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A Review on in Vitro and in Vivo Models of Antiulcer Agents: Mechanisms, Efficacy, and Translational Challenges

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ABSTRACT:

This review explores the in vitro and in vivo models used to study antiulcer agents, highlighting their mechanisms, efficacy, and translational challenges. Various models, including histamine H4 receptor antagonists, methylene blue-induced lesions, serotonin-induced ulcers, and ethanol-induced gastric damage, have been instrumental in understanding ulcer pathogenesis and evaluating potential therapies. In vitro assays, such as DPPH and FRAP, and in vivo studies using rodent and rabbit models, provide comprehensive insights into the antioxidant and gastroprotective effects of novel compounds. The findings demonstrate the role of oxidative stress, inflammation, and molecular pathways in ulcer development and healing. Despite the advances, limitations such as genetic variability among animal strains and differences in human gastric physiology pose challenges to the direct translation of preclinical results to clinical practice. Future research should focus on refining these models to better mimic human conditions and improve the predictive value of preclinical studies.

Keywords: in vitro models, in vivo models, antiulcer agents, gastric ulcers, oxidative stress, inflammation, translational research.

1. INTRODUCTION

The Greek word peptikos, which means to digest, is the source of the English word peptic. Peptic ulcers (PUs) are sores on the mucosa that reach the layer of the muscularis mucosae and make a hole surrounded by either short-term or long-term inflammation. Najm (2011) [1] classifies these ulcers as either duodenal or stomach ulcers, depending on their location. This is also the most common digestive problem, affecting 40% of industrialized countries and 80% of developing countries [2]. It is caused by the careless use of non-steroidal anti-inflammatory drugs (NSAIDs) and Helicobacter pylori infections. According to Mousavi et al. (2020), [3] the incidence of other causes has also increased, including pollutants, pesticides, heavy metals, alcohol, tobacco, cigarette smoking, physical and psychological stress, and pollution. Peptic ulcer disease has been a major cause of gastrointestinal surgery with a high morbidity and death rate for over a century. According to some experts, the sickness is so common that it is the new epidemic of the twenty-first century and affects individuals all over the globe [4]. Furthermore, the illness interferes with everyday activities, causing significant suffering and mental misery for patients. The majority of peptic ulcer sufferers experience pain, discomfort, or nausea in their abdomens. The most typical sign of gastric and duodenal ulcers is abdominal discomfort. A burning feeling after eating is one of the symptoms of this discomfort [5].

The efficacy of the therapeutic therapy now used for stomach ulcers is poor, and the recurrence rate is significant. As a result, treating stomach ulcers is still very difficult, necessitating the urgent development of new medications as well as complementary and alternative treatments. Researchers [6] have demonstrated antiulcer characteristics in many medicinal herbs, and given their widespread use, these plants could potentially serve as a viable source for drug manufacturing. Recently, researchers have undertaken numerous efforts to identify medications with anti-ulcer and protective properties [7]. Researchers have conducted numerous investigations into the preventive properties of plants and medications in animals, utilizing the ethanol-induced stomach ulcer model.

To assess whether plants and medications have anti-ulcer (or ulcer prevention) properties, reliable experimental models are required. Models are helpful resources for learning more about the pathophysiological processes of wounds, the antioxidant mechanisms of necessary medications, and the mechanisms of compounds with antioxidant effects. As a result, there are several models available for assessing anti-ulcer medications, making the choice of a suitable model difficult. Local sources, study aims, test hypotheses, or the researcher's queries often influence the selection of a specific model, as each model offers a variety of benefits and drawbacks [8]. As a result, an unskilled researcher must carefully analyze and compare a large number of models, which is expensive, time-consuming, and requires a wealth of knowledge in order to choose an experimental model from the many available models.

2. IN VITRO METHODS

2.1 Gastrin Binding assay

Gastrin was initially a focal point in gastrointestinal research, notably around 1970, amid high hopes that it played a pivotal role in peptic ulcer disease [9, 10]. There was also anticipation that gastrin could be implicated in the flawed incretin mechanism of type 2 diabetes [11]. Furthermore, discoveries linking gastrin gene expression to major cancers suggested it might act as a carcinogenic growth factor [12]. The identification of gastrinomas as the cause of Zollinger-Ellison syndrome (ZES) underscored the necessity for gastrin measurements in diagnosis [13].

However, current understanding has shifted significantly: Helicobacter pylori infection is now recognized as the primary cause of peptic ulcers, diminishing the role of gastrin measurement

in diagnosis. Moreover, the focus in diabetes research has largely shifted to incretins like glucagon-like peptide 1 and gastric inhibitory polypeptide, reducing interest in gastrin [14]. While gastrin was once considered a potential major growth factor in cancer, it has not achieved prominence in this regard [15]. Nonetheless, there has been an increase in the detection of small gastrinomas causing mild ZES symptoms, necessitating gastrin measurements in a growing number of cases [16, 17, 18].

2.1.1 Method: The experiment uses a fundic gland suspension from the stomach of guinea pigs. In order to conduct the binding and competition tests, the gland suspension is incubated for 90 minutes at 37°C with 50 µl of [1] gastrin in the presence of test chemicals, buffer alone (for total binding), unlabeled gastrin (for non-specific binding), or both. The incubated combination is then put on top of ice cold buffer in microcentrifuge tubes, and the mixture is centrifuged for five minutes at 10,000 g. After discarding the supernatant, we use the pellet to measure radioactivity.

2.1.2 Evaluation: We determine the amounts of total, non-specific, and specific binding. The percentage of specifically bound [1251] gastrin displaced by a certain concentration of the test drug is calculated, as well as the values of its dissociation constant (Ki) and IC [19, 20]

2.2 [H] Tiotidine Binding Assay for Histamine H, Receptors

In individuals with duodenal ulcers, the effects of cimetidine and tiotidine, novel histamine H2-receptor antagonists, on food-stimulated stomach acid production were assessed. In vivo intragastric titration was employed to evaluate food-stimulated acid secretion, and homogenized steak meals were infused immediately after, one hour after, five hours after, and ten hours after an oral drug dosage. The initial action of tiotidine and cimetidine was comparable, but tiotidine was more effective and lasted longer [21]. Two indicators of increased potency were observed: 150 mg of tiotidine inhibited acid secretion approximately as much as 300 mg of cimetidine did 1 to 2 hours after medication, and the concentration of plasma tiotidine was eight to nine times lower than the concentration of plasma cimetidine for a given percent inhibition of acid secretion. 150 and 300 mg of tiotidine reduced acid secretion by 80% and 97%, respectively, from 5 to 7 hours after therapy, while 300 mg of cimetidine only reduced acid secretion by 22%, suggesting a longer duration of impact. Additionally, 150 and 300 mg of tiotidine reduced acid secretion by 22% and 53%, respectively, 10–12 hours after therapy, while 300 mg of cimetidine had no such impact. Tiotidine's plasma levels plateaued between two and six hours after treatment, which contributed to the prolonged duration of impact. Tiotidine and cimetidine did not significantly affect the release of gastrin induced by food or the emptying of a nonabsorbable marker from the stomach [21].

