13, 14].

Stress ulceration is caused due to stress and the conditions of stress depend upon various factors such as psychological factors, emotional factors, stressful events like burns, trauma, surgery etc. In the mucosal layer of stomach esophagus, the intestine lining of lesions occurs due to stress. This type of ulcer is commonly seen in very critically ill patients in the intensive care unit and sometimes it also causes gastrointestinal bleeding which is linked with a highly increased morbidity and mortality despite aggressive acid suppression treatment. A better understanding of the mechanisms of stress ulcer formation will help to develop useful prophylactic or therapeutic interventions that may lead to a decrease in morbidity and mortality [13, 14]. As we know that emotional stress and psychological factors are also responsible for an ulcer, these are also identified as important contributors for ulcer pathogenesis. After a severe earthquake in Japan, stress works as a contributory factor which rises the bleeding in gastric ulcers in elderly people [15]. It is commonly known that the patient with the severe disease, burn, traumas and others, develops severe intestinal bleeding or perforation is caused due to stress. Later, endoscopy also revealed that the stress is a major factor which is responsible for the ulcer formation [16-18].

The pros associated with this model is the animals that respond to centrally acting agents such as diazepam and imipramine suggesting a role of central neurotransmission in the production of gastric ulcer. Hence, this is a major advantage of using this model. This model helps to develop useful prophylactic or therapeutic interventions that lead to a decrease in morbidity and mortality rates. Cons associated with this model is that this method is time-consuming and needs continuous supervision of the animals in the activity cages, and another drawback of this model is, in some of the cases, animals developing activity stress gastric lesions do not respond to histamine H2 blockers which lead to deviation of the expected result.

There are four stress ulcer methods available for in-vivo studies.

3.1.1.1. Restraint Induced Ulcer

Restrain stress induced methodology is used mostly in the area of drug development or discovery of new compounds. Many agents like acetylcholine, atropine, ascorbic acid, ranitidine, clonidine, and many other agents have their predominant action but they also possess antiulcer, antacid anticholinergic and anti-histaminic activity and these agents directly affect gastrointestinal tract and despite all these agents, many centrally acting drugs have been also used in restraint stress methodology research including sedative hypnotic anti-psychotic and anti-depression. In all of these agents, anti-depressant drugs or psychoactive drugs are preferred mostly because in many studies, it was found that the psychological factors and depression factors also increased gastric secretion which directly affects the gastrointestinal tract [19, 20].

An interesting example of stress drug interaction is illustrated by Tanaka et al. In their study, they found that morphine increases noradrenaline release in non-stressed rats. While in restraint-stressed rats, morphine possesses opposite effect and decreases noradrenaline release. Another scientist named, Appelbaum and Holtzman found in their study that in restraint-stressed rats, morphine possesses higher analgesic effect while the non-stressed rats were very sensitive to the analgesic effect of morphine. The opiate receptor affinity was similar in both stressed and non-stressed ulcer [19, 20]. It shows that restraint stress may increase the release of endogenous opioids and when morphine is administered, then it reduces noradrenaline and decreases gastric stress ulcer formation.

Another study is conducted by Nakane et al. which illustrates that by using various centrally administered neuropeptides, they found that none of the peptides stimulates ulcer formation in restrained stressed rats. These restraint studies illustrate both the usefulness of the methodology as well as it also describes the complexity of drug effect and stress. Role of the central nervous system in case of the restraint stressed gastric ulcer is described in various studies. A scientist name Henke observed in his study that when stimulation was applied to the central nucleus of the amygdala, gastric erosions were produced. Thus, he concluded that this amygdala area of the brain responded to the emotional component of restraint stress [21]. In Fig. 2, procedure of the restrained stress induced ulcer followed in laboratories for the detection of antiulcer activity [22-24].

3.1.1.2. Coldwater Immersion Induced Ulcer

Cooling of rats in water during the restraint period accelerates the occurrence of gastric mucosal lesions formation and shortens the time of necessary immobilization. Water immersion stresses also known as stress which are responsible for the formation of gastric ulcer in rats. Heat shock or stress protein are synthesized by Eukaryotic cells in the response of physiological or environmental stress like ethanol, heat, heavy metals amino acid analogs and these proteins may play a very important role in thermotolerance and cytoprotective action [25].

When the rats are restrained in a cold environment thus due to the cold environment, mucosal lesions rapidly develop in animal's stomach. Yet the mechanism is not fully understood. Still previous studies shows that the changes in acid secretion, as well as gastric motility, are affected and lesions formation occurred in cold restrain fasted rats. In case of cold water immersion induced ulcer, the increasing frequency and amplitude are monitored with the help of intragastric balloons which are acutely implanted. Many scientists have also reported that acid hypertension is also associated with cold restraint. While some other authors also reported that no changes occur in restrain procedure [26].

