Pharmacological Screening Techniques for Evaluation of Gastric Ulcers: Principles, Mechanism and Procedures

Shivam*, Neetu Sachan, Phool Chandra
School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Delhi Road (NH-24), Moradabad (UP)-244 192, India.
*Corresponding author’s E-mail: shivamdmohit@gmail.com

Received: 12-09-2020; Revised: 24-10-2020; Accepted: 29-10-2020; Published on: 15-11-2020.

ABSTRACT
A gastric ulcer is the most common disease in the world. Different allopathic medicine or drugs are used for the treatment of gastric ulcers. These are effective in the treatment of ulcers but produce various side effects. These days use alternative medicine for the treatment of gastric ulcers. The natural drugs produce lower side effects in the living system therefore these are best from allopathic drugs. Identification of plants for the treatment of gastric ulcers the screenings of plants are requiring. Various methods are used to check the activity of natural drugs or herbs. These methods are called experimental animal’s models. In pursuing medical knowledge and alleviating human suffering, animal models have provided invaluable details. Using different animal models, the basis of our basic knowledge of disease pathophysiology and human anatomy can primarily be traced to preclinical studies.

Keywords: Pharmacological Screening; Models; Gastric ulcers; Principles; Mechanism.

INTRODUCTION
A gastric ulcer is the mucosal lesions in which damage the mucosal layer and form a cavity covered by chronic and acute inflammation. Gastric ulcers may locate at the stomach or duodenum. Stomach ulcers are called peptic ulcers and intestine ulcers called duodenal ulcers. Duodenal ulcers are located at the duodenal bulb. A peptic ulcer is the most predominant gastric disease. 10 % of the world population suffered from gastric ulcer disorder. H. pylori induce the damage gastric mucosa layer and increase skin lesions. Regular intake of NSAIDS cause inhibits prostaglandin secretion and result development of gastric ulcers. Peptic ulcers are caused by a disturbance of the balance between gastric protective factors and gastric aggressive factors. If the secretion of the pepsin and hydrochloric acid from parietal cells increases then increase the chances of development of gastric ulcers. Peptic ulcer disease may be identified based on the presence of clinical symptoms such as acidity, dyspepsia, nocturnal pain, reduced pain after food and epigastric burning pain. Some other symptoms such as vomiting, weight loss, anorexia and gastric bleeding observe in some patients. Enzyme-linked immunosorbent assay is used for the identification of H. pylori infection.

Different types of antiulcer drugs can use for the management of gastric ulcers. If patients suffer from peptic ulcer disease then not administers alcohol, Smoking and NSAIDs. Drugs such as antihistamine, Proton pump inhibitors, Antacids, anti-microbial and anticholinergic commonly use for the management of peptic ulcers. Proton pump inhibitors are more effective than H2 receptor antagonists. Plants are the natural source of the drugs that show different pharmacological activities and traditional uses. Some plants are traditionally used for the treatment of gastric ulcers or duodenal ulcers with minimum side effects. For the identification of the property of the meditational plants, screening is required. Different animal experimental models are used for the screening of the plant activities.

Several antiulcer animal models were used for the screening of antulcer activity of the Phytochemicals of the plants. The choice of the experimental model depends on the experimental design and objective of the studies.

EXPERIMENTAL ANIMAL MODELS
Gastric ulcers can be produced by surgical procedure, physiological methods and pharmacological or drugs in rats, mice, and other several species. There are following screening methods that are used for evaluation of the antiulcer activity of the drugs.

Acetic acid-H. pylori-induced ulcers
Acetic acid and H. pylori use for the induction of gastric ulcers in the experimental animals. This process was performed under anesthesia and laparotomy in rats.

Procedure
Firstly, the male experimental rats (200-250 g) select for this experiment. The animals divide into groups. Group first (normal control) administer the normal saline solution, group second with H. pylori (ulcer control)
administer test drug and group third administer the standard drug (clarithromycin 25 mg/kg).

The rats anesthetized intramuscularly by a mixture of zoletil (12.5 mg/kg), Ketamine (25 mg/kg) and Xylazine (25 mg/kg) at the ratio 1:1:1 with 0.1 ml/100 g body weight. The stomach of the experimental animal exposes by laparotomy with anesthetized. 0.03 ml 20 % acetic acid injects into the subserosal layer of the glandular part of the stomach with the help of a micro-syringe. The animals should be free from food for 8-9 hours. The animals receive H. pylori inoculums and drugs. Rats inoculated with 1 ml of pathogenic serum of H. pylori suspended in MHB after 24 hours acetic acid induce ulceration.12, 13 After treatment, the animals are sacrificed after 4 hours of administration of the test drug. The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index. The area and diameter of the ulcer measure with help of a ruler. The ulcer score calculates on the base of the severity of gastric ulcer lesion. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.14

**Acetic Acid Induce Gastric Ulcers**

This model is used to develop chronic gastric ulcers. Acetic acid interferes with the pH of gastric fluid. This model suitable for screening the effect of potential drugs and the evaluation of test drugs that use for healing chronic ulcers. This model also uses anti-secretary and cytoprotective evaluation 11. Different animals maybe use for this experimental model.15

**Procedure**

Albino rats (150-200 g) use for experimental work in his model. The experimental animals are anesthetized with a general anesthetic such as pentobarbitone (35 mg/kg). When an animal is completely anesthetized then open the abdomen and stomach is visualized. 50 % acetic acid solution (0.06 ml/rat) is dropped into the tube that has a 6 mm diameter is tightly put on the gastric (stomach) wall and allowed to remain for only 1 minute. The abdomen of the rat folds in two layers after removal of acid solution and animals are put in the cage for normal fed. After 4 hours petition of acetic acid the test and standard drug administered through oral route for 9 days after the induction of gastric (peptic) ulcers.16 The last test and standard-dose administered 10th day and after 18 hours the animals anesthetized and sacrificed. The abdomen open and dissected its stomach and determine the ulcer index and ulcer scores.17