Recent studies have expanded our understanding of tiotidine, a histamine H2-receptor antagonist. One notable study highlights tiotidine's function as an inverse agonist, binding with high affinity to an inactive G-protein-coupled form of the receptor. This behavior supports the cubic ternary complex model, which suggests that tiotidine biases the receptor system to a G-protein-coupled state unable to evoke a response. These findings are based on theoretical simulations and experimental results showing tiotidine's unique mechanism of action compared to traditional H2 antagonists [22]

Receptors Histamine H, receptor blockers have been the mainstay of anti-ulcer therapy since the early 1970s due to their potent acid-suppressing properties. Tiotidine is an H, receptor blocker that Is used for H, receptor binding assay. Procedure: The assay is done using cerebral cortex homogenate obtained from male White leghorn chicks or from guinea pigs. The cerebral cortex homogenate is incubated with [H] tiotidine for 90 min at 4°C in the presence of Na,HPO,/KH, PO, buffer (pH 7.4) alone to determine total binding or in the presence of unlabeled ranitidine and buffer to determine non-specific binding or in the presence of test compound in buffer for competition assay. 5 ml of ice-cold phosphate buffer is added to

terminate the incubation. Subsequently the reaction mixture is filtered under vacuum through glass fiber filters that are pre-soaked with buffer. Filters are then washed with 5 ml of ice-cold buffer twice and radioactivity measured by liquid scintillation counting.

2.2.1 Evaluation: Specific binding, Le. total binding minus non-specific binding and IC, are determined. In another study, the [PH]-tiotidine has also been used as a specific ligand for H, receptors in dispersed mucosal cells from guinea pig stomach and it was found to have limited binding to H, receptors and as such [H]-tiotidine was not a suitable ligand for labelling the H,-receptor on gastric mucosal cells."

2.3 H/K-ATPase Inhibition

To evaluate H+/K+-ATPase inhibition, a well-defined procedure and rigorous assessment are essential. The process begins with the preparation of a tissue homogenate from gastric tissue, which can be sourced from experimental animals or human samples. The homogenate is prepared in a buffer, such as Tris-HCl, to maintain physiological conditions. Next, the tissue homogenate is incubated with the inhibitor of interest, such as proton pump inhibitors (PPIs) like omeprazole, esomeprazole, or pantoprazole, known for their ability to inhibit H+/K+-ATPase activity.

To measure the ATPase activity, a colorimetric assay is typically used. This assay detects the hydrolysis of ATP, which results in the release of inorganic phosphate (Pi). The amount of Pi released is directly proportional to the ATPase activity. Control experiments, where the tissue homogenate is incubated with only the vehicle (buffer), are also necessary to account for non-specific effects.

The percentage inhibition of H+/K+-ATPase activity by the inhibitor is then calculated using the formula:

Percentage Inhibition=(ATPase activity without inhibitorATPase activity without inhibitor−ATPase activity with inhibitor)×100.

A higher percentage indicates stronger inhibition. To validate the results, statistical analyses such as t-tests or ANOVA are performed to determine the significance of the differences observed between the inhibitor-treated and control groups. This method is well-documented in scientific literature, providing a robust framework for evaluating the efficacy of H+/K+-ATPase inhibitors [23, 24]

3. IN VIVO METHODS

3.1 Pylorus Ligation in Rats

Pylorus ligation in rats is a widely used in vivo method to study gastric acid secretion. The procedure begins with the selection and fasting of experimental rats to standardize conditions. The rats are then anesthetized, and a midline abdominal incision is made to expose the stomach and pyloric region. The pylorus, which is the outlet of the stomach, is carefully identified and ligated using sutures or clips to prevent the passage of gastric contents into the duodenum. After closing the abdominal incision, the animals are monitored post-surgery for recovery from anesthesia and any signs of distress.

Following a specified period, typically four hours or more, to allow gastric acid secretion to accumulate, the animals are euthanized using approved methods. Gastric secretions are then collected from the stomach for analysis. The volume and pH of the collected gastric secretions are measured, and further analysis can be performed to assess the content of acid and other components, such as pepsin. The percentage inhibition of H+/K+-ATPase activity can be calculated and statistical tests, such as t-tests or ANOVA, are performed to compare results between experimental groups and controls.

This method is essential for evaluating the efficacy of acid-reducing drugs like proton pump inhibitors and H2-receptor antagonists, studying the regulation of gastric acid secretion in response to various stimuli or conditions, and assessing the impact of dietary factors, hormones, and neural inputs on gastric acid production [25, 26].

3.2 Stress Ulcer Models

The study described an experiment involving adult and piglet models to investigate stress ulceration in swine. Adult Yorkshire-Hampshire pigs subjected to various stressors such as restraint, corticosteroids, portacaval shunting, arteriovenous fistulae, and hemorrhage did not reliably develop gastric ulcers [27]. In contrast, piglets subjected to hemorrhagic shock, simulating severe stress, exhibited a significant propensity for gastric ulceration within 72 hours post-retransfusion. The study noted that gastric acid secretion did not consistently change during stress and is unlikely to be a primary factor in stress ulcer development. Instead, localized decreases in juxtamucosal pH were observed in areas where ulcers subsequently formed, suggesting a potential role in ulcer pathogenesis. Overall, the piglet model of hemorrhagic shock was deemed a reliable tool for studying stress ulcer pathophysiology, offering insights into mucosal blood flow, mucus secretion, and other factors contributing to ulcer formation [27].

