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Review Article

In Vivo Models Used for Evaluation of Potential Antigastroduodenal Ulcer Agents

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Peptic ulcer is among the most serious gastrointestinal diseases in the world. Several orthodox drugs are employed for the treatment of the disease. Although these drugs are effective, they produce many adverse effects thus limiting their use. In recent years, there has been a growing interest in alternative therapies, especially those from plants due to their perceived relative lower side effects, ease of accessibility, and affordability. Plant medicines with ethnomedicinal use in peptic ulcer management need to be screened for their effectiveness and possible isolation of lead compounds. This requires use of appropriate animal models of various ulcers. The limited number of antiulcer models for drug development against gastric and duodenal ulcer studies has hindered the progress of targeted therapy in this field. It is, therefore, necessary to review the literature on experimental models used to screen agents with potential antigastroduodenal ulcer activity and explain their biochemical basis in order to facilitate their use in the development of new preventive and curative antiulcer drugs. Clinical trials can then be carried out on agents/drugs that show promise. In this paper, current *in vivo* animal models of ulcers and the pathophysiological mechanisms underlying their induction, their limitations, as well as the challenges associated with their use have been discussed.

1. Introduction

Peptic ulcer diseases comprise heterogeneous disorders, which manifest as a break in the lining of the gastrointestinal mucosa bathed by acid and pepsin. It is the most predominant of the gastrointestinal diseases [1, 2] with a worldwide prevalence of about 40% in the developed countries and 80% in the developing countries. It is generally recognized that peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors [3]. Based on site of attack, peptic ulcer may be classified as oesophageal, duodenal, or gastric. The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermal growth factors) [4]. According to Peckenpaugh and Poleman [5], some other factors, such as bad dietary habits, excessive intake of nonsteroidal anti-inflammatory agents, stress, hereditary predisposition and *Helicobacter pylori* infection, which is reported to account for more than 70% of cases, are responsible for the development of peptic ulcer diseases [6].

Several orthodox pharmaceutical drugs such as anticholinergic drugs, histamine H2-receptor antagonists, antacids, and more recently, proton-pump inhibitors have been employed in the management of peptic ulcers, but they provoke many adverse effects. In recent years, there has been growing interest in alternative therapies especially from plant sources due to their perceived lower side effects, ease of accessibility and affordability [7]. Plants are some of the most attractive sources of new drugs, and some have been shown to have promise for the treatment of gastroduodenal ulcer

with minimum side effects [8, 9]. Plants with traditional ethnomedicinal uses in peptic ulcer management thus need to be screened for potential antiulcer activity and as sources of antiulcer lead compounds. It is, therefore, necessary to have credible experimental models that can be used to screen such phytomedicines with potential antigastroduodenal ulcer activity.

There are several models that are used to evaluate antiulcer medicines. However, the choice of a suitable model has proven to be difficult as each model has significant advantages as well as disadvantages. The choice of a particular model is sometimes influenced by local resources, the objectives of the study, the hypothesis being tested, or research questions being answered by the researcher. The choice of model may also depend on the relevance to the type of peptic ulcer disease under investigation. Generally, preclinical experiments should be carried out *in vivo* and supported, when possible, with *in vitro* studies to explore the mechanisms of action of drug candidates with antiulcer activity.

Some challenges associated with the various models for peptic ulcers are that, apart from information on their pathophysiological and biochemical bases being scanty, they are also scattered in the literature and not easy to find. Thus the aim of this paper is to provide a comprehensive overview on available peptic ulcer models with the objective of making known their scientific bases and their relevance in pharmacological research. These models could also serve as tools that could help to better understand the pathophysiological mechanisms, such as antisecretory, gastrocyto-protective, gastrohealing and antioxidant mechanisms underlying medicines or agents that have antiulcer effects.

Currently, a comprehensive paper is available for an *in vitro* model for *Helicobacter pylori* [10], and can be employed to evaluate medicinal plants for anti-peptic ulcer activity. The current paper, focuses on discussions of models for *in vivo* peptic ulcers, the mechanisms underlying their induction and indices used to measure the extent of the induced ulcers.