**Cysteamine-induced duodenal ulcers**

It is a model that is used to induce duodenal ulcers in rats. This model firstly describes by Selye and Szabo. This model widely uses as induction of duodenal ulcer for the screening of antiulcer drugs.18-20

**Principle and Mechanism**

Cysteamine stimulates gastric acid secretion and inhibits the production of alkaline mucous from Brunner’s gland.21

**Procedure**

Firstly, animals select for experimental work. Male Wistar rats (200-250 g) use for this model.21 All animals free from food for 24 hours but not water. The experimental animals were divided into groups. Group first administers only normal saline solution and group second administers standard (gastro-protecting) drug. The cystamine hydrochloride (450 mg/kg) administers to the animals.22 Animals are sacrificed after 4 hours of administration of the test drug. The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.

**Diethyldithiocarbamate- (DDC)-induced Ulcers**

The diethyldithiocarbamate model is used to produce lesions in the stomach of rats. This model also uses for the screening of antioxidant activity.

**Principle and Mechanism**

Diethyldithiocarbamate generates free radicals such as superoxide and hydroxyl radicals that suppressed gastric mucosal copper-zinc superoxide dismutase (Cu, Zn-SOD) activity result in gastric lesions. Diethyldithiocarbamate also decreases blood flow in gastric mucosal that causes gastric ulcers.23

**Procedure**

Animals select for experiments and divide them into groups. All animals fast for 24 hours. Animals should free from water four two hours. Acute glandular lesions produced 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. Test drug administers to the animals after 2 hours. 24, 25 Animals are sacrificed after 4 hours of administration of the test drug. The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.26, 27

**Ethanol Induced Ulcers**

Absolute ethanol uses for induction gastric ulcers in the experimental animals. Alcohol penetrate the mucous of the stomach, therefore, cause gastric ulcers.

**Principle and Mechanism**

Ethanol disturbs the gastric secretion through gastric mucous depletion, damage the mucosa, alteration in permeability and generation of free radicals. When ethanol metabolizes then produce free radicals such as hydroperoxy free radicals and superoxide free radicals.28 Alcohol is responsible for penetrating the gastric mucosa.
Therefore damage the cells and increase the permeability to sodium and water. Intracellular calcium also accumulates and causes pathogenesis of gastric injury that is responsible for the death of cells and exfoliation of the surface of gastric layers. Alcohol also increases the levels of malondialdehyde that is responsible for increase lipid peroxidation.

**Procedure**

Generally experimental rats (150-200 g) use for ethanol induce ulcer model. Firstly, weigh either sex rats then divide into groups. Animals should fast for 24 hours with free access to water. The animals are administered test drug or standard drugs. After 1 hour 1 ml absolute ethanol (99.80 %) administer orally to the experimental rats. The rats are anesthetized with ether 1 hour after alcohol dose. When animals anesthetized then dissected their stomach with greater curvature and calculates estimate gastric contents, pH of contents, total acidity and also calculates ulcer index. Finally, stomach tissues prepare for histopathology and evaluate the histochomical section by light microscope. For histopathology, a small fragment of the stomach wall of each animal is fixed with 10 % formalin buffer solution followed by tissue dehydration with xylene and alcohol. The section of the stomach wall is embedded in paraffin wax and sectioned (3-5 µm) slide before staining. Haematoxylin and eosin dye used for staining. The histochemical section was observed under a light microscope.

**Ferrous iron-ascorbic acid-induced gastric ulcers**

In this model the solution of ascorbic acid and ferrous iron use for the induction of gastric ulcer with direct local injection at the gastric wall.

**Principle and Mechanism**

In this model, the solution of ferrous iron and ascorbic acid interfere in the lipid peroxidation, therefore, generate free radicals (oxygen radicals) that causes ulceration in the stomach wall.

**Procedure**

Male albino rats select for this experimental work. The weight of the animal should not less than 150 g. The animals divide into groups. All animals free from fed for 18 hours before start the experimental work but not free from water. 25 µl of the drug dissolved in the normal saline and prepare a solution. This solution injects into the submucosa anterior wall of the stomach with the help of a microsyringe (Naito, et al 1995). Animals are sacrificed after 4 hours of administration of the test drug. The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.

**Histamine Induced Gastric Ulcers**

Histamine is amine of tissue that is responsible for the secretion of gastric acid with disturbance of gastric mucosa. Generally, histamine present in the animal within storage granules of mast cells. Histamine rapidly stimulates the secretion of (HCl) hydrochloric acid with disturb in the gastric mucosa. Histamine not only increases secretion of hydrochloric acid in animals but also results in an abnormality in the gastric motility, decrease mucous production, mucosa and microcirculation in the stomach.

**Principle and Mechanism**

Most cells present in the wall of the stomach that secretes histamine. Histamine binds with histamine receptor (H₂) are present on the surface of parietal cells. Histamine stimulates adenyl cyclase enzyme that converts adenosine triphosphate into c-AMP, which in turn activates the membrane proton pump (H⁺K⁺ATPase) then stimulates the secretion of hydrochloric acid.

**Procedure**

Mostly guinea pigs (300 – 450 gm) use as an experimental animal for this model. The animal does not administer food for 48 hours before the experiment or administration of histamine. 1 ml histamine acid phosphate solution (50mg/ml/ip) administer to the guinea pig and after one hour administer test drug dose to the animal. After four hours of histamine treatment, the animal anesthetized and dissected out the stomach. All content of stomach estimates and the score of ulceration compared with control animals.