3.2.1 Preparing a model of an ulcer. The experimental rats were placed in a separate room and given a 24-hour fast before being evaluated. The sound of submachine gun firing, which was captured on tape and delivered to the rats via a loudspeaker at a distance of 20–30 cm, served as an instigating element. Using a frequency spectrum analyzer and precision pulse counter, the firing noise was examined, and its frequency was found to be between 0.25 kHz and 4.00 kHz, with an intensity of 110 dB(A). The stomachs of the rats were opened, and the development of their stress ulcer was noted after a 12-hour period of consecutive stimulation by the sound of a gunshot. Three groups of rats were randomly assigned to the rats: Eight rats made up the control group, which was neither protected nor stimulated; eight rats made up the stimulation group, which was further split into groups A, B, and C. These subgroups were split into groups A, B, and C, each with eight rats, and the prevention group was also split into groups A, B, and C. Blood was drawn for testing immediately, 12 hours, and 24 hours after stimulation, respectively. Group B received 2 mg ip of anisodamine 30 min before and 6 h after stimulation; group C received the above-mentioned combination medication. Group A received an injection of 40 mg ip of cimetidine 30 minutes before the stimulation. FolAfter the treatment, we anesthetized the abdominal cavities of all the rats used in this investigation with 1.2 mg of 5-g/L amobabital sodium, and opened their abdomens to observe any alterations in the stomach mucosa. er decapitation, we extracted two blood samples: one for serum centrifugation and the other for plasma separation using EDTA and retardant peptidase. The samples were then stored at -40 °C in a refrigerator so that hormones, hepatic functions, and renal functions could be determined.

Assessments of mucosal injury. Guth's method [28] assigned a score of one point to a stomach mucosal injury region that was less than 1mm; 2 mm to 3 mm; 4 mm or more received a segmental score. The ulcer index was the sum of all the stomach scores. All groups' tissue and cell structures were examined and photographed using an electroscope and a light microscope, respectively [29].

The study involved the detection of hepatic-renal functioning, ET, NO, and plasma gas. Using the Northern Immunity Agents Research Institute equipment and following the directions, gas was found both before and after stimulation. As stated in the operating instructions, the East Asian Immunity Technique Research Institute used the kit to detect ET. The handbook indicates that the Military Medical Academy provided the equipment for testing NO. The laboratory assessed hepatic and renal function using the blood samples we submitted [29].

3.2.1 Examining unsightly specimens

The control group's mucosa appeared intact, neat, and smooth following the opening of the stomach wall, with the exception of a few bleeding areas underneath the mucosa of two rats Fig. 1.

A significant difference ($P < 0.01$) was observed in the ulcer index between group B (ulcer index, 8.4 ± 0.6) and the prevention group (ulcer index, 0.3 ± 0.1), as well as the control group (ulcer index, 0). The stimulating group displayed varying degrees of congestion and edema in the seruous layers of the abdominal cavity and on the gastric walls [29, 30, 31].

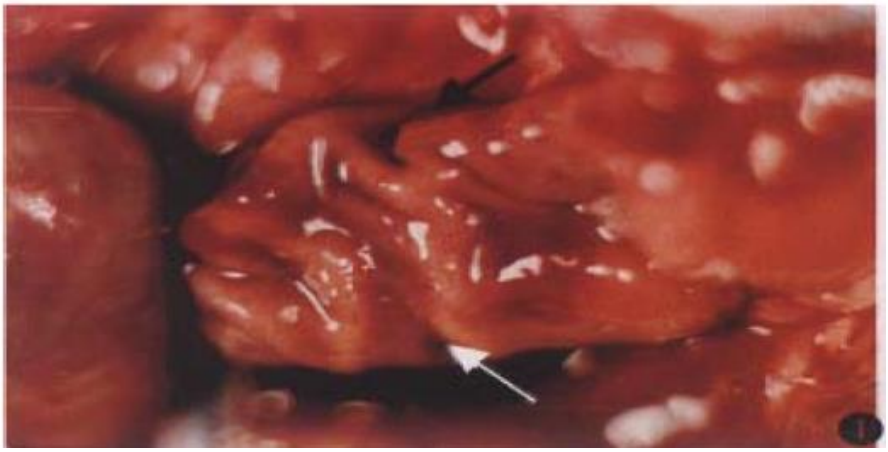


Figure 1 Gross specimens in stimulating group congestion, edema, erosion and ulcer formation.

3.2.3 Inspection using a light microscope

The control group exhibited neat and smooth mucosal layers, systematic gland placement, and no signs of submucosal hemorrage or inflammatory cell infiltration. In subgroups A, B, and C of the stimulation group, there were problems with the mucosa, bigger gaps between the glands, and damaged gland parts. In the meantime, the glands were accumulating a significant number of RBCs. Infiltration and capillary thrombosis were seen inside submucous eosinophils (Figure 2). All three of the subgroups in the preventive group had intact mucosa, well-structured glands that had slightly widened gaps, and a few RBCs scattered underneath the mucosa [29, 32, 33].

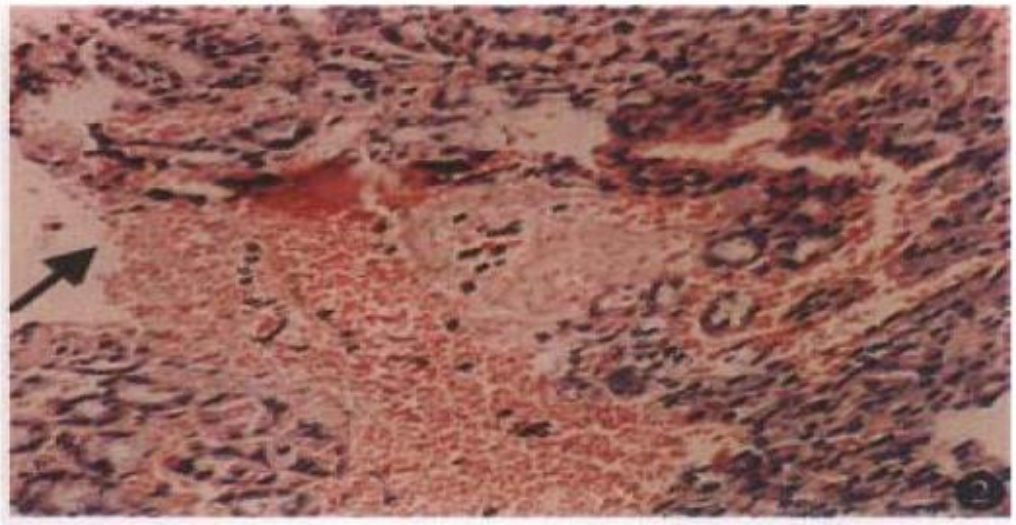


Figure 2 Stimulating group: interruptions of mucosa, enlarged glandular gaps, damaged parts and a large amount of RBCs accumulated among glands. HE × 10 [29]

3.3 Cold Water Immersion-induced Ulcers

It has been observed that when the restrained animals are subjected to additional cold water immersion, the occurrence of gastric ulcers is accelerated, and this also shortens the immobilization time [34, 35]

Procedure: Wistar rats (150 to 200 g) are used for the experiments. After fasting the animal for 16 hours, the test compound is administered orally. The animals are placed individually in restraint cages vertically and then immersed in water at 22°C for 1 h. After removing the rats from the cage, we inject Azovan blue (Evan's blue) intravenously via the tail vein at a dose of 30 mg/kg. Ten minutes later, they are sacrificed. The stomach is removed and ligated at both ends. It is filled with formol saline and kept overnight. On the next day, the stomach is opened along the greater curvature, washed in warm water, and examined for ulcerative lesions. Evan's blue helps in the evaluation of the lesion score, which is calculated by adding the lengths of the longest diameters of the lesions. Two hours of cold restraint stress can also induce gastric mucosal lesions in rats [34, 35].