2. Experimental Peptic Ulcer Models

Peptic ulcers can be induced by physiological, pharmacological or surgical manipulations in several animal species. However, most experiments in peptic ulcer studies are carried out in rodents. Several models are used experimentally for testing or evaluating antipeptic ulcer activity of drugs/agents, and these include the following:

(i) water-immersion stress or cold-water-restraint or cold-restraint stress [11–13],

(ii) NSAIDs- (indomethacin, aspirin, and ibuprofen) induced gastric ulcers [14–16],

(iii) ethanol-induced gastric ulcers [17, 18],

(iv) acetic acid-induced gastric ulcers [19–21],

(v) histamine-induced gastric ulcers [22],

(vi) reserpine-induced gastric ulcers [23],

(vii) serotonin-induced gastric ulcers [24, 25],

(viii) pylorus-ligated-induced peptic ulcers [26],

(ix) diethyldithiocarbamate- (DDC)-induced peptic ulcers [27],

(x) methylene blue-induced ulcers [28],

(xi) ischemia-reperfusion- (I-R-) induced gastric ulcers [29],

(xii) cysteamine-induced duodenal ulcers [30],

(xiii) indomethacin-histamine-induced duodenal ulcers [31],

(xiv) ferrous iron-ascorbic acid-induced gastric ulcers [32],

(xv) acetic acid-*H. pylori*-induced ulcers [33].

All *in vivo* animal models can be used to investigate the preventive or curative properties of medicines or drugs depending on the time of induction of the peptic ulcer. For preventive studies, it is advisable to pretreat animals for at least two weeks before the ulcerogen is administered to induce the peptic ulcer, after which measurement of the degree of ulcers is taken with the appropriate index to determine the extent of ulcer prevention achieved. In the case of healing or curative models, the ulcers are induced after which the animals are treated for at least two weeks followed by measurement of the extent of ulcers with the appropriate index to determine the degree of healing of the ulcers [34]. In employing these models, it is advisable to decide on an appropriate experimental design. Nyarko et al. [34] recommend that, for *in vivo* studies, animals should be placed in a minimum of seven groups of at least six animals in each group. Generally, Group 1 is designated as negative control (where animals treated with ulcerogen are not treated with any reference drug or test material such as plant extract except for water). Group 2 serves as positive controls (animals treated with ulcerogen are given a reference drug). Group-3 animals are normal controls (the animals are not treated with any ulcerogen except with the vehicle used to prepare the test agent. In this design, animals in Group-4 to 7 treated with the ulcerogen are also treated with different doses of the extract test material. In all experiments, it is important to keep the animals in cages with raised floors of wire mesh to prevent coprophagy. In addition, for preventive models, it is advisable to compare the potential drug or test material with cytoprotectant reference drugs such as misoprostol and sucralfate that are known to prevent peptic ulcers [35]. In the case of healing, or curative studies, the use of histamine receptor antagonists such as cimetidine or ranitidine, and proton-pump inhibitors such as omeprazole, is recommended as reference drugs. A combination of proton-pump inhibitors with antibiotics such as clarithromycin could be used when *H. pylori* is employed as an ulcerogen.

2.1. Water-Immersion-Stress-Induced Ulcer Model. Various physical and psychological stressors cause gastric ulceration in humans [36], and rat models have been developed to mimic the disease condition in humans. This model employs the restraint technique developed by Brodie and Hanson [37] coupled with the cold-water or ordinary-water immersion method by Levine [12]. The combination of these methods

is reported to be synergistic in inducing acute stress lesion in rats [11], arising mainly from physiological discomfort. Gastric ulcers induced by cold-water-restraint stress (CWRS) or cold-restraint stress (CRS) or water-immersion stress (WIS) in rats or mice are known to resemble human peptic ulcers, both grossly and histopathologically [33]. The model is widely used and is reported to be useful for assessing or studying the effects of agents/medicines on the healing of ulcers in rats.

Stress-induced ulcers manifest as single or multiple mucosal defects. The pathophysiology of stress-induced ulcers is complex. The ulcers are produced due to the release of histamine, leading to an increase in acid secretion, a reduction in mucus production [38], pancreatic juice reflux, and poor flow of gastric blood [39]. Stress also causes an increase in gastrointestinal motility resulting in folds in the stomach [40] that are more susceptible to damage when they come in contact with acid [37]. Furthermore, stress has also been found to decrease the quality and amount of mucus adhering to the gastric mucosa. It has been suggested that, in conditions of emotional tension, there is not only a greater destruction of mucus and decreased synthesis of its components, but also a quality change that affects the translation, acylation, and glycosylation of the ribosomal peptides [40]. Implicitly, the stomach wall mucus plays an important role in stress-induced glandular lesions. Increased vagal activity has also been reported to be one of the factors involved in stress-induced ulcers [37]. Due to the critical role that mucus plays in protecting the stomach and also enhancing healing in the stomach walls, the model is recommended for use when evaluating mucosal and cytoprotective agents.