**Hydrochloric Acid Induce Gastric Ulcers**

Hydrochloric acid induces peptic ulcers with a direct increase in the acidity in the stomach. This animal model uses for experimental rats and mice.

**Procedure**

Experiment rats (weight about 150-180 gm) may be used for this experiment. Rats divide into groups and deprived of food for 24 h in a cage. Test, control and standard drug administer to the rats after 30 minutes HCl with ethanol (98% ethanol containing 150 mM HCl) the dose 5 ml/kg administer to each animal. After 1 hour each animal anesthetized with a general anesthetic such as ether. When an animal completely anesthetized the open its abdomen and dissects the stomach with the help of greater curvature. The inner surface of the stomach washes with normal saline solution and observed the ulceration. The gastric tissue fixes for 24 hours in 10% formalin for the examination of histopathological study. The section of the stomach wall is embedded in paraffin wax and sectioned (3-5 µm) slide before staining. Hematoxylin and eosin dye used for staining. The histochemical section was observed under a light microscope.

**Ischemia-reperfusion- (I-R-) induced gastric ulcers**

Ischemia-reperfusion model generally uses for the induction of gastric ulcers. The mucosa of the gastrointestinal tract is sensitive to ischemia.
Methylenedi blue is a synthetic drug that is used to induce lesions in the gastric mucosa. Methylene blue generally uses for screenings of antiulcer drugs.

**Principle and mechanism**

Methyl blue activate H+/K+-ATP-ase therefore the secretion of hydrochloric acid in the stomach increase and causes gastric lesions. Methyl blue also generates free radicals such as superoxide dismutase that cause oxidative stress and result in gastric ulcers. Methyl blue also interferes with the blood supply that causes acidity. Methyl blue show affinity for (M receptors) muscarinic or cetylcholine receptors and inhibit the activity of cholinesterase.

**Procedure**

Generally, experimental rats use for this experiment. Adults’ albino rats (weight 150-250 g) select and divides into groups. Test dose and standard drug dose (such as ranitidine 50 mg/kg and omeprazole 200 microgram/ml) administered to the animals. The animals fast for 24 hours and methyl blue (125 mg/kg) is administered to the animal through an oral route. Animals are sacrificed after 4 hours of administration of methyl blue. The dissect stomach wash with saline solution and observe ulcer score and calculate the ulcer index. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.

**NSAIDs Induced Peptic Ulcers**

Non-Steroidal Anti-inflammatory Drugs such as Aspirin, Ibuprofen and Indomethacin commonly used for produced peptic ulcers in experimental animals. It is the most common experimental animal model use for the anti-ulcer activity.

**Principle and Mechanism**

Nonsteroidal Anti-inflammatory Drugs (NSAIDs) are considered one of the most uses medications for patients for the treatment of pain and inflammation. NSAIDs administration displays the main cause of peptic ulcers. NSAIDs cause gastric ulcers by inhibiting prostaglandin secretion, inhibiting the formation of lipid peroxidation and generate a reactive oxygen species (ROS). Indomethacin promotes apoptosis and necrosis of cells of the stomach. NSAIDs block the activity of COX-I and COX-II (Cyclooxygenase enzyme) hence leading to inhibit mucosal blood flow, inhibit mucus and bicarbonate secretion, interfere in platelets aggregation and disturbing in microvascular structure. Indomethacin induces gastric motility, inhibits mucous secretion and bicarbonate and disrupting production of nitric oxide production in stomach tissues. It was found in various studies that indomethacin inhibits the release of protective factors, inhibiting antioxidant parameters while stimulating oxidant parameters.

**Procedure**

Indomethacin and aspirin are the most common frequently use for the induction of gastric ulcers. The experimental animals (rats) fasted for about 36 hours before start the experiment. The experimental animal’s treats with test drug 30 minutes before the administration of ulcer induce drugs like aspirin (200 mg/kg suspension in carboxymethyl cellulose) two-dose at an interval of 15 hours, after 6 hrs animals sacrificed. Phenylbutazone administers 100 mg/kg to the experimental animal at the 15 hrs dose interval and sacrificed after 6 hours. The dose of reserpine is 5 mg/ kg for the experimental animal after administration 24 hours of animal scarrifices. The dose of indomethacin is 10 mg/kg for the animal after 36 hours of fasting and after the treated dose, 15 hours animal scarrifices.

**Pylorus Ligation Model**

The Pylorus ligation model is a surgical procedure that is used for the induction of gastric ulcers. In this model, stomach pylorus ligates with the help of surgery without damage blood vessels.

**Principle and Mechanism**

When the lower part of the stomach ligates, then automatic digestion starts and breakdown of the stomach wall because gastric juice such as pepsin and gastric acid secretes for digestion. When pylorus ligated then the blood supply of the stomach disturbs and free radicals generated that are responsible for gastric juice production therefore induced gastric ulcers through this model.

**Procedure**

In this model, mainly rodent animal (Rat 140-165 gm) selects for the experiment. Firstly administered the
anesthesia to the animal then open the abdomen and ligate its stomach without damage blood vessels and then sutured it. Testing a drug or agent administered to the animal after operation within two days. Dissected the stomach after 18 hours and collect its content for estimation of acidity and pH measurement. Check the ulcer at the wall of the stomach of the treated group and standard drug and compare gastric volume, acidity, pH and ulcer index with the control group.