3.4 Histamine induced Gastric Ulcers

While the duration of treatment is primarily responsible for gastric ulcer healing, duodenal ulcer healing is contingent upon the extent and duration of gastric secretion suppression. At 4 weeks, 77–92% of duodenal ulcers are healed by currently available dosages of histamine H2-receptor antagonists; adjuvant therapy to remove Helicobacter pylori accelerates this rate. Administration once daily has the same therapeutic effects as more frequent dose schedules and may even accelerate the process of recovery. Although they heal more slowly, 75 to 88% of gastric ulcers resolve after 8 weeks of therapy. Although ulcers heal somewhat faster with more recent, stronger acid suppressors like omeprazole, H2-receptor antagonists have an unmatched safety record spanning more than 15 years. It seems improbable that prostaglandin analogs would surpass the effectiveness of H2-receptor antagonists while posing a lower risk of adverse events [36].

In this study, researchers investigated the role of histamine H4 receptors (H4Rs) in gastric ulcer formation using a mouse model of gastric damage. They administered the H4R antagonist JNJ7777120 and H4R agonists VUF8430 and VUF10460 to fasted CD-1 mice alongside indomethacin (IND) and bethanechol (BET), which are known to induce gastric lesions. The study also explored strain-related differences by testing JNJ7777120 in NMRI, BALB/c, and C57BL/6J mice [37].

The results showed that neither JNJ7777120 nor the H4R agonists had effects on the normal stomach at any tested dose (10 and 30 mg/kg s.c.). However, IND+BET administration led to significant lesions in the fundic mucosa, which were notably reduced by JNJ7777120 (at 10 and 30 mg/kg s.c.). This gastroprotective effect of JNJ7777120 was observed in CD-1, NMRI, and BALB/c mice, but not in C57BL/6J mice. Interestingly, VUF8430 did not modify the damage induced by IND+BET in CD-1 mice but prevented the gastroprotection induced by JNJ7777120.

In conclusion, the findings suggest a potential role for H4Rs in mouse gastric ulcerogenesis, highlighting H4R blockers as potentially beneficial anti-inflammatory drugs that spare the gastrointestinal tract. The differing response in C57BL/6J mice underscores the importance of considering strain-specific variations in pharmacological studies of H4R function and the development of selective ligands [37, 38, 39, 40].

3.5 Methyline Blue-induced Ulcers

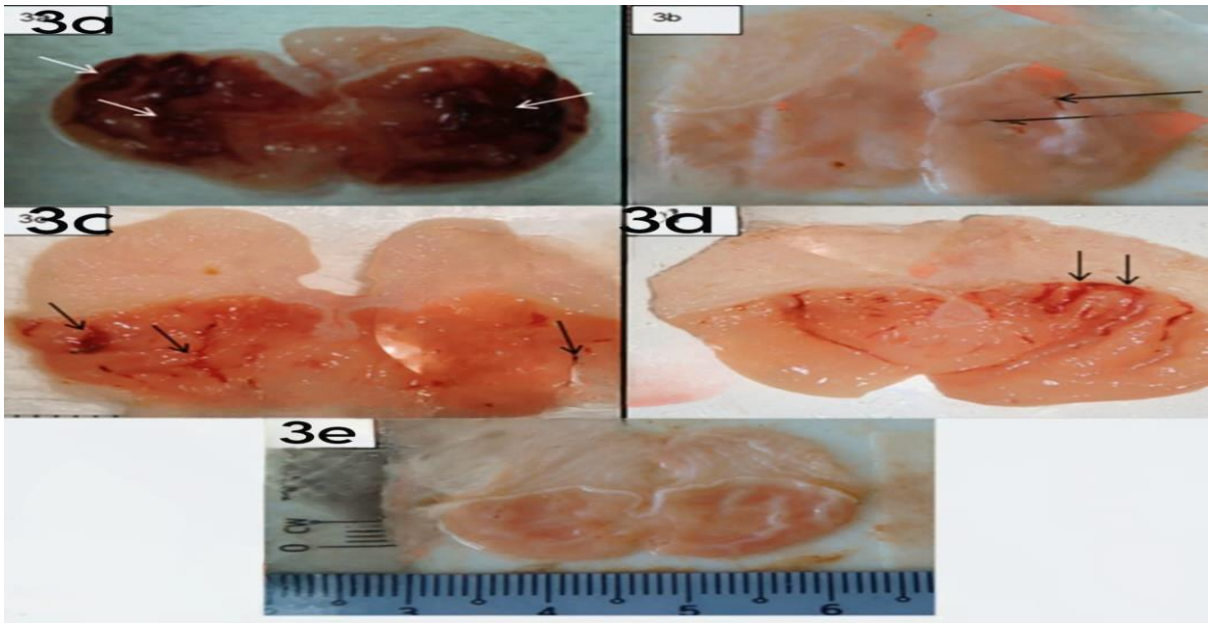
Methylene blue, administered orally at a dose of 100 mg/kg, causes severe lesions in the gastric mucosa by elevating levels of reactive substances like thiobarbituric acid. It also enhances activities of H+/K+-ATPase and superoxide dismutase. Additionally, methylene blue reduces blood flow to the gastric mucosa and lowers levels of glutathione through increased catalase activity. This compound activates H+/K+-ATPase, leading to heightened hydrochloric acid secretion, and induces local oxidative stress radicals, which contribute to mucosal ulceration. Researchers use methylene blue-induced ulcers to study the mechanisms of potential anti-ulcer agents [41].

3.6 Serotonin (5-HT)induced peptic ulcers

Serotonin, also known as 5-hydroxytryptamine (5-HT), is a monoamine derived from tryptophan, an essential amino acid. It is synthesized in serotonergic neurons within the central nervous system, where it functions as a neurotransmitter, and also in chromaffin cells of the digestive tract. Serotonin exerts its effect on blood vessels by causing vasoconstriction, which reduces blood flow to the gastric mucosa. This reduction in blood flow can lead to lesions in the mucosa. Serotonin is typically administered via gastric intubation following a 36-hour period of food deprivation [42].