Garrick et al. studied gastric motility is a major factor in cold restraint-induced lesion formation in rats and found that Physiological functions and gastric erosion production increases due to cold water immersion method [26]. Hamajima...
et al. studied FK506, An Immunosuppressive Agent, on water immersion model and found that stress due to immersion in water, in gastric mucosa myeloperoxidase activity increased due to water immersion stress and FKS06 reduced this myeloperoxidase activity [27]. Fernandez analyzed the cold-water restraint procedure in gastric ulceration and body temperature and find that hypothermia cause gastric ulcer during cold water emersion but the animal's conscious activity increase the severity of peptic ulcer disease [28]. By this method, Shian et al. studied the role of lipid peroxidation on gastric mucosal lesions and found that in the early phase of gastric lesions formation, lipid peroxidation plays a very important role while other factors are involved in the later phase of gastric ulcer formation [29]. In Fig. (3), procedure of the cold water immersion induced ulcer followed in laboratories for the detection of antiulcer activity [22-24]. Pros associated with this model are that rat models mimic the disease condition in humans, hence are suitable for analyzing the antiulcer activity of the drug on the basis of physiology. Gastric ulcers induced by cold water restraint stress or cold restraint stress or water immersion stress in rats or mice are known to resemble human peptic ulcer, both grossly and histopathologically. This model is reported to be successful. Cons associated with this model is that this model is specific for the detection or evaluation of mucosal and cytoprotective agents.

3.1.1.3. Stress and NSAIDs Immersion Induced Ulcer

As mentioned above, we have learned that stress is responsible for excessive acid secretion while NSAIDs are also responsible for gastric injury. If both are combined together, then their chances of gastric injury may increase also.

Nonsteroidal anti-inflammatory drugs are generally used for rheumatic disorder osteoarthritis and other different types of pain as well as inflammation [26, 30, 31]. Nowadays, out of all the population, one-third patients are taking NSAIDs since a very long period of time which increases the chances of gastric injury in those patients and it was also detected by endoscopy [32]. It is assumed that oxygen species production promoted by NSAIDs induces gastric mucosal apoptosis which is the main reason behind the gastric injury [33-36]. NSAIDs anti-inflammatory drugs cause a gastric ulcer or gastrointestinal injury through both topical as well as systemic effect which is caused by inhibition of the cyclooxygenase enzyme. Thus, prostaglandin formation is blocked which is responsible for the gastric injury. Still, there is a lack of evidence. Hence, we cannot say that it is a sole mechanism for gastric injury which is caused by NSAIDs [37].

Alsarra et al. studied the influence of cyclodextrin complexation with NSAIDs by this method and found that...
**Fig. (3).** Methodology of cold water immersion induced ulcers.

- Wistar Rats (150-200 gm)
- 16 hour fasting
- Test drug administered
- Animal placed individually in restraint cages and immersed in water at 22 °C for 1 hour
- Animals removed from cage
- Stomach is removed and ligated at both ends filled with formal saline and kept overnight.
- Animal sacrificed
- 10 min later
- Azovan blue (30 mg/kg) injected intravenously in tail vein

On the very next day, stomach is opened (greater curvature) and washed with warm water and examined ulcerative lesions. Azovan blue (Evans blue) helps in evaluation of lesions score, which is calculated by adding the length of longest diameter of the lesions.

**Fig. (4).** Methodology of stress and NSAIDs induced ulcers.

- Wistar Rat (Weight 150-170 gm. and starved for 24-36 hour)
- Test drug (in 1% carboxymethyl cellulose) given via gastric incubation and NSAIDs such as aspirin indomethacin or diclofenac
- Placed the rats in stress cage and immersed in water up to the level of xiphoid process at 23 °C for 7 hour.

The dose of NSAIDs require to increase gastric erosion by 100 % relative to immobilization is compared with that of NSAIDs require to produce 100 % increase in gastric erosion under the protective effect of drug.

The animals sacrificed and stomach is removed for the evaluation of ulcer index.
the cyclodextrin complex acts as a protective agent against gastrointestinal disorder which is induced by a combination of stress and NSAIDs [37, 38]. Pal et al. studied about Gallic acid which prevents non-steroidal anti-inflammatory drug-induced gastropathy and found that gallic acid inhibits mitochondrial dysfunction and activation of apoptosis in gastric mucosal cells which are induced by NSAIDs and responsible for gastric injury or gastropathy. Thus, it inhibits mitochondrial oxidative stress and prevents gastric injury [39]. While in the detection of the combined effect of stress and NSAIDs inducing ulcer in rats, we can follow the below procedure Fig. (4) and determine the effect of newly prepared preparation in the ulcer [22-24].

3.1.1.4. Swimming Stress Ulcer

As compared to the other stress methods, in swimming stress ulcer method, more ulcer formation occurs in the animal because it is a more depressed strain and a highly positive correlation is obtained between depression and ulcer severity scores [40].

In this method, animals are forced to swim in deep water. The individual animal is placed into a cylindrical chamber which is closed on both sides. Rats are forced to swim under 30 cm deep water at 23-28°C water so that feet of the animals cannot touch the surface of the cylinder. Minimum two swimming sessions were conducted and drug was administered between these two periods [41].

Armario A et al. studied the comparison between the behavioural and endocrine response of stress in rats by this method [42]. In Fig. (5), we have discussed the laboratory procedure for swimming stress ulcer method for rats.

Due to the release of histamine, acid concentration increases in the stomach and produces an ulcer. The gastrointestinal motility increases due to the result of folds in the stomach which is caused because of stress and when this gastrointestinal motility comes in contact with acid, damage chances increases. The quality and quantity of mucus also decrease due to stress and cause damage in the mucosal layer. It also decreases synthesis of the mucosal component and after the study of stress ulcer model, we can say that this experiment is useful for the antiulcer evaluation of prepared preparation because stress is a psychogenic factor so that not only anticholinergic, H₂ antagonist proton pump inhibitor but assessment of psychotropic drugs like neuroleptics, can be done by this method.

3.1.2. Pylorus Ligation Induced Ulcer in Rats

One of the easiest methods for the production of gastric ulceration in the rat based on ligation of the pylorus has been published by Shay et al. (1945). The mucosal lesions are caused by the accumulation of acidic gastric juice in the stomach.