**Reserpine Induced Gastric Ulcers**

Reserpine is an active chemical that is derived from the roots of the medicinal plant *Rauwolfia serpentine*. This chemical is an alkaloid in nature that has antipsychotic and antihypertensive activity. Reserpine is used for induction the gastric ulcer in experimental animals such as mice and rats.

**Principle and Mechanism**

Reserpine produces the gastric ulcer due to the degranulation of mast cells therefore increase the secretion of gastric acid by sympathetic activation. It was observed that reserpine causes gastric ulcer with disturbing in the serotonin, catecholamine and histamine store at (CNS) central nervous system and peripheral nervous system. Reserpine also generates free radicals and inhibits the production of prostaglandins. Reserpine disturbs the blood supply in the stomach mucosa and alters the gastric motility.

**Procedure**

Mice selected for this experiment and it was divided into groups. All animals are fasts for 24 hours after the test drug administers intraperitoneally to the animals. After 1 hour the ulcer induces agent reserpine (10 mg/kg) administers intraperitoneally to the animals. After 4 hours all animals anesthetized with ether and then scarify and dissects their stomach. The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.

**Serotonin Induced Gastric Ulcers**

Serotonin uses for the induction of gastric ulcers in the experimental rats. This model use screening of antiulcer drugs.

**Principle and Mechanism**

Serotonin reduces the blood flow in the gastric mucosa and acts as a vasoconstrictor therefore induces the ulcer in the stomach.

**Procedure**

Firstly, albino rats select for experiments and divide them into the group. All rats free from food for 24 hours but not water and it confirms that their stomachs are empty. Before 2 hours of ulcer, induction stops the receiving of water to rats. The control group administers only a normal saline solution. A single dose of serotonin (0.5 mL of 50 mg/kg) administers by subcutaneous injection to the rats. Serotonin administers to the rats by intra-gastric intubation with the help of an orogastric cannula. Animals are sacrificed after 6 hours of administration of test drug. The dissect stomach wash with saline solution and observe ulcer scores and calculate the ulcer index. The contents of the stomach used for estimation of acidity or pH. After this prepare the slide and observe pathophysiology.

**Water- Immersion Stress-Induced Ulcers**

This model was used for the develop gastric ulcer in rats. The technique of this model was developed by Hanson and Brodie. Levine developed ordinary water immersion or cold-water method.

**Principle and Mechanism**

This model induces gastric ulcers due to disturbance in the gastric mucosa causing an increase in the secretion of histamine therefore increase the acid secretion and inhibit mucus production. Water immersion stress induces the motility of gastric folds of the stomach and disturbs the blood supply.

**Procedure**

All animals were divided into groups and fasted for 24 hours with free from food and drinking water before starting the experiment. Each rat restrained individually in a cage and immersed up to its xiphoid in temperature-controlled water (about 23°C) for 10 hours. After this, the animals anesthetized and the stomach of each animal ligate at pylorus and cardia and fixed by paraformaldehyde for 24 hours. Then the stomach opens with the help of greater curvature and dissects it. The dissect stomach wash with cold saline solution to remove the gastric contents. The stomach flattened and dissects its photographs. After this calculate ulcer index, ulcer scores and prepare the slide for pathophysiology.

**PARAMETERS TO BE CALCULATED**

There are seven parameters i.e., pH, Volume of gastric contents, Total and Free Acidity, Lipid Peroxidation, ulcer index, % protection ratio and % curative ratio, calculated by using the method described by different scientist to evaluate the anti-ulcer activity of the drug in *in-vivo* models.

**pH**

pH of gastric content determines by dipping the electrode of the pH meter in a beaker containing gastric contents.

**The volume of gastric contents**

The volume of gastric contents measures pouring gastric Contents carefully in the graduated cylinder.

**Total and Free Acidity**

Collect one mL of gastric content and centrifuge it and filter for titration against 0.1 sodium hydroxide solution using
the Topfers reagent. This reagent uses as an indicator for the determination of free acidity. 1% solution of phenolphthalein indicator use for confirmed total acidity. The sum of two titrations will be total acidity. 83

Lipid Peroxidation

The glandular part of the stomach tissue will be homogenized in trichloroacetic acid (TCA) and the homogenate use to estimate malondialdehyde. Briefly, lipid peroxidation will be induced by adding ferric chloride (10 1d. 400mM) and 1-ascorbic acid (10d, 400mM) to a mixture containing stomach homogenate (0.3 ml) in phosphate buffer solution (5 ml pH 7.4. 0.2 M). After incubation for 1 h at 37 °C. the reaction will be stopped by adding hydrochloric acid (2 ml. 0.25 N) containing trichloroacetic acid (1 ml. 15% w/v ) and thiobarbituric acid (0.5 ml. 0.375% w/v) boiled for IS mm. cooled, centrifuged and absorbance of the supernatant % will be measured at 532 nm. 84

Scoring of ulcers based on ulcer severity.