4. ETHANOL-INDUCED MUCOSAL DAMAGE

Ethanol is recognized as a significant risk factor in the development of pathological changes associated with gastric ulcers [43]. A series of novel 2-(ethylthio)benzohydrazone derivatives (1–6) were synthesized and characterized using IR, 1H NMR, 13C NMR spectroscopy, and mass spectrometry. These compounds were evaluated for their antioxidant activities through in vitro assays including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays. Compound 1 exhibited the highest antioxidant potency among them, prompting further investigation into its potential anti-ulcer effects on ethanol-induced gastric mucosal lesions in rats. Sprague Dawley rats were divided into four groups: an ulcer control group treated with 10% Tween 20, a reference group treated with 20 mg/kg omeprazole, and experimental groups treated with 50 mg/kg and 100 mg/kg of compound 1. Macroscopic examination revealed extensive hemorrhagic lesions in the gastric mucosa of the ulcer control group compared to those treated with omeprazole or compound 1. Rats pre-treated with compound 1 showed increased gastric pH and enhanced gastric mucus production. Histologically, the ulcer control group exhibited severe damage to the gastric mucosa characterized by edema and infiltration of leukocytes in the submucosal layer. Immunohistochemical analysis showed that rats pre-treated with compound 1 exhibited up-regulation of HSP70 and down-regulation of Bax proteins in stained tissue sections. In conclusion, the gastroprotective effect of compound 1 may be attributed to its antioxidant properties, as well as its ability to modulate HSP70 and Bax protein levels in gastric tissue [43, 44, 45]



Photographs showing the gross appearance of the gastric mucosa in rats. (3a) Rats pre-treated with 5 ml/kg 10% Tween 20 (ulcer control). Severe injuries are seen in the gastric mucosa (arrow). Absolute ethanol produced extensive visible haemorrhagic necrosis of the gastric mucosa. (3b) Rats pre-treated with of omeprazole (20 mg/kg). Injuries to the gastric mucosa are very milder (arrow) compared to the injuries seen in the ulcer control rats. (3c) Rat pre-treated with compound 1 (50 mg/kg). Moderate injuries are seen in the gastric mucosa (arrow). The compound reduces the formation of gastric lesions induced by absolute ethanol. (3d). Rats pre-treated with (100 mg/kg) of compound 1, mild injuries are seen in the gastric mucosa (arrow). (3e) Rats in the normal control group showed intact gastric mucosa [43]

It penetrates the protective gastric mucus easily due to its ability to dissolve it, thereby exposing the gastric mucosa to the damaging effects of hydrochloric acid and pepsin [46]. Additionally, ethanol reduces blood flow in the vessels of the gastric mucosa, contributing to microvascular lesions. Typically, animals are fasted for 24 hours before the experiment but have access to water until 4 hours prior to the experiment. Gastric ulcers are induced by administering ethanol intragastrically in a solution with a concentration of 95-99%, at a volume of 0.5 mL per 100 g of body weight. This experimental model closely mimics the development of acute gastric ulcers observed in humans [46, 47].

5. ACETIC ACID-INDUCED GASTRIC ULCERS

The acetic acid-induced chronic gastric ulcer model in rabbits is established through the following procedure. Rabbits are fasted for 20 hours before the operation but allowed to drink water. Anesthesia is administered via an intramuscular injection of Rompun (11.7 mg/kg) and Ketamine (4.2 mg/kg). Throughout the procedure, the rabbits' body temperature is maintained at 37-38°C using a heating pad. A 7-cm median incision is made along the midline of the abdomen to expose the stomach. The stomach is then opened along the large curvature, and the inner cavity is rinsed with isotonic saline. Two ulcers are induced on opposite sides of the gastric cavity by applying 60% acetic acid solution for 15 seconds, followed by immediate rinsing with isotonic saline. The stomach and abdomen are then sutured closed. Post-surgery, the rabbits receive Cefazol and UNI Ketopro injections and are placed back in their cages. Food is restricted for an additional 12 hours. Rabbits are sacrificed one, three, and seven days after

the operation, and their stomachs are collected, opened along the greater curvature, washed with isotonic saline, and spread open for examination [48].

The rabbit endoscopic mucosal resection (EMR)-related ulcer model was experimentally developed as follows. A median laparotomy was performed to expose the gastric mucosal layer. Subsequently, 0.2 mL of isotonic saline was injected into the submucosal layer of the upper corpus, causing the mucosal layer to swell. A 7-10 mm diameter section of the swollen mucosal layer was then resected using scissors. Bleeding was controlled by pressing the resected area with gauze, followed by cleaning with sterile isotonic saline. After administering an antibiotic injection, the stomach and abdomen were sutured closed, and the rabbits were placed in individual cages. The stomachs were processed as previously described in related experiments [48, 49, 50].

The rat acetic acid-induced gastric ulcer model was developed with the following procedure. Rats were fasted for 16 hours with free access to water before the operation. Anesthesia was induced using an intramuscular injection of Rompun (9.0 mg/kg) and Ketamine (3.0 mg/kg). Body temperature was maintained between 37 to 38°C using a heating pad throughout the experiment [48, 51].

To create chronic gastric ulcers, two methods were modified from the procedure described by Okabe et al. For the intra-luminal application, 0.2 mL of 60% acetic acid solution was injected into the fundus, which was clamped with ring forceps, and the acetic acid was removed after 45 seconds. Two ulcers were induced on opposite sides of the gastric cavity. For the serosal application, a vial containing 75% acetic acid was directly placed on the serosal surface of the rat stomach for 20 seconds. The stomachs were then rinsed with isotonic saline, the abdomens sutured, and the rats returned to their cages. The rats were sacrificed one or five days after the intra-luminal operation or serosal application, respectively, and their stomachs were harvested. The harvested stomachs were opened along the greater curvature, rinsed with isotonic saline, and spread open on a board [48, 52].

5.1 Gastric Mucosal Injury by Local anesthesia ischemia-reperfusion in Rats

Stomach damage resulting from ischemia-reperfusion (I/R) is a significant clinical issue linked to various pathological conditions [53]. Hemorrhagic events such as peptic ulcer bleeding, hemorrhagic shock, vascular rupture, and surgery can lead to gastric I/R [54]. Ischemic damage refers to pathological changes in tissues or organs due to oxygen deprivation caused by reduced or halted blood flow [55]. Reperfusion is the restoration of blood flow to ischemic tissues. If blood flow is not restored, a sequence of pathological events leading to cellular dysfunction and necrosis may occur. Paradoxically, rapid reperfusion can cause more severe damage than ischemia alone. Reperfusion injury results from the overproduction of reactive oxygen species (ROS), which act as reperfusion mediators, produced by molecular oxygen delivered in large quantities to ischemic tissues by arterial blood [56]. These ROS generate toxic products like malondialdehyde (MDA) by oxidizing cell membrane lipids. Another damage mechanism is the activation of the cyclooxygenase-2 (COX-2) enzyme due to increased intracellular calcium during ischemia, leading to the release of pro-inflammatory prostaglandins and ROS from arachidonic acid [57, 58].