It is the oldest method or oldest animal model for gastric ulcer which is developed by Shay et al. in 1945. It is the most common method for the antiulcer drug development and investigates the efficacy or antiulcer activity of drugs on gastric secretions already done by many investigators. Accumulation of gastric acid in the stomach occurs due to the ligation of pyloric end of the stomach. Due to the autodigestion of gastric mucosa which leads to the breakdown of
gastrointestinal barrier and forms ulcer. The models are used to evaluate the anti-secretory drugs that reduce gastric acid secretion or reduce gastric aggressive factors such as acid and pepsin which are responsible for increasing acid concentration. For the determination of the cytoprotective effect of the drug, preparation can also be measured by this model, which increases the secretion of mucus in the stomach [11].

Rastogi et al. studied the involvement of free radicals in the pyloric ligation induced ulcer in rats and found that generation of free radiicle and depletion of antioxidant increase acidity and peptic activity in gastric juice [43]. Kandhare et al. studied the ameliorative effect of Fisetin, a bioflavonoid by this method, and found that Fisetin significantly reduces lipid peroxidase level and neutrophil infiltration and prevents gastric ulcer formation [44]. Khustar et al. studied the ability of ginger oil against aspirin and pylorus ligated induced gastric ulcer and on their study, they found that the ginger oil has a protective effect against both aspirin plus pylorus ligated induced ulcer in rats [45]. Brodie DA gave the mechanism of gastric hyperacidity, demonstrated the relative importance of nervous and hormonal factors in the stimulation of rat gastric secretion [46]. The methodology of pylorus ligation induced ulcer is given in Fig. (6) [22-24]. Pros associated with this model is that this is helpful in the evaluation of antiulcer activity of the drug with a different mechanism of action at different doses, but the limitations of this model are that the ulcers are localized in the antrum of the stomach and are not beneficial for the expected result.

Easiest and most followed method for the determination of an antiulcer property of drug molecule, nowadays, the "Shay-rat" is a valuable tool to evaluate the anti-ulcer activity of a drug molecule. For the evaluation of the antisecretory effect of prepared preparation, this model can be used because antisecretory drugs reduce secretion of gastric aggressive factors like acid and pepsin. For the evaluation of the cytoprotective effect of drugs which increases secretion of mucus, this model is also used.

3.1.3. Histamine-induced Gastric Ulcer in Rats

As explained above that histamine is one of the major transmitters which is responsible for gastric acid secretions, thus on this basis, histamine-induced gastric ulcer model is used for the determination of the effect of the drug in case of excessive gastric acid secretion as well as for the study of antihistaminic drugs. This transmitter histamine not only causes gastric acid secretion but also causes disturbances in other parts of the stomach like gastric mucosa abnormal motility reduction in mucus production as well as microcirculation [41, 47].

Alphin et al. investigated antihistaminic activity in gastric ulcer. They followed this method for the study and found that the antihistaminic drug provides a protective effect against gastric ulcer for not more than 6 hours [48]. Okabe et al. studied the effect of cimetidine on duodenal and gastric ulcer and found that cimetidine is an H2 blocker and possesses action against ulcer formation but the problem with cimetidine is that it provides a protective effect in selective circumstances [49]. The methodology of the histamine-induced gastric ulcer is described in Fig. (7) [22-24].

As histamine is one of the most responsible factors for the secretion of gastric juice in the body so it is a useful tool for the assessment of antiulcer activity because of rapid mucosal formation in the animal stomach. Histamine is responsible for acid secretion in the body and that is the advantage of this model so that we can say that this method is most suitable because it produces 100% gastric ulceration. It increases the volume of gastric acid secretion and a marked enhancement of free and total acidity is observed or calculated. After the study of the histamine-induced ulcer model, we can say that this model is most suitable for the Histamine H2 receptor antagonistic activity of prepared preparation.

3.1.4. Ethanol-induced Mucosal Damage in Rats

There is another antiulcer model named as ethanol-induced mucosal damage, in this model, we used ethanol in excess amount because ethanol excessive amount causes gastritis which is characterized by sub-epithelial haemorrhages, cellular exfoliation, inflammatory cell infiltration, and mucosal oedema. These lesions can be at least partially inhibited by various drugs, such as some prostaglandins. Time to time this method has been modified by several authors [47, 50, 51].

Various studies also focus on the pathogenesis of ethanol-induced gastric mucosal injury which suggests that increased in vascular permeability, oedema formation, and epithelial lifting cause disruption of the vascular endothelium. Mechanism of this model is still unclear but on the bases, several factors like arachidonate metabolite products, oxygen-derived radical and mast cell secretory products are responsible for the gastric injury [52, 53]. While ethanol is considered as a risk factor for the development of ulcer because it easily and readily penetrates the gastric mucosa due to mucous solubilizing ability and exposes it into acidic environment of stomach and pepsin, which damage the membrane. Alcohol stimulates and increases acid secretion and reducing blood flow which further leads to injury at microvascular level [52]. It increases the activity of xanthine oxidase through vascular endothelium disruption and by facilitating vascular permeability. Ethanol also imbalances cellular oxidation processes in human [11, 54, 55].