For calculating ulcer following may be considered: 13

| Score | Ulcer severity            |
|-------|---------------------------|
| 0     | No lesions                |
| 1     | mucosal edema             |
| 2     | 1-5 small lesions (1-2 mm in size) |
| 3     | > 5 small or intermediate (3-4 mm in size) lesions |
| 4     | ≥ 2 intermediate lesions or 1 gross (> 4 mm in size) lesion |
| 5     | Perforated ulcers         |

Calculation of Ulcer Index (UI) based on ulcer score

By using the ulcer score as described above, the ulcer index can be calculated as follows:

\[
\text{Ulcer Index (UI)} = \frac{\text{Total ulcer score}}{\text{Number of animals ulcerated}}
\]

Calculation of % protection ratio and % curative ratio by using the Ulcer Index

The following formula may be used for the calculation of percentage protection and percentage curative ratio 85

\[
\text{% protection ratio} = \frac{\text{UI of ulcerogen treated group}}{\text{UI of ulcerogen treated group}} \\
\text{UI of drug pre treated group} \\
\text{UI of ulcerogen treated group}
\]

\[
\text{% Curative Ratio} = \frac{\text{UI of ulcerogen treated group}}{\text{UI of ulcerogen treated group}} \\
\text{UI of drug treated group} \\
\text{UI of ulcerogen treated}
\]

CONCLUSION

It is undeniable that animal models have led to human science. Without a high fidelity, highly reproducible model, with the added advantage of avoiding potential human damage, many modern developments would simply not have been made possible. However, a closer look at the present environment poses concerns. We must reexamine the use of sentient animals in human research with the implementation of alternatives such as simulation, with an eye to reduction, enhancement, and finally replacement if possible.

ACKNOWLEDGEMENTS

Authors are thankful to Prof. M.P. Pandey, Vice-Chancellor, IFTM University, Moradabad for providing all facilities to carry out research work

REFERENCES

1. Aro P, Storskrubb T, Ronkainen J, Bolling-Sternevald E, Engstrand L, Vieth M, Stolte M, Talley NJ, Agréus L, Peptic ulcer disease in a general adult population: the Kalixanda study: a random population-based study, American journal of epidemiology, 163(11), 2006, 1025-1034.
2. Malfertheiner P, Chan FK, McColl KE, Peptic ulcer disease, Lancet, 374(9699), 2009, 1449-1461.
3. Shimoyama AT, Santin JR, Machado ID, de Oliveira e Silva AM, de Melo IL, Mancini-Filho J, Farshy SH, Antulcerogenic activity of chlorogenic acid in different models of gastric ulcer, Naunyn-Schmiedeberg's archives of pharmacology, 386(1), 2013, 5-14.
4. Meyer-Rosberg K, Scott DR, Rex D, Melchers K, Sachs G, The effect of environmental pH on the protox motive force of Helicobacter pylori, Gastroenterology, 111(4), 1996, 886-900.
5. Bardou M, Barkun AN, Preventing the gastrointestinal adverse effects of nonsteroidal anti-inflammatory drugs: from risk factor identification to risk factor intervention, Joint bone spine, 77(1), 2010, 6-12.
6. Lanas A, García-Rodríguez LA, Polo-Tomás M, Ponce M, Quintero E, Perez-Aisa MA, Gisbert JP, Bujanda L, Castro M, Muñoz M, Del-Pino MD, García S, Calvet X, The changing face of hospitalisation due to gastrointestinal bleeding and perforation, Alimentary pharmacology & therapeutics, 33(5), 2011, 585-591.
7. Chan FK, Leung WK, Peptic-ulcer disease, Lancet, 360(9337), 2002, 933-941.
8. Lazzaroni M, Porro GB, Management of NSAID-induced gastrointestinal toxicity: focus on proton pump inhibitors, Drugs, 69(1), 2009, 51-69.
9. Alkofahi A, Atta AH, Pharmacological screening of the anti-ulcerogenic effects of some Jordanian medicinal plants in rats, J Ethnopharmacol, 67(3), 1999, 341-345.
10. Schmeda-Hirschmann G, Yesilada E, Traditional medicine and gastroprotective crude drugs, J Ethnopharmacol, 100(1-2), 2005, 61-66.
11. Takagi K, Okabe S, Saziki R, A new method for the production of chronic gastric ulcer in rats and the effect of several drugs...
on its healing, Japanese journal of pharmacology, 19(3), 1969, 418-426.

12. Konturek PC, Brzozowski T, Konturek SJ, Stachura J, Karczewsk E, Pajdo R, Ghiara P, Hahn EG, Mouse model of Helicobacter pylori infection: studies of gastric function and ulcer healing, Alimentary pharmacology & therapeutics, (13), 1999, 333-346.

13. Bhattacharia SK, Yean Van VL, Koh Lee C, Hui Kuean C, Candasamy M, Liew YK, Sahu PS, Protective activity of geraniol against acetic acid and Helicobacter pylori-induced gastric ulcers in rats, J Tradit Complement Med, 9(3), 2019, 206-214.

14. Bhattacharia S, Hooi L, Shyan L, Chieh L, Candasamy M, Sahu P, Effect of geraniol and clarithromycin combination against gastric ulcers induced by acetic acid and Helicobacter pylori in rats, Pharmacognosy Reseach, 11(4), 2019, 356-362.

15. Okabe S, Amagase K, An overview of acetic acid ulcer models—the history and state of the art of peptic ulcer research, Biological & pharmaceutical bulletin, 28(8), 2005, 1321-1341.

16. Bhattacharia S, Hooi L, Shyan L, Chieh L, Candasamy M, Sahu P, Effect of geraniol and clarithromycin combination against gastric ulcers induced by acetic acid and Helicobacter pylori in rats, Pharmacognosy Research, 11(4), 2019, 356-362.

17. Okabe S, Roth JL, Pfeiffer CJ, A method for experimental, penetrating gastric and duodenal ulcers in rats. Observations on normal healing, The American journal of digestive diseases, 16(3), 1971, 277-284.

18. Selye H, Szabo S, Experimental model for production of perforating duodenal ulcers by cysteamine in the rat, Nature, 244(5416), 1973, 458-459.

19. Nakamura T, Yoshida M, Kitagawa Y, Jin L, Ishikawa H, Kameyama K, Wakabayashi G, Tanabe M, Kawachi S, Shinoda M, Saikawa Y, Wada N, Kubota T, Kumai K, Sano K, Kitajima M, Intravenous injection of micafungin counteracts Candida albicans-induced aggravation of duodenal ulcers caused by cysteamine in rats, Digestive diseases and sciences, 53(9), 2008, 2422-2428.