5.1.1 Experimental Procedure

The DGIR animal group was given intraperitoneally (i.p.) 50μg/kg dexmedetomidine in order to conduct this experiment. The GIR and HG groups were given distilled water as a solvent using the same volume and procedure. All rat groups received an intraperitoneal (i.p.) injection of 25 mg/kg of thiopental sodium thirty minutes after receiving dexmedetomidine and distilled water. The rats were then given xylazine to breathe at predetermined intervals to induce anesthesia. Following the injection of thiopental sodium, the rats were kept in wait until the right time for surgery [59]. When animals remain still in the supine position, it is considered

appropriate to perform surgery. After that, the rats underwent a sterile laparotomy with a 2.5 cm midline incision. The celiac artery was constricted with clips to produce ischemia for one of the DGIR and GIR groups in order to generate ischemia reperfusion lesions. The HG group's abdominal area was sutured shut after being opened without the celiac artery being clipped. After one hour, we removed the clip and maintained reperfusion for three hours. After the third hour of reperfusion, all animals were put to death using a high dosage of thiopenteal anesthetic (50 mg/kg). Subsequently, the animal stomach tissue was subjected to biochemical and histological analyses [60, 61].

5.1.2 Determination of MDA:

The determination of malondialdehyde (MDA) is performed by measuring the absorbance of a pink complex formed by thiobarbituric acid (TBA) and MDA at a high temperature (95 °C) using a spectrophotometer at 532 nm. Homogenates are centrifuged at 5000g for 20 minutes, and the supernatants are used to quantify MDA. The procedure involves adding 250 µL homogenate, 100 µL 8% sodium dodecyl sulfate (SDS), 750 µL 20% acetic acid, 750 µL 0.08% TBA, and 150 µL distilled water to capped test tubes, which are then vortexed. After incubating the mixture at 100 °C for 60 minutes, 2.5 mL of n-butanol is added. The absorbance of the resulting red color is measured at 532 nm using 3-mL cuvettes. The MDA content is determined using a standard curve created from a pre-prepared MDA stock solution, taking into account the dilution factors [62].

5.1.3 Determination of Myeloperoxidase (MPO) Activity:

MPO activity is measured following a modified method by Bradley et al. [63] Homogenized samples are frozen and then centrifuged at 1500g for 10 minutes at 4 °C. To determine MPO activity, 100 µL of the supernatant is mixed with 1.9 mL of 10 mmol/L phosphate buffer (pH 6.0) and 1 mL of 1.5 mmol/L o-dianisidine hydrochloride containing 0.0005% hydrogen peroxide. Changes in absorbance at 450 nm are recorded using a UV-Vis spectrophotometer.

6. CONCLUSION

In conclusion, the review comprehensively addresses the various in vitro and in vivo models utilized in the study of antiulcer agents. These models have significantly contributed to our understanding of the mechanisms underlying ulcer formation and the potential therapeutic benefits of various compounds. The studies on histamine H4 receptors, methylene blue-induced ulcers, serotonin-induced peptic ulcers, and ethanol-induced mucosal damage provide insights into the complex interactions and pathways involved in gastric ulceration. The findings underscore the importance of these models in elucidating the gastroprotective effects of potential antiulcer agents and their mechanisms of action.

7. LIMITATIONS

Despite the valuable insights gained from these studies, several limitations need to be addressed. The variability in responses observed among different animal strains highlights the necessity of considering genetic and physiological differences in pharmacological research. Additionally, while in vivo models offer a closer approximation to human physiology, they cannot entirely replicate the complexity of human gastric pathology. Furthermore, the extrapolation of results from animal models to human conditions remains a challenge due to interspecies differences. Future research should aim to develop more sophisticated models that better mimic human gastric ulcers and consider the translational aspects of preclinical findings to clinical applications

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Ethical Declaration
As it's a review hence no ethical approval required and all the studies included in review has been approved by their own country.

8. REFERENCES

1. Najm, W. I. (2011). Peptic ulcer disease. *Primary Care: Clinics in Office Practice*, *38*(3), 383-394.
2. Ajeigbe, K. O., Onifade, A. A., Omotoso, D. R., Enitan, S. S., & Olaleye, S. B. (2014). Anti-ulcerogenic activity of Aspilia africana leaf extract: roles of gastric acid, oxidative stress and neutrophil infiltration. *African Journal of Biomedical Research*, *17*(3), 193-201.
3. Mousavi, T., Hadizadeh, N., Nikfar, S., & Abdollahi, M. (2020). Drug discovery strategies for modulating oxidative stress in gastrointestinal disorders. *Expert Opinion on Drug Discovery*, *15*(11), 1309-1341.
4. Sumbul, S., Ahmad, M. A., Mohd, A., & Mohd, A. (2011). Role of phenolic compounds in peptic ulcer: An overview. *Journal of pharmacy and bioallied sciences*, *3*(3), 361-367.
5. Sharifi-Rad, M., Fokou, P. V. T., Sharopov, F., Martorell, M., Ademiluyi, A. O., Rajkovic, J., ... & Sharifi-Rad, J. (2018). Antiulcer agents: From plant extracts to phytochemicals in healing promotion. *Molecules*, *23*(7), 1751.
6. Srinivas[1], R. L., Lakshmi[1], S. M., Shama[1], S. N., Reddy[1], G. K., & Prasanna, K. R. (2013). Medicinal plants as anti-ulcer agents. *Journal of Pharmacognosy and Phytochemistry*, *2*(4), 91-97.
7. Karampour, N. S., Arzi, A., Rezaie, A., Pashmforosh, M., & Rad, H. (2019). Gastroprotective effects of betanin against ethanol-induced gastric ulcer in rats. Jundishapur Journal of Natural Pharmaceutical Products, 14(3).
8. Adinortey, M. B., Ansah, C., Galyuon, I., & Nyarko, A. (2013). In vivo models used for evaluation of potential antigastroduodenal ulcer agents. *Ulcers*, *2013*(1), 796405.
9. McGuigan, J. E., & Trudeau, W. L. (1968). Immunochemical measurement of elevated levels of gastrin in the serum of patients with pancreatic tumors of the Zollinger-Ellison variety. *New England journal of medicine*, *278*(24), 1308-1313.
10. Hansky, J., & Cain, M. D. (1969). Radioimmunoassay of gastrin in human serum. *The Lancet*, *294*(7635), 1388-1390.
11. Yalow, R. S., & Berson, S. A. (1970). Radioimmunoassay of gastrin. *Gastroenterology*, *58*(1), 1-14.
12. Stadil, F., & Rehfeld, J. F. (1971). Radioimmunoassay of gastrin in human serum. *Scandinavian Journal of gastroenterology. Supplement*, *9*, 61-65.
13. Bralow, S. P. (1969). Current concepts of peptic ulceration. *The American Journal of Digestive Diseases*, *14*, 655-677.
14. Walsh, J. H., & Grossman, M. I. (1975). Gastrin: (First of Two Parts). *New England Journal of Medicine*, *292*(25), 1324-1334.
15. Dupre, J., Curtis, J. D., Unger, R. H., Waddell, R. W., & Beck, J. C. (1969). Effects of secretin, pancreozymin, or gastrin on the response of the endocrine pancreas to administration of glucose or arginine in man. *The Journal of clinical investigation*, *48*(4), 745-757.
16. Rehfeld, J. F., & Van Solinge, W. W. (1994). The tumor biology of gastrin and cholecystokinin. *Advances in cancer research*, *63*, 295-347.