Hollander et al. studied the effect of sucralfate against gastric ulcer by ethanol-induced gastric ulcer method and on the basis of their study, they concluded that sucralfate provides protection against ulcer and confirm that it provides protection in another method also [56]. Mizui et al. studied the effect of polyamines and nonprotein sulfhydryl by this method and on the basis of their study, they suggest that polyamine has a protective effect against ulcer [57]. Park et al. studied the preventive effect of flavonoid wogonin against ulcer by the ethanol-induced method and on the basis of their study, they concluded that flavonoid Wogonin which has strong anti-inflammation and apoptosis induction action can be used in case of ethanol-induced gastric ulcer which means it has antiulcer property [58]. For laboratory purpose, the methodology of ethanol-induced mucosal damage is elaborated in Fig. (8) [22-24].

As we know that alcohol stimulates acid secretion and reduces blood flow leading to microvascular injuries, through disruption of vascular endothelium which facilitates...
**Fig. (6).** Methodology of pylorus-ligated-induced peptic ulcers.
vascular permeability. Hence it is choice of model for antiulcer activity evaluation and reproducible method to produce gastric lesions in experimental animals. Despite all of the pros, there are some cons also associated with this model like ethanol is considered a risk factor for developing gastric ulcers as it penetrates the gastric mucosa due to its ability to solubilize and hydrolytic actions of hydrochloric acid and pepsin, causing damage to the membrane.

During the study of this model in a minimum dose range, several types of prostaglandin provide cytoprotection in rats and in the clinical study of prostaglandin show ulcer healing effect at antisecretory doses. Ethanol-induced model is not appropriate or useful for the determination of antisecretory drugs or prepared preparations due to the absence of gastric acid secretion despite this model is useful for the determination of the efficacy of drugs which have a cytoprotective or antioxidant effect.

3.1.5. Acetic Acid-induced Gastric Ulcer in Rats

Chronicity of peptic ulcer disease is characterized by repetitive events of healing and exacerbation. Without scar formation, most ulcerative lesions heal quickly and easily in

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**Fig. (7).** Methodology of histamine induced gastric ulcer.
Male Wistar Rat (Weight 250-300 gm.) Are deprived of food for 18 hours prior to the experiment but allowed to free access of water. Kept in cages to prevent cannibalism & coprophagy. Administered the appropriate vehicle of cytoprotective drug to rats like – Prostanoid before the administration of 1% absolute ethanol. And untreated animal are used as control group.

Subjective scores of the treated tissue are recorded. Stomach stretched in a piece of foam core-mat for mucosal site up. Excised stomach with greater curvature and gently rinsed with water. 1 hour after ethanol administration, Animals are anaesthetized with CO₂.

Circular full thickness area (13 mm. diameter) cut from each lobe of fundus just below the rigid portion dividing the glandular portion from the non-glandular portion of the stomach. Excised pair of tissue from each stomach placed in a Plexiglas tempeltem burnished on one side with emery cloth in which six holes are present in four rows. The template is positioned on Aristo model T16 cold cathode transilluminator (38/38 cm.) containing a w45 b/w white lamp and a camera mounted on a copy stand directly above the tempeltem. The optical density is displayed on a digital read out and recorded. Damaged tissue appears bright while undamaged tissue appears dark. Photographs are taken on the film in a stand manner. The optical density of the test tissue is determined by placing each area of the negative sequence.

Lower optical density value indicates more damage while higher optical density indicates little damage or no damage in case of control.

**Evaluation:**
The significance of difference in optical densities between control and ethanol treated is evaluated by non-paired single tail test.

Fig. (8). Methodology of ethanol induced mucosal damage.

A few days and spontaneously does not re-ulcerate. In 1969, this model is developed by Takagi and his co-workers, they induce chronic gastric ulcer by injecting acetic acid in the submucosa of rat and after that, recorded healing time after ulcer preparation. An ulcer formed by this procedure considered as chronic due to consumption of long time and resemblance to a human chronic ulcer. Since 1969, the required modification was also done in this method time to time by different other scientists [59, 60].

This method is useful for the chronic peptic ulcer and also used for their antisecretory and cytoprotective effects [60, 61]. This model is not only used in case of rats, instead, but we can also use it in other animals like cats, dogs, guinea pigs, Mongolian gerbils, miniature pigs, and monkeys. This model easily produces round, deep ulcers in the animal's stomach and duodenum of mice, rats [62]. The choice of this model is frequentas the chronic ulcer model due to the following reasons - (1) this model follows a simple procedure and gives the rapid result of ulcer severity of the incidence of this model which is 100%. (2) In terms of both pathological features and healing mechanisms, it resembles human ulcers and frequently relapse of healed ulcer is observed. (3) Ulcers of this model respond very well to antiulcer drugs such as proton pump inhibitor sucralfate, etc. [62, 47]. The ulcer models are used for screening of antiulcer drugs and the methodology of this model is explained in Fig. (9) [22-24].
Chenga studied the healing effect of Centella extract and asiaticoside against ulcer formation by this method. Centella extract stimulates antioxidant production in wound site and possesses healing effect and in wound tissues, these also increase the tensile strength and increase matrix components secretion such as collagen. It is also effective in the case of glycosaminoglycan's synthesis [63]. Wang studied the effect of delayed action of indomethacin on the acetic acid-induced ulcer and in their study, they found that it inhibits prostaglandin and in the healing process of gastric ulcers, endogenous prostaglandins may play an important role [64].

The main pros of this model are that it is a simple procedure resulting in ulcers of consistent size and severity at an incidence of 100%. It resembles human ulcers in terms of both pathological features and healing mechanisms and relapse of healed ulcers are frequently observed, just as in peptic ulcer patients. Cons of this model are that submucosal injection produced ulcers penetrating entire gastric wall & adherence of ulcer based on adjacent organs (mainly liver) and it is considered as a pitfall of this model. This model is most suitable for chronic peptic ulcer and used for the evaluation of the healing capability of the prepared preparation and also used to evaluate their antisecretory and cytoprotective effects.