The procedure for inducing ulcers with the water-immersion-stress-induced ulcer model, include animals being fasted for a period of 24–36 hours prior to the experiment. Ulcers are then induced by placing animals individually in a restricted cage and immersing them vertically in water tank, (15–20°C) gradually to the level of the xiphoid for 17 hours in the case of "water-immersed model", or 2–4 hours in cold water when employing the "cold water-immersed model" or in restraint cold ventilated refrigerator at a temperature of 2-3°C for 2–4 hours in the case of "cold restraint stress model" [11, 13, 41].

2.2. NSAIDs Induced Mucosal Damage. Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, aspirin and ibuprofen are known to cause gastric ulcers, especially when abused. This phenomenon has been employed in the development of NSAIDs-induced gastric ulcer models in rats. The model is important in investigating the potential usefulness of anti-secretory and cytoprotective agents since the underlying pathophysiology involves gastric acid secretion and mucosal prostaglandin synthesis. It is the most commonly used ulcer model in antiulcer studies. The frequency of usage could be attributed to the fact that NSAID induced peptic ulcers are the second most common etiology of peptic ulcers aside those caused by *Helicobaceter pylori*.

NSAIDs are known to induce ulcers by inhibiting prostaglandin synthetase in the cyclooxygenase pathway [42]. Prostaglandins are found in many tissues including the stomach, where they play a vital protective role via stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal cell turnover and repair [43]. Thus, the suppression of prostaglandin synthesis by NSAIDs results in increased susceptibility to mucosal injury and subsequently gastric ulceration.

The pathogenesis of NSAIDs-induced gastric ulceration includes the NSAID blocking the activities of the cyclooxygenase enzymes (COX-1 and COX-2) hence leading to reduced mucus and bicarbonate secretion, decreased mucosal blood flow, impaired platelet aggregation, alteration of microvascular structures leading to epithelia damage, reduced angiogenesis, and increased leukocyte adherence [44]. Increased production of reactive oxygen species (ROS), increased lipid peroxidation, and neutrophil infiltration also play a role in oxidative mucosal damage by NSAIDs [45, 46]. NSAIDs also inhibit gastric peroxidases and may increase mucosal hydrogen peroxide and hydroxyl ion levels that will cause oxidative mucosal damage. NSAIDs, particularly those of acidic nature, can directly kill epithelial cells. Various mechanisms have been proposed for this cytotoxic action of NSAIDs, including the induction of osmotic lysis subsequent to trapping of charged NSAIDs with the epithelial cells and death of the epithelial cell subsequent to uncoupling of oxidative phosphorylation [15]. NSAIDs can also reduce mucus and bicarbonate secretion, thus decreasing the effectiveness of the juxtamucosal pH gradient in protecting the epithelium [14]. Furthermore, NSAIDs disrupt the layer of surface-active phospholipids on the mucosal surface, independent of effects on prostaglandin synthesis. Such an action would render the mucosa less able to resist damage induced by luminal acid [16, 47].

Aspirin and indomethacin are the most frequently used ulcerogen in ulcer induction. The ulcerogen is usually administered through an appropriate route in an appropriate vehicle after fasting selected animals for 24–36 hours. The dose of aspirin orally administered is usually in the range of 125–150 mg/kg body weight in rats, and the animals sacrificed after 4 hours [48]. In the case of indomethacin, the dose is 40–100 mg/kg body weight, and the ulcers are scored after 4–8 hours [49]. In case of ibuprofen-induced ulcer model, usually, a dose of 400 mg/kg body weight, p.o. administered to the animals, which are sacrificed 5 hours after administration. It is usually recommended that a pilot assessment be undertaken to determine the effective dose needed to produce the gastric ulcerations.

2.3. Ethanol-Induced Peptic Ulcer Model. Ethanol is considered a risk factor for developing gastric ulcers. It readily penetrates the gastric mucosa due to its ability to solubilize the protective mucous and expose the mucosa to the proteolytic and hydrolytic actions of hydrochloric acid and pepsin [18], causing damage to the membrane [50]. Moreover, alcohol stimulates acid secretion and reduces blood flow leading to microvascular injuries, through disruption of the vascular endothelium and facilitating vascular permeability; it also increases activity of xanthine oxidase. Ethanol also triggers imbalances in cellular antioxidant processes. For example,

it causes the release of superoxide anion and hydroperoxy-free radicals, and hence increased oxidative stress in the tissues, evidenced by increased levels of malondialdehyde, a marker of increased lipid peroxidation [51–53]. The harmful effects of ethanol thus manifest either through direct generation of reactive metabolites, including free radical species that react with most of the cell components, therefore changing their structures and functions, or by contributing to other mechanisms that finally support oxidative damage [54, 55]. Ethanol also produces necrotic lesions in the gastric mucosa of animals by a direct toxic effect thereby reducing the secretion of bicarbonates and depleting gastric mucus production in animals [56].