17. Gregory, R. A., Grossman, M. I., Tracy, H., & Bentley, P. H. (1967). Nature of the gastric secretagogue in Zollinger-Ellison tumours. *The Lancet*, *290*(7515), 543-544.
18. Gregory, R. A., Grossman, M. I., Tracy, H., & Bentley, P. H. (1967). Nature of the gastric secretagogue in Zollinger-Ellison tumours. *The Lancet*, *290*(7515), 543-544.
19. Praissman, M., Walden, M. E., & Pellecchia, C. (1983). Identification and characterization of a specific receptor for cholecystokinin on isolated fundic glands from guinea pig gastric mucosa using a biologically active 125I-CCK-8 probe. *Journal of receptor research*, *3*(5), 647-665.
20. Gully D, Fréhel D, Marcy C, Spinazzé A, Lespy L, Neliat G, Maffrand JP, Le Fur G. Peripheral biological activity of SR 27897: a new potent non-peptide antagonist of CCKA receptors. Eur J Pharmacol. 1993 Feb 23;232(1):13-9. doi: 10.1016/0014-2999(93)90722-t. PMID: 7681406.
21. Richardson CT, Feldman M, Brater C, Welborn J. Tiotidine, a new long-acting histamine H2-receptor antagonist: comparison with cimetidine. Gastroenterology. 1981 Feb;80(2):301-6. PMID: 6108895.
22. Monczor, F., Fernandez, N., Legnazzi, B. L., Riveiro, M. E., Baldi, A., Shayo, C., & Davio, C. (2003). Tiotidine, a Histamine H2 Receptor Inverse Agonist That Binds with High Affinity to an Inactive G-Protein—Coupled Form of the Receptor. Experimental Support for the Cubic Ternary Complex Model. Molecular pharmacology, 64(2), 512-520.
23. Valenzuela, J. E., Strecker, R. B., & Douglas, A. P. (1981). Tiotidine, a new H 2-receptor antagonist, is a potent inhibitor of nocturnal acid secretion in duodenal ulcer patients. *Digestive Diseases and Sciences*, *26*, 433-437.
24. Smeulders, L., Bunkens, L., Vereycken, I., Van Acker, K., Holemans, P., Gustin, E., ... & Dams, G. (2013). A Homogeneous Time-Resolved Fluorescence Assay to Identify Inhibitors of HIV-1 Fusion. *Antiviral Methods and Protocols*, 11-17.
25. Sachs, G., Wen, Y., & Scott, D. R. (2009). Gastric infection by Helicobacter pylori. *Current gastroenterology reports*, *11*(6), 455-461.
26. Wyle, F. A., Tarnawski, A., Schulman, D., & Dabros, W. (1990). Evidence for gastric mucosal cell invasion by C. pylori: an ultrastructural study. *Journal of clinical gastroenterology*, *12*(1), S92-S98.
27. Norton, L., Nolan, P., Sales, J. E. L., & Eiseman, B. (1972). A swine stress ulcer model. *Annals of surgery*, *176*(2), 133-138.
28. Guth PH, Aures D, Paulsen G. Topical aspirin plus HCl gastric lesions in the rat. Cytoprotective effect of prostaglandin, cimetidine, and probanthine. Gastroenterology. 1979;**76**:88–93.
29. Liu GS, Huang YX, Li SW, Pan BR, Wang X, Sun DY, Wang QL. Experimental study on mechanism and protection of stress ulcer produced by explosive noise. World J Gastroenterol. 1998 Dec;4(6):519-523. doi: 10.3748/wjg.v4.i6.519. PMID: 11819360; PMCID: PMC4723443.
30. Zhang XY, Zhang NZ. Modern field of internal medicine (in Chinese) Beijing: People's Military Medicine Publishing House. 1997:249–251
31. Wan JL. Mechanism of formation of stress ulcer (in Chinese) Chin J Pathophysiol. 1993;**9**(5):664–666.
32. Huang XQ, Wang YZ. Damage of noise to body and mind of patient (in Chinese) Med Information. 1995;**8**(6):279–280
33. Shi XY. Medical experimental zoology (in Chinese) Xi'an: Shaanxi Sci. & Tech Pub House. 1989;(6):56–62