3.1.6. Cystamine Induced Duodenal Ulcer

Cystamine induced duodenal ulcer were first introduced by Seley and Szabo in 1973. Cystamine and propionitrile cause ulcer in rats, the cysteamine ulcer model is very similar to human duodenal ulcer disease like increase fasted gastric serum level which is supersensitive to lunch or dinner, increase gastric acid secretion and rigid localization of gastric lesions on the walls of duodenum respectively anterior or posterior [65, 66]. Nowadays, animal models by using chemical duodenal ulcerogenic the most useful tool to determine the ulcer formation mechanism. The cysteamine-induced duodenal ulcer model is used by the different scientists for the evaluation of antiulcer drugs. A mouse is very convenient for cysteamine-induced duodenal ulcer model because of smaller size and low in cost and it requires few treatments for rapid production of ulcer which are similar to human disease [67]. Striking of duodenal ulcer in stomach and food sensitive hypergastrinemia in patients and animals (rats) is similar [68]. The cysteamine-induced ulcer is formed together with adrenocortical necrosis.

The histology of glucocorticoids in humans is almost impossible which is also documented in various studies [66]. After sialoadenectomy, Bedekovic et al. studied the effect of the different antiulcer agent by cystamine induced ulcer and found that different antiulcer agents (like ranitidine, atropine, and omeprazole) decrease and inhibit duodenal ulcer incidence by salivary glands in the rats [69]. Minaiyan et al. studied the effect of Zatariamultiflora Boiss. By Cystamine induced ulcer method and on their study, they found that the plant Zatariamultiflora Boiss possesses protective effect against duodenal ulcer and its higher dose possesses the
Male Wistar or Sprague Dawley Rats
(200 gm)

Test drug or standard drug administered on the first day of experiment to fed rats

After 30 minutes, first dose of Cysteamine HCL administered in the dose of 280 mg/kg orally, three times a day

After 24 hours from first dose of cysteamine, test drug or standard drug administered

A small ulcer usually presents on the posterior wall of the duodenum and it invariably penetrates in pancreas.

The duodenal ulcer develops 2-4 mm away from pylorus on the anterior wall of duodenum and frequently penetrates in liver

After 48 hours from first dose of cysteamine, test drug or standard drug rats are sacrificed.

The intensity of the duodenal ulcer is evaluated using the scores from 0 to 3
0 = no ulcer
1 = superficial mucosal erosion
2 = deep ulcer usually with transmural
3 = perforated or penetrated ulcer

Fig. (10). Methodology of cysteamine induced duodenal ulcers.

same effect in comparison to reference drug [70]. Saghaei et al. studied the captopril effect by this method against ulcer formation and on the bases of their study, they found that the captopril inhibits the decrease in superoxide dismutase and glutathione peroxidase activities and by increasing antioxidant defences, it also causes lipid peroxidation. So that captopril provides a protective effect against ulcer which is induced by the Cysteamine method. The methodology of the cysteamine-induced ulcer model is described in Fig. (10) [22-24].

Pros of this model is that in this method, ulcerogenesis is seen with one of the cysteamine. It is an easily reproducible method and is an acceptable method for scrutinizing the pathogenesis of duodenal ulcer. This method is one of the successful methods for anti-ulcerogenic regimes detection. As some of such chemicals might have a role in the aetiology of human duodenal ulcers as well, therefore the structure-activity conclusions may help in the identification of ulcerogenic chemicals in human diet and environment. Cons associated with this model is that the ulcers are located on the anterior wall, the walls frequently get perforated resulting in peritonitis or penetrate into the liver and a small ulcer is usually present on the posterior wall of the duodenum which penetrates the pancreas and results in unfavourable conditions. For the determination of activity of prepared preparation in case of duodenal ulcer, we can use this model.

3.1.7. Indomethacin + Histamine-induced Duodenal Ulcer

As mentioned above, we have studied how histamine control and regulate gastric acid secretion in our body and most of the time, it is also responsible for the formation of ulcer despite many models like cysteamine NSAIDs and other models. Various scientists have also studied other models in which indomethacin is given with histamine and both are responsible for gastric lesions formation. For prostaglandin synthesis, Indomethacin is a potent inhibitor which is also responsible for the development of an ulcer in animals as well as human. Gastric lesions which are induced by indomethacin are inhibited by antacids, anticholinergic and by using vagotomoy [71-74].

Single dose administration of indomethacin and after that dosing with histamine produce gastric lesions in rat's proximal duodenum at the opposite site of mesenteric attachment [75]. Levine et al. studied the effect of indomethacin by this method and on the basis of the study, they are concluded that indomethacin reduces endogenous prostaglandin level which is responsible for gastric ulcer formation in the stomach and increase gastric acid directly affect mucosa of the stomach.
Takeuchi et al. performed indomethacin plus histamine-induced duodenal ulcer experiment in dog and found that indomethacin constantly causes duodenal ulcer while histamine increases gastric acid secretion and both works together so as to increase ulcer formation in the stomach of the dog [77]. In case of duodenal ulcer, Takeuchi et al. studied the healing process in this method and concluded that the agent which are used for the determination of antiulcer activity if they decrease acid secretion and stimulate alkaline secretion to possess beneficial effect on the healing of duodenal ulcer. This method is one of the useful methods for the determination of antiulcer activity [78]. The methodology of indomethacin plus histamine-induced duodenal ulcer is described in Fig. (11) [22-24].