Furthermore, ethanol-induced membrane damage is associated with increased permeability of the plasma membrane by sodium and water. It also produces massive intracellular accumulation of calcium, which represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium [57]. Also, ethanol-induced ulceration is linked to reduced mucosa microcirculation and to increased apoptosis [58].

The damaging effects of ethanol have been exploited in developing the ethanol model of peptic ulcers. The model is independent of gastric acid secretion and resembles acute peptic ulcers in humans [59]. As a model, ethanol-induced ulcer may not be appropriate or useful for the assessment of the usefulness of antisecretory drugs or testing materials due to the absence of gastric acid secretion where acid secretion underlies the development of the ulcer. Instead, the ethanol-induced ulcer model is useful for studying the efficacy of potential drugs or testing agents that have cytoprotective and/or antioxidant activities.

To induce ulcers with ethanol, animals are fasted for 24–36 hours following which absolute ethanol (95%–99%) is administered at a dose of 1 mL/200 g body weight to each animal and after 1 hr the animals are sacrificed. It is recommended that for every study, a preliminary assessment be done to determine the effective dose required for optimum induction of ulcers [60].

2.4. Acetic Acid-Induced Gastric Ulcers. One of the least understood aspects of peptic ulcer is the chronicity of the disease that is characterized by repeated episodes of healing and re-exacerbation, a phenomenon which is a challenge to both patients and physicians. Most experimental ulcerative lesions heal quickly in a few days without scar formation and do not reulcerate spontaneously. Takagi et al. [19] developed a model for inducing chronic gastric ulcer in rats by means of submucosal 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. Since its development in 1969, modifications have been made to acetic-acid-induced ulcer model in order to circumvent certain pitfalls such as consistent adherence of ulcer base to the adjacent organs such as the liver. A new method was developed by Okabe and Pfeiffer, which involves intraluminal application of acetic acid solution [20]. The model has become well established and is now used throughout the world by basic and clinical scientists.

The acetic acid model is suitable for chronic peptic ulcers. It is used to evaluate the effect of potential drugs or to test materials on the healing process of chronic peptic ulcers and also to screen for their antisecretory and cytoprotective effects [19, 61]. 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 [21, 62]. Due to the model's resemblance to human ulcers, its been found suitable for use in assessing agents with potential ulcer healing effects in chronic peptic ulcers.

The procedure for inducing ulcers by acetic acid involves fasting rats for 24–36 hours with access to water *ad libitum*. The animals are observed to ensure good health before induction of ulcers. First, the animals are put under light ether anesthesia. A flexible plastic catheter with an outside diameter of 2 mm is inserted 8 cm into the colon via the anus through which dilute acetic acid (4%) (2 mL) is introduced into the colon. The rats are then maintained in a head-down position for 2 minutes to prevent leakage of the acetic acid solution. After 24 hours of acetic acid administration, the animals are sacrificed [61]. It is prudent to conduct a preliminary dose-finding study to determine optimum dose for ulcer induction.

2.5. Histamine-Induced Gastric Ulcer. Gastric ulceration is mediated by several factors including the release of histamine, and this is the basis for the histamine model for producing ulcers. 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 [63, 64]. These pharmacological effects of histamine underlie the histamine-induced ulcer model and hence its usefulness in evaluating antisecretory effects of potential drugs against ulcers and agents that function as H2-receptor antagonists [22].

To induce ulcers with histamine, the selected animals are fasted for 18 hours prior to the beginning of the experiment. Ulcers are induced by administering histamine phosphate (40–100 mg/kg body weight) subcutaneously. After 2 hours, the animals are sacrificed. Pilot studies to determine the effective dose for ulcer induction are usually needed.

2.6. Reserpine-Induced Peptic Ulcer. Scientists have also used reserpine to induce ulcers. Reserpine-induced gastric ulceration has been attributed to the degranulation of gastric mast cells consequent to liberation of histamine, believed to be mediated by the cholinergic system [65]. Rats fasted for 36 hours are administered reserpine dissolved in 10% Tween 80 (5–8 mg/kg, i.p.) in [66]. Although the model is acid dependent, hypermotility seems to be more important than hypersecretion for the induction of gastric mucosal lesion [23, 66]. Normally, drugs or plant extracts to be evaluated

are administered to the test animals, at least, 30 minutes before the administration of the reserpine. The test animals are then sacrificed 24 hours later.