34. Landeira-Fernandez J. Analysis of the cold-water restraint procedure in gastric ulceration and body temperature. Physiol Behav. 2004 Oct 15;82(5):827-33. doi: 10.1016/j.physbeh.2004.06.016. PMID: 15451646.
35. Guo S, Gao Q, Jiao Q, Hao W, Gao X, Cao JM. Gastric mucosal damage in water immersion stress: mechanism and prevention with GHRP-6. World J Gastroenterol. 2012 Jun 28;18(24):3145-55. doi: 10.3748/wjg.v18.i24.3145. PMID: 22791951; PMCID: PMC3386329.
36. Deakin M, Williams JG. Histamine H2-receptor antagonists in peptic ulcer disease. Efficacy in healing peptic ulcers. Drugs. 1992 Nov;44(5):709-19. doi: 10.2165/00003495-199244050-00003. PMID: 1280563.
37. Adami, M., Pozzoli, C., Menozzi, A., Bertini, S., Passeri, B., Cantoni, A. M., ... & Coruzzi, G. (2012). Effects of histamine H4 receptor ligands in a mouse model of gastric ulceration. *Pharmacology*, *89*(5-6), 287-294.
38. Satapathy, T., Sen, K., Sahu, S., Pradhan, B., Gupta, A., Khan, M. A., ... & Yadav, N. (2024). Experimental animal models for gastric ulcer/peptic ulcer: An overview. *Journal of Drug Delivery and Therapeutics*, *14*(1), 182-192.
39. Takeuchi, K., Furukawa, O., Tanaka, H., & Okabe, S. (1986). A New Model of Duodenal Ulcers Induced in Rats by Indomethacln Plus Histamine. *Gastroenterology*, *90*(3), 636-645.
40. Kath, R. K., & Gupta, R. K. (2006). Antioxidant activity of hydroalcoholic leaf extract of Ocimum sanctum in animal models of peptic ulcer. *Indian Journal of Physiology and Pharmacology*, *50*(4), 391.
41. Shah, D. I., Santani, D. D., & Goswami, S. S. (2006). A novel use of methylene blue as a pharmacological tool. *Journal of pharmacological and toxicological methods*, *54*(3), 273-277.
42. Pérez, S., Taléns-Visconti, R., Rius-Pérez, S., Finamor, I., & Sastre, J. (2017). Redox signaling in the gastrointestinal tract. *Free Radical Biology and Medicine*, *104*, 75-103.
43. Nazarbahjat, N., Kadir, F. A., Ariffin, A., Abdulla, M. A., Abdullah, Z., & Yehye, W. A. (2016). Antioxidant properties and gastroprotective effects of 2-(ethylthio) benzohydrazones on ethanol-induced acute gastric mucosal lesions in rats. *PLoS One*, *11*(6), e0156022.
44. Konturek, P. C., Brzozowski, T., Konturek, S. J., Pajdo, R., Konturek, J. E., Kwiecien, S., ... & Hahn, E. G. (1999). Apoptosis in gastric mucosa with stress-induced gastric ulcers. *Journal of Physiology and Pharmacology*, *50*(2).
45. Maity, P., Bindu, S., Choubey, V., Alam, A., Mitra, K., Goyal, M., ... & Bandyopadhyay, U. (2008). Lansoprazole protects and heals gastric mucosa from non-steroidal anti-inflammatory drug (NSAID)-induced gastropathy by inhibiting mitochondrial as well as Fas-mediated death pathways with concurrent induction of mucosal cell renewal. *Journal of Biological Chemistry*, *283*(21), 14391-14401.
46. Mohamed Yousif Ibrahim, M. Y. I., Najihah Mohd Hashim, N. M. H., Dhiyaaldeen, S. M., Al-Obaidi, M. M. J., El-Ferjani, R. M., Adam, H., ... & Hapipah Mohd Ali, H. M. A. (2016). Acute toxicity and gastroprotection studies of a new Schiff base derived manganese (II) complex against HCl/Ethanol-induced gastric ulcerations in rats.
47. Sidahmed, H. M., Hashim, N. M., Amir, J., Abdulla, M. A., Hadi, A. H. A., Abdelwahab, S. I., ... & Mohan, S. (2013). Pyranocycloartobiloxanthone A, a novel gastroprotective compound from Artocarpus obtusus Jarret, against ethanol-induced acute gastric ulcer in vivo. *Phytomedicine*, *20*(10), 834-843.
48. Maeng, J. H., Lee, E., Lee, D. H., & Yang, S. G. (2013). Rabbit gastric ulcer models: comparison and evaluation of acetic acid-induced ulcer and mucosectomy-induced ulcer. *Laboratory animal research*, *29*(2), 96-102.

49. Blaser, M. J. (1992). Hypotheses on the pathogenesis and natural history of Helicobacter pylori-induced inflammation. *Gastroenterology*, *102*(2), 720-727.
50. KURATA, J. H., & HAILE, B. M. (1984). Epidemiology of peptic ulcer disease. *Clinics in Gastroenterology*, *13*(2), 289-307.
51. Okabe, S., & Amagase, K. (2005). An overview of acetic acid ulcer models—the history and state of the art of peptic ulcer research—. Biological and Pharmaceutical Bulletin, 28(8), 1321-1341.
52. Groenen, M. J., Kuipers, E. J., Hansen, B. E., & Ouwendijk, R. J. T. (2009). Incidence of duodenal ulcers and gastric ulcers in a Western population: back to where it started. *Canadian Journal of Gastroenterology and Hepatology*, *23*(9), 604-608.
53. Peña Mercado, E., Garcia Lorenzana, M., & Beltran, N. E. (2018). Histomorphometric analysis with a proposed tissue lesion index in ischemia-reperfusion induced gastric mucosa damage.
54. Keshavarzi, Z., Mohebbati, R., Mohammadzadeh, N., & Alikhani, V. (2018). The protective role of estradiol & progesterone in male rats, following gastric ischemia-reperfusion. *Acta Endocrinologica (Bucharest)*, *14*(1), 30.
55. Kisaoglu, A., Borekci, B., Yapca, O. E., Bilen, H., & Suleyman, H. (2013). Tissue damage and oxidant/antioxidant balance. *The Eurasian journal of medicine*, *45*(1), 47.
56. Yapca OE, Borekci B, Suleyman H. Ischemia-Reperfusion Damage. Eurasian J Med. 2013;45(2):126-7. https://doi.org/10.5152/eajm.2013.24
57. Semenza, G. L. (2000). Cellular and molecular dissection of reperfusion injury: ROS within and without. *Circulation research*, *86*(2), 117-118.
58. Parks, D. A., & Granger, D. N. (1983). Ischemia-induced vascular changes: role of xanthine oxidase and hydroxyl radicals. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *245*(2), G285-G289.
59. Demiryilmaz, I., Turan, M. I., Kisaoglu, A., Gulapoglu, M., Yilmaz, I., & Suleyman, H. (2014). Protective effect of nimesulide against hepatic ischemia/reperfusion injury in rats: effects on oxidant/antioxidants, DNA mutation and COX-1/COX-2 levels. *Pharmacological Reports*, *66*(4), 647-652.
60. Wada, K., Kamisaki, Y., Kitano, M., Kishimoto, Y., Nakamoto, K., & Itoh, T. (1996). A new gastric ulcer model induced by ischemia-reperfusion in the rat: role of leukocytes on ulceration in rat stomach. *Life sciences*, *59*(19), PL295-PL301.
61. Sharma, A. K., Bhat, M., Singh, S., Gill, K., Zaid, M., Tantray, J., ... & Garg, A. (2023). New Drug Discovery of Anti-inflammatory Drugs: Insights in Animal Models.
62. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, *95*(2), 351-358.
63. Bradley, P. P., Priebat, D. A., Christensen, R. D., & Rothstein, G. (1982). Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *Journal of investigative dermatology*, *78*(3), 206-209.