For rapid ulcer formation and determination of the antiulcer activity of prepared preparation, we can use this method. According to some investigator stress, an induced ulcer can be treated by H2 receptor antagonist while indomethacin and an aspirin-induced ulcer cannot be treated by H2-receptor antagonists.

Pros of this model are that it is a simple procedure with a high incidence of ulcer formation and no mortality. The physiological factors involved in this model appear to be relevant to the pathogenesis of human duodenal ulcer disease and for screening anti-ulcer agent. This model shows that both an increase in gastric acid secretion and an impairment of HCO3 secretion are responsible for ulcer production. One of the major cons of this model is that the submucosal injection produced ulcers penetrating entire gastric wall & adherence of ulcer base to adjacent organs. Both histamine and Indomethacin are responsible for increased acid secretions in stomach further which causes mucosal damage so that we can say that if a researcher wants to evaluate the effect of prepared preparation against both (indomethacin and histamine), then this method is most suitable for screening.

3.1.8. NSAIDs Induced Gastric Mucosal Damage

Non-steroidal anti-inflammatory drugs (NSAIDs) are used for the treatment of inflammation but somewhere these NSAIDs also cause gastrointestinal disease like ulcer formation gastric acid secretion. NSAIDs drugs such as indomethacin, aspirin, and ibuprofen are also known for the formation of gastric ulcers. Due to this phenomenon, we used NSAIDs drugs for the ulcer formation in models for screening purpose. The model is useful for the determination of cytoprotective anti-secretory and antiulcer activity of drugs [78, 79].

NSAIDs drugs increase gastric lesions formation in the animal's stomach if other factors are present as if we use NSAIDs in case of depression then it increases gastric acid secretion and if Helicobacter pylori is present, then in combination with NSAIDs, it also increase gastric secretion which is responsible for ulcer formation or lesions formation in animal as well as human's stomach. In cyclooxygenase, pathway NSAIDs inhibit prostaglandin synthetase which is lead to the formation of ulcer because of prostaglandin if found in many tissues of body as well as also present in the stomach and in stomach, they play a very important role by stimulating the bicarbonate, and mucus secretion prostaglandin also maintain mucus blood flow and repair and regulate mucosal cell. By blocking prostaglandin secretion, these NSAIDs are also decreasing bicarbonate and mucus secretion which is further lead ulcer formation epithelia damage reduce angiogenesis and increase leukocyte adherence. These NSAIDs also increase mucosal hydrogen peroxide and hydroxyl ion by inhibiting gastric peroxide which is respon-
sible for oxidative mucosal damage. Drugs which are acidic nature directly affect and kill an epithelial cell [11, 78, 79].

Graham et al. studied the effect of misoprostol on the prevention of NSAIDs induced ulcer and on their experiments, they concluded that misoprostol possesses a protective effect against gastric ulcer and duodenal ulcer [79]. Hence, we can conclude that NSAIDs are also responsible for the gastric lesions. The procedure of the gastric mucosal damage by NSAIDs drug is described in Fig. (12) [22-24].

Pros associated with this model is that this method gives an effective result in the detection of the effect of the antiulcer drug on gastric blood flow. It is a suitable method for the detection of the effect of COX inhibitors on leukocyte adhesion. Cons associated with this model is that inhibition of both COX-1 and COX-2 is required for NSAIDS - induced gastric injury in rats because neither a COX-1 nor a COX-2 inhibitors cause macroscopically or histologically detectable gastric damage when given individually in most of the cases. If we want to see the effect of particular prepared preparation

Fig. (12). Methodology of NSAID induced gastric mucosal damage.
Acidity is one of the major gastrointestinal problems which cause functional disorder like peptic ulcer and ultimately effect mucosal layer of stomach. To the neutralization of this type of acidity, we use various types of antacids either allopathic antacids or herbal antacids which are easily available in your nearest shop nowadays and these antacids are neutralizing gastric acids by reducing the gastric pH. For the assessment of antiulcer drugs in the laboratory, following In vitro methods have been developed which are helpful to determine the capacity and effect of the drug in peptic ulcer or gastric ulcer or other diseases [83].

For the herbal extracted drug and for antacids, these In-vitro model is most suitable for the determination of an antiulcer property of extract. These models are easy to use and less time consuming which are its pros. But the major cons associated with these models are that we can't totally depend upon the result which is obtained from these models because there are so many laboratory conditions which can affect the result of these models like if proper procedure is not followed during the preparation of artificial gastric juice then it leads major errors in results.

3.2.2. Determination of the Duration of Neutralization Capacity of Prepared Preparation on Artificial Gastric Acid

In this method, we prepare laboratory acid and pH is adjusted according to the stomach conditions like pH - 1.2 - 2.5. This is a highly acidic condition at which damage of mucosal layer starts and if this pH is not controlled in time then it is responsible for the cause of the gastric ulcer. The plant extract or prepared preparation (drug) and reference drug is added separately in artificial gastric juice at pH 1.2 - 2.5 and then this mixture examined the neutralizing effect on artificial gastric juice by titration method. Excess acid is neutralized in titration time and we determined that the exact amount of prepared preparation neutralizes the artificial gastric juice. For the preparation of artificial gastric juice, take NaCl (2 gram) and pepsin (3.2 mg) and dissolve them in distilled water (500 ml). Then after that, hydrochloric acid (7.0 ml) is added and the sufficient amount of water is added to increase the volume [11, 84].