2.7. Serotonin-Induced Gastric Ulcer. 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 [25]. In this model, rats are fasted for 24–36 hours. 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 intragastric intubation with the aid of an orogastric cannula. The animals are sacrificed by cervical dislocation 6 hours later.

2.8. Pylorus-Ligated-Induced Peptic Ulcer (Shay's Method). Ligation of the pylorus induces ulcers that serve as a useful model for investigating the efficacy of drugs on gastric secretions. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach that produces ulcers. These ulcers result from autodigestion of the gastric mucosa leading to a breakdown of the gastric mucosal barrier. So, basically an increase in acid-pepsin accumulation due to pylorus obstruction may cause subsequent mucosal digestion. The model is useful for evaluating the effects of anti-secretory drugs that reduce secretion of gastric aggressive factors such as acid and pepsin. The model is also useful for assessing the cytoprotective effects of drugs that increase secretion of mucus. Animals are fasted for 36–72 hours prior to pylorus ligation. In this model, the pylorus is ligated by means of the "Shay" technique under ether anaesthesia [26]. The drug or test material is administered orally 1 hour before the pylorus is ligated. The animals are killed 18–20 hours later and ulcers are assessed.

2.9. Diethyldithiocarbamate-Induced Gastric Ulcer. The diethyldithiocarbamate model is used to assess the antioxidant activities of drugs in the prevention of gastric damage [27]. 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 [67]. Acute glandular lesions are induced by subcutaneous administration of 1 mL of diethyldithiocarbamate in saline (800 mg/kg body weight) followed by 1 mL oral dose of 0.1N HCl. In this model, food is also withdrawn 24 hours and water 2 hours just before the commencement of the experiment.

2.10. Methylene Blue-Induced Ulcer. Methylene blue (MB), a synthetic drug is known to uncouple ATPases [28] 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 [68]. Additionally, MB has affinity for acetylcholine or muscarinic receptors and has been reported to inhibit cholinesterase activity [69]. 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. To induce ulcers with MB, animals are fasted 24 hours before MB administration at a dosage of 125 mg/kg body weight p.o. followed by the administration of the drug(s) or substances under investigation. Animals are sacrificed after 4 hours of MB administration, and ulcer index determined.

2.11. Ischemia-Reperfusion (I-R) Gastric Ulcer Model. The gastrointestinal (GI) mucosa is very sensitive to ischemia. Reperfusion of the GI following ischemia causes erosion and ulceration in the gastric mucosa due to the formation of free radicals [70]. In this model, rats are fasted for 24 hours after which they are anesthetized with ketamine (100 mg/kg), intramuscular injection (i.m.) and xylazine (16 mg/kg, i.m.). Laparatomy is performed, and the esophageal and pyloric ends of the stomach are clamped using bulldog clips. The celiac artery is then clamped at a point 0.5 cm distal from the branch to the aorta for 30 minutes. The GI is then reperfused for 20 minutes [71]. The rats are then sacrificed, and ulcer index is calculated. This model can be used to evaluate antiulcer drugs in a preclinical setting [29].

2.12. Cysteamine-Induced Duodenal Ulcers. A duodenal ulcer in rats induced by cysteamine HCl was first described by Selye and Szabo [72]. Cysteamine induced duodenal ulcer in rat has been widely used as a model of peptic ulcer disease. This chemically induced ulcer resembles duodenal ulcer in man with respect to its location, histopathology and some aspects of pathophysiology. Although the mechanism involved in ulcer production has not been fully elucidated, generally it is reported that 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 [73, 74], with a decrease in defensive processes that lower levels of bicarbonate, mucus, and epidermal growth factors [75, 76]. Studies have demonstrated that cysteamine also reduces somatostatin bioavailability and markedly elevates serum gastrin levels, with an associated increase in gastric acid secretion [77], a significant decrease in the neutralization of acid in the proximal duodenum, decreased dopamine levels in glandular stomach and duodenum, and inhibition of gastric emptying and motility [61, 78]. It is also known that certain transcription factors (e.g., early growth response factor-1, hypoxia-inducible factor-1) and their target genes play a key role in the pathogenesis of cysteamine-induced duodenal ulcers. *In vitro* studies have

also demonstrated that the cytotoxic effects of cysteamine depend primarily on the generation of H_2O_2 in the presence of transition metals (Mn) such as Fe^{+3} [79–81]. The thiol-derived H_2O_2 reacts with the reducing transition metals to produce hydroxyl radicals via the Fenton reaction. These radicals are the likely final mediators of cysteamine-induced cytotoxicity as evidenced by the fact that the effect of cysteamine is diminished by catalase, and catalase inhibits cysteamine-induced duodenal ulcers in rats [82, 83]. Cysteamine also promotes focal intracellular accumulations of iron, which may exacerbate its cytotoxic effects [77]. It is not clear whether such iron accumulations have a role in the pathogenesis of cysteamine-induced tissue damage although iron is inherently toxic as a result of its propensity to promote oxidation through the Fenton reaction [84].