20. Rezvanjoo B, Rashidi S, Jouyban A, Beheshtihis SH, Samini M, Effects of vitamin C and melatonin on cysteamine-induced duodenal ulcer in a cholestatic rat model: A controlled experimental study, Current therapeutic research, clinical and experimental, 71(5), 2010, 322-330.

21. Warzeca Z, Ceranowicz D, Dembiński A, Ceranowicz P, Cieszkowski J, Kuwahara A, Kato I, Dembiński M, Konturek PC, Ghrelin accelerates the healing of cysteamine-induced duodenal ulcers in rats, Medical science monitor : international medical journal of experimental and clinical research, 18(5), 2012, Br181-187.

22. Saghafi F, Karimi I, Jouyban A, Samini M, Effects of captopril on the cysteamine-induced duodenal ulcer in the rat, Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie, 64(4), 2012, 373-377.

23. Ogino K, Hobara T, Kawamoto T, Kobayashi H, Iwamoto S, Oka S, Okazaki Y, Mechanism of Diethyldithiocarbamate-Induced Gastric Ulcer Formation in the Rat, Pharmacology & Toxicology, 66(2), 1990, 133-137.

24. Chen SH, Pan S, Okita K, Takemoto T, Role of superoxide dismutase in mechanism of diethyldithiocarbamate-induced gastric antral ulcer in rats: protective effect of prostaglandin, cimetidine and pirenzepine, Journal of gastroenterology and hepatology, 8(5), 1993, 457-461.

25. Hung CR, Modulation of gastric hemorrhage and ulceration by oxidative stress and histamine release in Salmonella typhimurium-infected rats, Inflammopharmacology, 13(1-3), 2005, 235-248.

26. Salim AS, Protection against stress-induced acute gastric mucosal injury by free radical scavengers, Intensive care medicine, 17(8), 1991, 455-460.

27. Salim AS, Oxygen-derived free radical scavengers: a new approach to the problem of refractory peptic ulceration, The medical journal of Malaysia, 48(4), 1993, 392-396.

28. Okkon JE, Nwafor PA, Antiulcer and anticonvulsant activity of Croton zambesicus, Pakistan journal of pharmaceutical sciences, 22(4), 2009, 384-390.

29. Dwiwedi V, Semwal CB, Yadav NH, Evaluation of anti-ulcer activity of Clitoria ternatea Leaves (Linn) extract in Wistar rats., Indian J Res Pharmacy Biotech, (3), 2014, 1225-1229.

30. Marotta F, Tajiri H, Safran P, Fesce E, Ideo G, Ethanol-related gastric mucosal damage: evidence of a free radical-mediated mechanism and beneficial effect of oral supplementation with bionormalizer, a novel natural antioxidant, Digestion, 60(6), 1999, 538-543.

31. Potrich FB, Allemend A, da Silva LM, Dos Santos AC, Baggio CH, Freitas CS, Mendes DA, Andre E, Werner MF, Marques MC, Antiulcerogenic activity of hydroalcoholic extract of Achillea millefolium L.: involvement of the antioxidant system, J Ethnopharmacol, 130(1), 2010, 85-92.

32. Njar VC, Adesanwo JK, Raji Y, Methyl angolensate: the antiulcer agent of the stem bark of Entandrophragma angolense, Planta medica, 61(1), 1995, 91-92.

33. Dadahmed HMA, Vadivelu J, Loke MF, Arbab IA, Abdul B, Sukri MA, Abdelwahab SI, Anti-ulcerogenic activity of dentatin from clausena excavata Burm.f. against ethanol-induced gastric ulcer in rats: Possible role of mucus and antioxidant effect, Phytomedicine, 55, 2019, 31-39.

34. Naito Y, Yoshikawa T, Yonetra Y, Yagi N, Matsuyama K, Arai M, Tanigawa T, Kondo M, A new gastric ulcer model in rats produced by ferrous iron and ascorbic acid injection, Digestion, 56(6), 1995, 472-478.

35. Hiraiishi H, Terano A, Razandi M, Sugimoto T, Harada T, Ivey KJ, Role of iron and superoxide in mediating hydrogen peroxide injury to cultured rat gastric cells, Gastroenterology, 104(3), 1993, 780-788.

36. Tripathi KD, Essentials of Medical Pharmacology Sixth ed. New Delhi, India: Jaypee Brothers Medical Publishers (P) Ltd, 2008.

37. Hay LJ, Varco RL, Code CF, Wangensteen OF, Experimental production of gastric and duodenal ulcers in laboratory animals by intramuscular injection of histamine in beeswax, The Journal of Surgery, Gynecology and Obstetrics, 74, 1942, 70-182.

38. Cho CH, Pfeiffer CJ, Gastrointestinal ulceration in the guinea pig in response to dimaprit, histamine, and H1- and H2-
blocking agents, Digestive diseases and sciences, 26(4), 1981, 306-311.

39. Zaidi SH, Mukerji B, Experimental peptic ulceration. I. The significance of mucus barrier, The Indian journal of medical research, 46(1), 1958, 27-37.

40. Zhao X, Cheng Q, Qian Y, Yi R, Gu L, Wang S, Song JL, Insect tea attenuates hydrochloric acid and ethanol-induced mice acute gastric injury, Experimental and therapeutic medicine, 14(5), 2017, 5135-5142.