3.2.1. Neutralization Effects of Prepared Preparation on Artificial Gastric Acid

In 1952 Reserpine is isolated from the root of the Rauwolfia serpentina, which is used for the treatment of hypertension but it produces an adverse effect like gastric mucosal lesions (GMLs), sexual dysfunctions and depression due to overdose and if it is used in long term. Reserpine induces gastric lesions by decreasing sympathetic tone and increasing cholinergic tone which is responsible for excessive gastric acid secretion [80].

Xiu-Juan MA et al. reviewed this method and concluded that mucosal lesion induced by this method are dose-dependent and lesions healed spontaneously in 3 weeks. Reserpine-induced gastric mucosal lesions were dose-dependent. The mucosal lesions which are formed by this method healed spontaneously within 3 weeks. While treatment with compound hypotensive tablets will not induce gastric mucosal lesion [80]. Calcitonin possesses an antulcerogenic effect by directly acting on the central nervous system. Calcitonin is present in the thyroid cell. Indirect synthesis and releasing of prostaglandin by calcitonin are helpful and contribute to the maintenance of gastric mucosa [81]. The methodology of reserpine-induced duodenal ulcer is described in Fig. (13) [82]. Pros of this model are that this model can be preferentially used for studying the acute as well as chronic ulcers. This method is most suitable for analyzing the ulcer inhibition mechanism of calcitonin. Cons of this model are that the method is dose-dependent and acid-dependent. Hypermotility is seemed to be more important than hypersecretion.

3.2. In-vitro Models

Acidity is one of the major gastrointestinal problems which cause functional disorder like peptic ulcer and ultimately effect mucosal layer of stomach. To the neutralization of this type of acidity, we use various types of antacids either allopathic antacids or herbal antacids which are easily available in your nearest shop nowadays and these antacids are neutralizing gastric acids by reducing the gastric pH. For the assessment of antiulcer drugs in the laboratory, following In vitro methods have been developed which are helpful to determine the capacity and effect of the drug in peptic ulcer or gastric ulcer or other diseases [83].
parts, an artificial stomach, a peristaltic pump for creating the environment same as the human body and a pH recording system for the determination of duration neutralization of acid. In apparatus, the artificial stomach is made up of 3 portions (secretary flux, reservoir, and gastric emptying flux). In the reservoir portion of the stomach 90 ml of prepared preparation or distilled water or reference, the drug is added in 100 ml of gastric juice where pH is 1.2 at 37°C. Continuous stirring at 30 rpm is done by the help of 2.5 cm. magnetic apparatus. Artificial gastric juice at 3ml/min is pumped out and 3 ml/min is again added in a reservoir at the same time. In the reservoir of an artificial stomach, a pH meter is also attached which is helpful to determine the changes of pH [1, 3, 11, 85].

3.2.3. Using a Titration Method of Fordtran’s Model for the Determination of Neutralizing Capacity In-vitro

250 ml beaker is taken and 90 ml of prepared preparation and reference drug in different beakers are placed at 37°C, then continuous stirring is done with the help of magnetic stirrer at 30 rpm for creating stomach movements or stomach environment. Then these (prepared preparation and standard reference) are titrated separately with artificial gastric juice to the endpoint of pH 3. The consumed volume of artificial gastric juice is measured as a reference drug. We take sodium bicarbonate or we can use the combination of magnesium hydroxide and aluminium hydroxide [11, 86]. In place of synthetic preparation, we also take plant extract for the determination of neutralizing capacity of extract and some of the plant extract like Tephrosia maxima Linn [87], Tephrosia purpurea Linn. [88] Garcinia indica. [2] Tephrosiacaalopolphylla Bedd. [87] and Laporteaestuans Linn. [89] are already used in the study [1, 4].

As mentioned above all these three In-vitro methods, we see that neutralization of gastric acid is one of the most followed method and the increased gastric acid concentration is responsible for the formation of gastric mucosal lesions. So here we also discuss the easiest laboratory method for the determination of the acid neutralizing capacity of prepared preparation.

3.2.3.1. Acid Neutralizing Capacity

After food ingestion or stress, stomach release gastric acid somewhere which is a most responsible factor for the formation of a gastric ulcer. When the stomach produces gastric acid in a very excess amount, this condition is known as acid indigestion or acid reflux. Commercial antacids or other allopathic preparation like H2 blocker or other containing one or more bases as well as different basic chemical material which are available to treat the excessive gastric acid condition by neutralizing acid in the stomach. The Acid Neutralizing Capacity (ANC) of a drug is the amount of acid that it can neutralize by the drug. In laboratory acid, neutralizing capacity can be measured by a process known as back titration. In this process, prepared preparation dissolves in the excess of artificial gastric acid and then this acidic solution titrated against a known amount of base until the endpoint is reached. The number of moles of acid neutralized is equalized to the difference between the moles of base required and the moles of acid added for the back titration [90, 91].