There are two types of duodenal ulcers, namely, acute and chronic. Acute duodenal ulcers are produced experimentally in rats by a single administration of cysteamine hydrochloride (400 mg/kg p.o.) [72]. In the case of chronic duodenal ulcer, cysteamine is administered twice, first 400 mg/kg (p.o.) at an interval of 4 hours [85] and by adding cysteamine-HCl to drinking water for a period of time [30]. After induction of ulcers, the animals are sacrificed 24 hours later, and the duodena are excised carefully, cut opened along the antimesenteric side, and the ulcer areas are measured.

2.13. Indomethacin, Plus Histamine-Induced Duodenal Ulcer. Another method for inducing duodenal ulcers described by Takeuchi et al. [31] involves administering indomethacin and histamine to rats. In this model, indomethacin (5 mg/kg) is first given subcutaneously to rats fasted for 24 hours followed 30 minutes later by histamine dihydrochloride (40 mg/kg also subcutaneously) three times at 2.5-hour intervals. After 3 hours, duodena are excised carefully, cut opened along the antimesentric side, and the ulcer area(s) is measured. 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 antiduodenal ulcer drugs or agents [31].

2.14. Ferrous Iron-Plus Ascorbic Acid-Induced Gastric Ulcer Model. 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 [32].

2.15. Acetic Acid-Plus H. pylori-Induced Ulcer Model. Rats can also be ulcerated with acetic acid according to the method described by Takagi et al. [19]. Under anesthesia, laparotomy is performed in rats through a midline epigastric incision, the stomach is exposed, and 20% of acetic acid (0.03 mL) is injected into the subserosal layer of the glandular portion, using a microsyringe (0.05 mL). After closing the abdominal incision, the animals are maintained in individual cages, with daily access to commercial food restricted to the time periods of 9-10 a.m. and 5-6 p.m. This allows for adequate fasting for administration of *Helicobacter pylori* drugs or agents under investigation as well as standard drugs (amoxicillin (AMX) 50 mg/kg + clarithromycin (CLR) 25 mg/kg with a proton-pump inhibitor such as omeprazole 20 mg/kg).

According to Konturek et al. [86], 24 hours after ulcer induction by acetic acid, animals are inoculated intragastrically with 1 mL of confirmed pathogenic strain of *Helicobacter pylori* such as ATCC 43504 (9×10^8) suspended in Mueller-Hinton broth or Brain-Heart Infusion Broth by using a cannula appropriate for orogastric gavage. For the animals in the control, and Acetic Acid-induced ulcer groups without *Helicobacter pylori* infection, only Mueller-Hinton or Brain Heart Infusion Broth is administered orally. The orogastric inoculation with *Helicobacter pylori* is done twice a day for 7 days, whereas the test drugs/agents, control and standard drugs, are administered twice a day, for 14 consecutive days, starting from the third day after ulcer induction by acetic acid. After treatment, the animals are sacrificed by cervical dislocation, blood is collected from the inferior *vena cava,* and the stomachs are removed for evaluation of gastric lesions.

3. Measurement of Gastric Lesions

Measurement of gastric ulcerations following their induction is done by first dissecting the stomach along its greater curvature and fixing on a board or transparent glass [87]. Examination can be carried out macroscopically with a hand lens and by tracing on a transparent paper after which the transparent paper is placed onto a graph sheet and sizes of ulcers are measured. Examination can also be carried out microscopically using a light or scanning microscope [88]. The stomachs can also be scanned using a camera, and later the presences of ulcers are quantified using computer-assisted image analysis programmes such as Scion, Image J, EARP Image analysis software, or by any other appropriate software [89]. Several methods have been designed to assess the extent of ulcerations and subsequently the calculation of an ulcer index as well as the protective and/or curative ratios for the ulcers [90–96].

The following scores/ratings as described by Takagi and Okabe [90] can be used to evaluate the ulcer index as well as the severity of gastric lesions:

0 = no lesion,

1 = mucosal oedema and petechiae,

2 = one to five small lesions (1-2 mm),

3 = more than five small lesions or one intermediate lesion (3-4 mm),

4 = two to more intermediate lesions or one gross lesion (>4 mm),

5 = perforated ulcers.