41. Oyagi A, Ogawa K, Kakino M, Hara H, Protective effects of a gastrointestinal agent containing Korean red ginseng on gastric ulcer models in mice, BMC complementary and alternative medicine, 10, 2010, 45.

42. Zhao X, Wang Q, Qian Y, Song JL, Ilex kudingcha C.J. Tseng (Kudingcha) prevents HCl/ethanol-induced gastric injury in Sprague-Dawley rats, Molecular medicine reports, 7(5), 2013, 1613-1616.

43. Kumar V, Abbas AK, Aster JC, Robbins & Cotran Pathologic Basis of Disease. 10th Edition ed. India: Elsevier, 2020.

44. Onen A, Kanay Z, Guzel C, Kurt D, Ceylan K, The effects of allopurinol on stomach mucosal barrier of rats subjected to ischemia-reperfusion, Turk J Medical Sci, 30(5), 2000, 449-452.

45. Wada K, Kamisaki Y, Kitano M, Kishimoto Y, Nakamoto K, Itoh T, A new gastric ulcer model induced by ischemia-reperfusion in the rat: role of leukocytes on ulceration in rat stomach, Life Sci, 59(19), 1996, PI295-301.

46. Mard SA, Nikraftar Z, Farbood Y, Mansouri E, A preliminary study of the anti-inflammatory and anti-apoptotic effects of crocin against gastric ischemia-reperfusion injury in rats, Brazilian Journal of Pharmaceutical Sciences, 51, 2015, 637-642.

47. Yonezawa D, Sekiguchi F, Miyamoto M, Taniguchi E, Honjo M, Masuko T, Nishikawa H, Kawabata A, A protective role of hydrogen sulfide against oxidative stress in rat gastric mucosal epithelium, Toxicology, 241(1-2), 2007, 11-18.

48. Paffendorf M, Bruning TA, Batnik HD, van Zwieten PA, The interaction between methylene blue and the cholinergic system, Br J Pharmacol, 122(1), 1995, 97-98.

49. Shah DI, Santani DD, Goswami SS, A novel use of methylene blue as a pharmacological tool, Journal of pharmacological and toxicological methods, 54(3), 2006, 273-277.

50. Hatware KV, Sharma S, Patil K, Shete M, Karri S, Gupta G, Evidence for gastroprotective, anti-inflammatory and antioxidant potential of methanolic extract of Cordia dichotoma leaves on indomethacin and stress induced gastric lesions in Wistar rats, Biomedicine & Pharmacotherapy, 103, 2018, 317-325.

51. Suleyman H, Albayrak A, Biliç M, Cadirci E, Halici Z, Different mechanisms in formation and prevention of indomethacin-induced gastric ulcers, Inflammation, 33(4), 2010, 224-234.

52. Redlak MJ, Power JJ, Miller TA, Role of mitochondria in aspirin-induced apoptosis in human gastric epithelial cells, American journal of physiology Gastrointestinal and liver physiology, 289(4), 2005, G731-738.

53. Wallace JL, McKnight W, Reuter BK, Vergnolle N, NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2, Gastroenterology, 119(3), 2000, 706-714.

54. Jiang GL, Im WB, Donde Y, Wheeler LA, EP4 agonist alleviates indomethacin-induced gastric lesions and promotes chronic gastric ulcer healing, World journal of gastroenterology, 15(41), 2009, 5149-5156.

55. Ozbaksi Dengiz G, Gürsan N, Effects of Momordica charantia L. (Cucurbitaceae) on indomethacin-induced ulcer model in rats, The Turkish journal of gastroenterology : the official journal of Turkish Society of Gastroenterology, 16(2), 2005, 85-88.

56. Williamson E, Okpado D, Evans F, Pharmacological Methods in Phytotherapy Research. Chichester, UK: John Wiley & Sons, 1986.

57. Guidobono F, Pagani F, Ticozzi C, Sibilia V, Pecile A, Netti C, Protection by amylin of gastric erosions induced by indomethacin or ethanol in rats, Br J Pharmacol, 120(4), 1997, 581-586.

58. Sairam K, Rao Ch V, Babu MD, Kumar KV, Agrawal VK, RK KG, Antilulcerogenic effect of methanolic extract of Emblica officinalis: an experimental study, J Ethnopharmacol, 82(1), 2002, 1-9.

59. De Sales IRP, Formiga RO, Machado FDF, Nascimento RF, Pessoa MMB, Barros M, Vieira GC, Gadelha F, Marinho AF, Barbosa Filho JM, Júnior RFA, Antunes AA, Batista LM, Cytoprotective, antioxidant and anti-inflammatory mechanism related to antilulcer activity of Cissampelos sympodium Eichl. in animal models, J Ethnopharmacol, 222, 2018, 190-200.

60. Deshpande MN, Balekar N, Evaluation of Anti-Ulcer Activity of the Ethanolic Extract of Phyllanthus Urinaria in Experimental Animals, Asian Journal of Pharmaceutical and Clinical Research, 11(12), 2018.

61. Zaghool SS, Abo-Seif AA, Rabeh MA, Abdelmohsen UR, Messiha BAS, Gastro-Protective and Anti-Oxidant Potential of Althaea officinalis and Solanum nigrum on Pyloric Ligation/Indomethacin-Induced Ulceration in Rats, Antioxidants (Basel), 8(11), 2019.

62. Shah H, S. A. Komarov, Fike SS, Meranze D, Grunstein M, H. S, A simple method for the uniform production of gastric ulceration in the rat, Gastroenterology, 5, 1945, 43–61.

63. Kulkarni SK, Hand Book of Experimental Pharmacology. 3rd Revised and Enlarged Edn ed. New Delhi: Vallabh Prakashan, 1999.