For this Investigation.

\[
\text{Moles of acid neutralized} = \text{moles of HCl added} - \text{moles of NaOH required} \\
= (\text{Volume of HCl X Molarity of HCl}) - (\text{Volume of NaOH X Molarity of NaOH})
\]

Acid neutralizing capacity per gram of antacid = moles of HCl neutralized grams of antacid

3.2.4. H+/K+-ATPase Inhibition Assay

Several H+/K+-ATPase inhibitors contain a sulphydryl group. At lower pH-values, the compounds are protonated and rearrange to a sulphenic acid and a sulphenamide that reacts with sulhydroyl groups in the enzyme. Therefore, the In-vitro assays are performed both at neutral and at acidic pH levels. H+/K+-ATPase also called a proton pump is responsible for acid secretion. In canaliculi of parietal cell, it exchanges intracellular H+ with extracellular K+ in response to the stimulation by histamine acetylcholine and gastrin. Proton pump inhibitors are now established antulcer drugs [90]. The examples of herbal by this method include Cleome viscosae Linn. Piper tuberculatum Jacq [91-93]. In Fig. (14), procedure of the H+/K+ ATPase inhibition assay was followed in laboratories for the detection of antiulcer activity [23, 24].

4. MEASUREMENT OF GASTRIC LESION

The measurement of gastric lesions is done after dissecting the stomach (greater curvature) from the animal body and is placed into the glass board and fixed it so that the stomach movement inboard is prohibited. The examination of ulcer lesions is done by microscope with a hand lens, and transparent paper is placed in a graph paper to measure the size of lesions. In place of microscopic or hand lens, we can use a light microscopic or scanning microscope. There are some methods also present for the determination or calculation of the ulcer index as well as protective and/or curative ratios for the ulcers. The different investigators have described a different method for the determination of ulcer index percentage, protective percentage, curative like Takagi and Okabe described method for the evaluation of the ulcer index as well as the severity of gastric lesions in their study. Unlike these scientists, there are some other methods which are used to determine the ulcer index [89, 92-95].

There are different scoring systems available for the measurement of gastroduodenal ulcers or ulcer index calculations. If we look forward to the scoring methods of the particular systems, we’ll then find that some methods developed by researchers like Takagi and Okabe, consider ulcer number rather than ulcer size which describes that the severity of ulcer in this scoring system may be statically correct but biologically irrelevant [96]. Some researchers try to reduce biological incorrectness level in ulcer quantifications like Nwafor et al. calculate ulcer index without considering total ulcer area in relation to total mucosal area, measure only the length of erosive ulcer while some researchers differentiate lesions types such as perforated ulcer, deep ulcer, haemorrhagic streaks, and spot ulcer but these different type of lesions cannot give information according to all parameters [97, 98]. On the basis of size, Andrade et al. character-
UI = UN + US + UP X 10⁻¹
UI = Ulcer index
UN = Average of the number of ulcer per animal
US = Average of severity score
UP = Percentage of an animal with ulcer
0.0 normal colored stomach, 0.5 red coloration
1.0 spot ulcers, 1.5 hemorrhagic streaks
2.0 ulcers with area >3 but ≤5mm², 3.0 ulcers >5mm².

The method described by Ganguly is that after each experiment, stomach is removed from the body and opens with greater curvature and spread it into clean cardboard. The area of ulceration erosions is traced by placing the tracing paper on the stomach which is then superimposed on a graph paper having a millimetre scale. The total area of stomach mucosa and the ulceration area are measured.

Relative Area = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}

For standard drugs like omeprazole which produces active metabolite at acidic pH. The microsomal homogenate is initially suspended in buffer at pH 6.1 along with standard drug for 30 minutes followed by homogenate transfer buffer in pH 7.4 and procedure from the addition of Malachite green colorimetric reagent is followed and % inhibition of H⁺/K⁺ ATPase is calculated.

The relative area is used to determine the ulcer index according to the scale which is given into Table 1.

**Table 1. The relative area and corresponding ulcer index.**

<table>
<thead>
<tr>
<th>Relative Area/mm²</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ulcer</td>
<td>0</td>
</tr>
<tr>
<td>91–100</td>
<td>0.1</td>
</tr>
<tr>
<td>81–90</td>
<td>0.2</td>
</tr>
<tr>
<td>71–80</td>
<td>0.3</td>
</tr>
<tr>
<td>61–70</td>
<td>0.4</td>
</tr>
<tr>
<td>51–60</td>
<td>0.5</td>
</tr>
<tr>
<td>41–50</td>
<td>0.6</td>
</tr>
<tr>
<td>31–40</td>
<td>0.7</td>
</tr>
<tr>
<td>21–30</td>
<td>0.8</td>
</tr>
<tr>
<td>11–20</td>
<td>0.9</td>
</tr>
<tr>
<td>1–10</td>
<td>1.0</td>
</tr>
<tr>
<td>Perforation</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**CONCLUSION AND DISCUSSION**

For validation of the presence of antiulcer activity in the newly synthesized drug which is going to be used by the practitioner to treat gastric mucosal lesions or ulcer, then it is essential to investigate the antiulcer property of the synthesized drug. The various number of antiulcer screening mod-
els have been developed over the past years in a different part of the world in which some are very good and some are not. Each and every model has its own pros and cons. The main objective for doing this review is to present an overview of antiulcer models which can be most frequently used by investigators for their gastrointestinal protection studies. After the study of antiulcer models, we found that the above mention models are widely used nowadays as per drug requirement and choice of the model also depends upon their result and timing. in-vitro models are less used in comparison with in-vivo but as per our study, we suggest that if both in-vitro and in-vivo models are used together then there is a chance to increase the efficacy of result and it will help to find the better antiulcer activity of new drug molecules.

CONSENT FOR PUBLICATION
Not applicable.

FUNDING
None.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS
We would like to thank Robert Roskoski jr. for the preparation of guidelines entitled “Guidelines for preparing color figures for everyone including the colorblind”.

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