The ulcer index, the percentage protective ratio, and the percentage curative ratio are, respectively, given by the following equation:

$$\begin{aligned}&\text{ulcer index (UI)}\\&\quad=\frac{\text{total ulcer score}}{\text{no. of animals ulcerated}},\\&\text{percentage protective ratio}\\&\quad=\left(\frac{\text{UI of ulcerogen treated group}}{\text{UI of ulcerogen treated}}\right.\\&\qquad\left.-\frac{\text{UI of drug pretreated group}}{\text{UI of ulcerogen treated}}\right),\\&\text{percentage curative ratio}\\&\quad=\left(\frac{\text{UI of ulcerogen treated group}}{\text{UI of ulcerogen treated}}\right.\\&\qquad\left.-\frac{\text{UI of drug treated group}}{\text{UI of ulcerogen treated}}\right).\end{aligned}\tag{1}$$

Another method is where the total ulcerative area in relation to the total area of each stomach is used in determining the ulcer index as described by Ganguly [91]:

$$\text{relative area}=\frac{\text{total mucosal area}}{\text{total ulcerated area}}.\tag{2}$$

The relative area is used to assign the ulcer index according to the scale shown in Table 1.

If the ratio is 101 and above, the ulcer index can be calculated in the order of 0.09, 0.08, and so forth.

Using the method described by Dekanski et al. [92], the severity of the mucosal lesions can also be assessed and the ulcer index is scored as follows:

0 = no damage,

1 = blood at the lumen,

2 = pinpoint erosions,

3 = one to five small erosions <2 mm,

4 = more than five small erosions <2 mm,

5 = one to three large erosions >2 mm,

6 = more than three large erosions >2 mm.

Table 1: The relative area and corresponding ulcer index.

Relative area/mm^2	Ulcer index
No ulcer	0
91–100	0.1
81–90	0.2
71–80	0.3
61–70	0.4
51–60	0.5
41–50	0.6
31–40	0.7
21–30	0.8
11–20	0.9
1–10	1.0
Perforation	1.0

The ulcer index, the percentage protection ratio, and the percentage curative ratio are, respectively, given by the following equation:

$$\begin{aligned}&\text{ulcer index (UI)}\\&\quad=\frac{\text{total ulcer score}}{\text{no. of animals ulcerated}}\\&\text{percentage protective ratio}\\&\quad=\left(\frac{\text{UI of ulcerogen treated group}}{\text{UI of ulcerogen treated}}\right.\\&\qquad\left.-\frac{\text{UI of drug pretreated group}}{\text{UI of ulcerogen treated}}\right)\\&\text{percentage curative ratio}\\&\quad=\left(\frac{\text{UI of ulcerogen treated group}}{\text{UI of ulcerogen treated}}\right.\\&\qquad\left.-\frac{\text{UI of drug treated group}}{\text{UI of ulcerogen treated}}\right).\end{aligned}\tag{3}$$

Another method used is described by Desai et al. [93]. In this method which is based on the intensity of lesions, ulcers are given scores as follows:

0 = no ulcer,

1 = superficial mucosal erosion,

2 = deep ulcer or transmural necrosis,

3 = perforated or penetrated ulcer.

The ulcer index, the percentage protective ratio, and the percentage curative ratio are, respectively, given by the following equations:

$$\begin{aligned}&\text{ulcer index (UI)}\\&\quad=\left(\frac{\text{arithmetic mean of Intensity in a group}}{\text{total number of animals}}\right.\\&\qquad\left.+\frac{\text{number of ulcer positive animals}}{\text{total number of animals}}\right)\times 2\\&\text{percentage protective}\\&\quad=\frac{\text{UI control}-\text{UI Pretreated}}{\text{UI control}}\times 100\\&\text{percentage curative}=\frac{\text{UI control}-\text{UI Treated}}{\text{UI control}}\times 100.\end{aligned}\tag{4}$$

According to the method by Nwafor et al. [94], the observation of erosions and scores made as 1–5 as follows; 1 = small round hemorrhagic erosion, 2 = hemorrhagic erosion <1 mm, 3 = hemorrhagic erosion of 2-3 mm, 5 = hemorrhagic erosion >4 mm. The scores are multiplied by 2 when the width of the erosion is larger than 1 mm.