64. Kaur D, Kumar S, Sharma R, Rana AC, Protective effects of Tinospora cordifolia against reserpine induced ulcer model, International Research Journal of Pharmacy, 3(8), 2012, 275-280.

65. Singh S, Evaluation of gastric anti-ulcer activity of fixed oil of Ocimum basilicum Linn. and its possible mechanism of action, Indian J Exp Biol, 37(3), 1999, 253-257.

66. Kim KS, Shore PA, mechanism of action of reserpine and insulin on gastric amines and gastric acid secretion, and the effect of monoamine oxidase inhibition, The Journal of pharmacology and experimental therapeutics, 141, 1963, 321-325.

67. Yusuf S, Adelaiye AB, Nok AJ, Ameh DA, Balogun EO, Effect of acute bilateral adrenalectomy and reserpine on gastric
mucus secretion and mucosal injury in pyloric ligated rats, African Journal of Biotechnology, 7(17), 2008, 3143-3148.

68. Gupta MB, Tangri KK, Bhargava KP, Mechanism of ulcerogenic activity of reserpine in albino rats, Eur J Pharmacol, 27(2), 1974, 269-271.

69. Qu XZ, Jin GZ, Effects of the artemisinin on gastric ulcer induced by ethanol and reserpine in mice. J Med Sci Yanbian University, 33, 2010, 96-97.

70. Li GJ, Sun P, Wang R, Zhou YL, Qian Y, Zhao X, Preventive Effect of Polysaccharide of Larimichthys crocea Swim Bladder on Reserpine Induced Gastric Ulcer in ICR Mice, The Korean journal of physiology & pharmacology : official journal of the Korean Physiological Society and the Korean Society of Pharmacology, 18(2), 2014, 183-190.

71. LePard KJ, Stephens RL, Jr., Serotonin inhibits gastric acid secretion through a 5-hydroxytryptamine1-like receptor in the rat, The Journal of pharmacology and experimental therapeutics, 270(3), 1994, 1139-1144.

72. Ismail IF, Golbabapour S, Hassandarvish P, Hajrezaie M, Abdul Majid N, Kadir FA, Al-Bayaty F, Awang K, Hazni H, Abdulla MA, Gastroprotective Activity of <i>Poligonum chinense</i> Aqueous Leaf Extract on Ethanol-Induced Hemorrhagic Mucosal Lesions in Rats, Evidence-Based Complementary and Alternative Medicine, 2012, 2012, 404012.

73. Ji C-X, Fan D-S, Li W, Guo L, Liang Z-L, Xu R-M, Zhang J-J, Evaluation of the anti-ulcerogenic activity of the antidepressants duloxetine, amitriptyline, fluoxetine and mirtazapine in different models of experimental gastric ulcer in rats, European Journal of Pharmacology, 691(1), 2012, 46-51.

74. Salem Sokar S, Elsayed Elsayad M, Sabri Ali H, Serotonin and histamine mediate gastroprotective effect of fluoxetine against experimentally-induced ulcers in rats, Journal of Immunotoxicology, 13(5), 2016, 638-651.

75. Brodie DA, Hanson HM, A study of the factors involved in the production of gastric ulcers by the restraint technique, Gastroenterology, 38, 1960, 353-360.

76. Levine R, A method for rapid production of stress ulcers in rats. Copenhagen, Denmark: Munksgaard, 1971.

77. Kitagawa H, Fujiwara M, Osumi Y, Effects of water-immersion stress on gastric secretion and mucosal blood flow in rats, Gastroenterology, 77(2), 1979, 298-302.

78. Peters MN, Richardson CT, Stressful life events, acid hypersecretion, and ulcer disease, Gastroenterology, 84(1), 1983, 114-119.

79. Lu S, Wu D, Sun G, Geng F, Shen Y, Tan J, Sun X, Luo Y, Gastroprotective effects of Kangfuxin against water-immersion and restraint stress-induced gastric ulcer in rats: roles of antioxidation, anti-inflammation, and pro-survival, Pharmaceutical biology, 57(1), 2019, 770-777.

80. Moore EW, Determination of pH by the glass electrode: pH meter calibration for gastric analysis, Gastroenterology, 54(4), 1968, 501-507.

81. Muniappan M, Sundararaj T, Antiinflammatory and antilulcer activities of Bambusa arundinacea, J Ethnopharmacol, 88(2-3), 2003, 161-167.

82. Ribeiro AR, do Nascimento Valença JD, da Silva Santos J, Boeing T, da Silva LM, de Andrade SF, Albuquergue-Júnior RL, Thomazzi SM, The effects of baicalein on gastric mucosal ulcerations in mice: Protective pathways and anti-secretory mechanisms, Chemico-biological interactions, 260, 2016, 33-41.

83. Hazarika I, Hussain M, Das A, Pylorus ligation induced gastric ulcer protection by <i>Sesamum indicum</i> ethanolic seed extract, Research & Reviews: A Journal of Pharmaceutical Science, 6(3), 2015, 42-49.

84. Duh PD, Yen GC, Yen WJ, Chang LW, Antioxidant effects of water extracts from barley (Hordeum vulgare L.) prepared under different roasting temperatures, J Agric Food Chem, 49(3), 2001, 1455-1463.

85. Sabiu S, Garuba T, Sunmonu T, Ajani E, Sulyman A, Nurain I, Balogun A, Indomethacin-induced gastric ulceration in rats: Protective roles of Spondias mombin and Ficus exasperata, Toxicology reports, 2, 2015, 261-267.

Source of Support: None declared.

Conflict of Interest: None declared.

For any question relates to this article, please reach us at: editor@globalresearchonline.net

New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com