The ulcer index, the percentage protective or inhibition ratio, and the percentage curative ratio are, respectively, given by the following equations:

$$\begin{aligned}&\text{ulcer index (UI)}\\&\quad=\frac{\text{total ulcer score}}{\text{no. of animals ulcerated}},\\&\text{percentage protective or inhibition}\\&\quad=\left(\frac{\text{UI of ulcerogen treated group}}{\text{Ul of ulcerogen treated}}\right.\\&\qquad\left.-\frac{\text{UI of drug pretreated group}}{\text{Ul of ulcerogen treated}}\right),\\&\text{percentage curative}\\&\quad=\left(\frac{\text{UI of ulcerogen treated group}}{\text{Ul of ulcerogen treated}}\right.\\&\qquad\left.-\frac{\text{UI of drug treated group}}{\text{Ul of ulcerogen treated}}\right).\end{aligned}\tag{5}$$

According to the method by Kulkarni [95], the ulcer index can be measured or registered using the following scores involving the number and severity of ulcers:

0.0 = normal colored stomach,

0.5 = red coloration,

1.0 = spot ulcers,

1.5 = hemorrhagic streaks,

2.0 = ulcers with area >3 but ≤5 mm^2,

3.0 = ulcers > 5 mm^2,

$$\text{ulcer index (UI)} = \text{UN} + \text{US} + \text{UP}\times 10,\tag{6}$$

where UI = ulcer index, UN = average number of ulcers per animal, US = average of severity score, and UP = percentage of animals with ulcer.

The percentage protective ratio, and the percentage curative ratio are, respectively, given by the following equation:

$$\begin{aligned}\text{percentage protective ratio} &= 100-\frac{[\text{UI pretreated}]}{[\text{UI control}]}\times 100,\\\text{percentage curative ratio} &= 100-\frac{[\text{UI treated}]}{[\text{UI control}]}\times 100.\end{aligned}\tag{7}$$

According to the method by Andrade et al. [96], ulcers are classified as;

level I ulcer area < 1 mm^2,

level II ulcer area = 1–3 mm^2,

level III ulcer area > 3 mm^2.

The following parameters are determined:

$$\begin{aligned}&\text{ulcerative lesion index (ULI)}\\&\quad=1\times(\text{number of ulcers level I})\\&\qquad+2\times(\text{number of ulcers level II})\\&\qquad+3\times(\text{number of ulcers level III}),\\&\text{percentage protective ratio}\\&\quad=100-\frac{[\text{ULI pretreated}]}{[\text{ULI control}]}\times 100,\\&\text{percentage curative ratio}\\&\quad=100-\frac{[\text{ULI treated}]}{[\text{ULI control}]}\times 100.\end{aligned}\tag{8}$$

In all of these ways of determining ulcer indices, attempts are made to find a solution to the problem of incomplete quantification of gastric and duodenal ulcers. Different scoring systems have been described for use to measure gastroduodenal ulcerations and calculate ulcer indices. In the method described by Takagi and Okabe [90], the number of ulcers rather than the size of ulcers is given importance for assessing ulcer severity. Implicitly, the approach may yield results that may be statistically correct in terms of the number of ulcers but will be biologically irrelevant. Several researchers, through their studies, have tried to reduce the level of biological incorrectness in ulcer quantification. For example, the method by Nwafor et al. [94] measures only the length of the erosive ulcers without considering the total area of ulcerations in relation to the total mucosal area. Others [93, 95] have tried to distinguish the different types of lesions or ulcerations such as spot ulcers, haemorrhagic

streaks, deep ulcers, and perforated ulcers. However, the areas of ulcerations fail to have consideration in most of the parameters used. Andrade et al. [96] in an attempt to improve on the biological correctness of ulcer indices factored into their assessment the areas of ulceration and categorized them into levels I, II and III based on size of the ulcers. Despite the improvement on the methods described by others [90, 93, 95], the approach suffers a pitfall as it does not consider the total area of the stomach in determining the ulcer index. The procedure would therefore, produce a statistically correct ulcer index that would not be biologically relevant. It would seem then that, the method described by Ganguly [91] although quiet old, would be better than others in the literature as it takes into consideration the total area of the stomach in relation to the total area of the ulceration in determining the ulcer index. Although this procedure also overlooks the level of erosion histopathologically in the quantification of ulcers, in our opinion, the method described by Ganguly produces ulcer indices that are close to being both biologically and statistically relevant.

4. Concluding Remarks and Future Perspectives

We have, in this paper, reviewed several experimental ulcer models, which can be used for testing potential antiulcer agents such as plant medicines that are reported to have ethnomedicinal uses against ulcers. In each case, we have discussed the pathophysiological mechanisms underlying the lesions produced to allow a better understanding of the processes involved. This will help investigators to make a sound scientific judgment when selecting a model for use to evaluate a test agent. We have also discussed currently available methods for scoring ulcers, pointing out the pitfalls in the various approaches. This paper could be a good resource for scientists who have interest in evaluating antiulcer agents.

Conflict of Interests

The authors report no conflict of interests.

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