International Standard Serial Number (ISSN): 2319-8141
International Journal of Universal Pharmacy and Bio Sciences 8(3): May-June 2019
INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES
*IMPACT FACTOR 4.018****
*ICV 6.16****
Pharmaceutical Sciences **REVIEW ARTICLE……!!!**

PHARMACOLOGICAL REVIEW OF ANTI-ULCER SCREENING

P.SUBATHIRADEVI[1*], Dr.P.AMUDHA[2]

[1] Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Rajiv Gandhi Salai, Old Mahabalipuram Road, Jyothinagar, Thorappaikam, Chennai -600 097, Tamil Nadu, India.

[2] Professor, Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Rajiv Gandhi Salai, Old Mahabalipuram Road, Jyothinagar, Thorappaikam, Chennai -600 097, Tamil Nadu, India..

KEYWORDS:

Peptic Ulcer, Symptoms, Ulcer models, Herbal Plants.

FOR CORRESPONDENCE:

P.SUBATHIRADEVI*

ADDRESS:

Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Rajiv Gandhi Salai, Old Mahabalipuram Road, Jyothinagar, Thorappaikam, Chennai -600 097, Tamil Nadu, India.

ABSTRACT

Peptic ulcer represents a serious medical condition, Approximately 500,000 new cases are reported each year, with 5 million people alone affected in United States alone. The highest risks of contracting peptic ulcer disease are those generations born around the middle of the 20th century. Ulcer disease has become a disease predominantly affecting the older population, with the peak incidence occurring between 55 and 65 years of age. Thirty – five percent of patients diagnosed with gastric ulcers will suffer serious complications. Patients with peptic ulcer disease may present with a range of symptoms, from mild abdominal discomfort to catastrophic perforation and bleeding. Most patients with peptic ulcer disease present with abdominal discomfort, pain, or nausea. The various herbal plants are claimed to posses anti ulcer. Preclinical experiments are done to screen the anti ulcer activity in various herbal drugs using various animal models discussed in screening assays.

INTRODUCTION:

Peptic ulcer indicates an interruption in the continuity of the intestinal mucosa as a result of the action of acids and pepsin. The ulceration can occur in the stomach, duodenum and sometimes in the jejunum. Ulcers may range in size from several millimeters to several centimeters. Ulcers are delineated from erosions by the depth of penetration (erosions are more superficial and do not involve the muscularis mucosae). Gastritis indicates inflammation of the gastric mucosa, without ulceration. Gastritis is usually a precursor of ulceration, but either condition can occur in isolation [1].

These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility. Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF", leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins(PG), nitric oxide)[3].

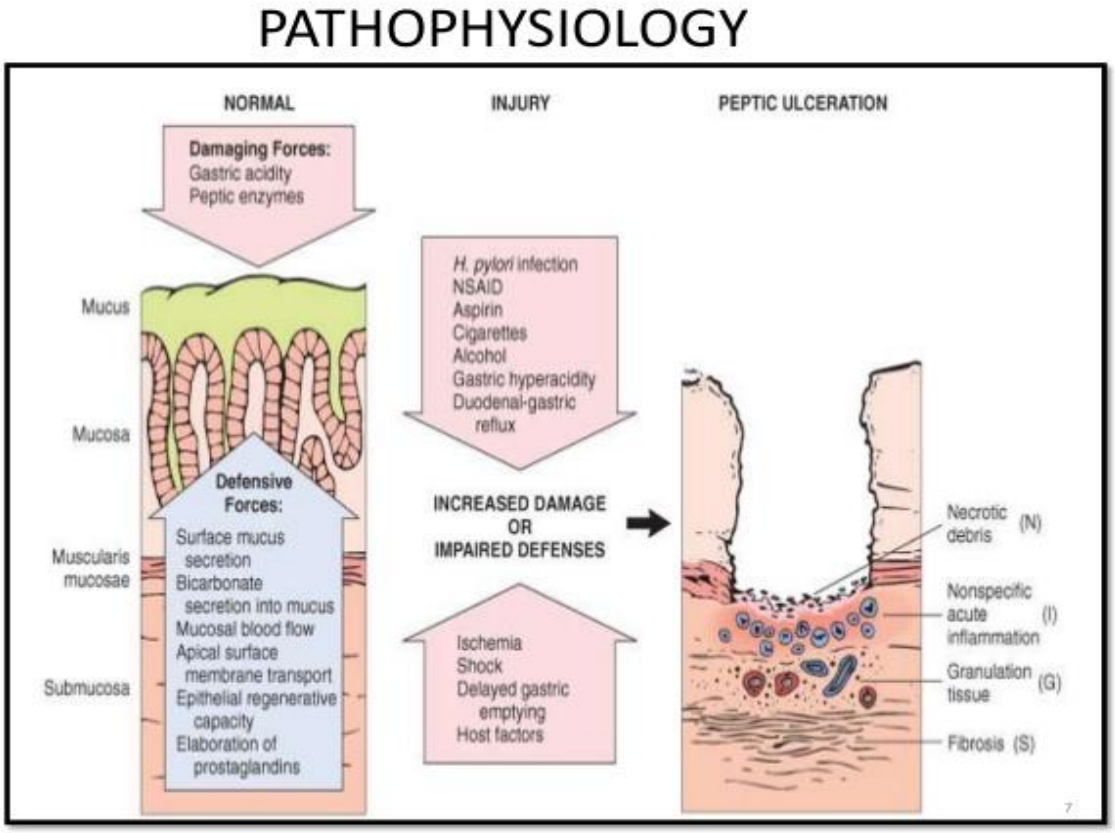
Epidemiology of peptic ulcer disease:

Ulcers can occur at any age, including infancy and childhood, but are most common in middle-aged adults. Peptic ulcer (gastric and duodenal) occurs most commonly in patients aged 30 to 50 years, although patients over the age of 60 years account for 80% of deaths even though they only account for 15% of cases. Prevalence has shifted from predominance in males to similar occurrences in both sexes. Lifetime prevalence is approximately 11% to 14% for men and 8% to 11% for women.

AETIOLOGY/PATHOPHYSIOLOGY:

H pylori and NSAIDs disrupt the normal mucosal defense and repair, making the mucosa more susceptible to acid. *H pylori* infection is present in 80% to 90% of patients with duodenal ulcers and 70% to 90% of patients with gastric ulcers. If *H.pylori* is eradicated, only 10% to 20% of patients have recurrence of peptic ulcer disease, compared with 70% recurrence in patients treated with acid suppression alone. Although the cause of peptic ulceration in some patients is apparent (such as aspirin usage), in most cases the pathogenesis is unknown.

 International Standard Serial Number (ISSN): 2319-8141



There are, however, a number of factors which have been identified as possibly leading to peptic ulceration, namely.

- ***Helicobacter pylori* infection:** As already mentioned, *H pylori* is present in the mucosa of 80% of patients with peptic ulceration and gastritis, while it is only present in 20% of the normal healthy population.
- **Genetic tendency:** A genetic tendency occurs especially in the case of duodenal ulceration.
- Furthermore, a family history exists in 50% to 60% of children with duodenal ulcer.
- **Medicine:** Medicine such as aspirin, NSAIDs and corticosteroids can cause peptic ulceration.
- **Alcohol:** Chronic drinkers of alcohol develop ulceration, while the occasional drinker normally only develops gastritis. Although alcohol is identified as a strong promoter of acid secretion, no definite data link moderate amounts of alcohol to the development or delayed healing of ulcers.
- **Cigarette smoking:** It is a risk factor for the development of ulcers and their complications. In addition, smoking impairs ulcer healing and increases the incidence of recurrence. Risk correlates with the number of cigarettes smoked per day.
- **Stress:** Severe physiologic stress can cause peptic ulcer disease, for example burns, central nervous system trauma, surgery and severe medical illness.

- **Bile salts and pancreatic enzymes:** They can cause ulceration when they leak back into the stomach on account of an inefficient pyloric sphincter, or when stasis of the intestinal bolus occurs as a result of partial obstruction.
- **Toxins secreted by micro-organisms:** e.g. toxins secreted in chronic gastroenteritis.
- Hypersecretory states. This is an uncommon cause. Examples include gastrinoma (Zollinger-Ellison syndrome), multiple endocrine neoplasia (MEN-I), antral G cell hyperplasia, systemic mastocytosis and basophilic leukemias. Very few patients have hypersecretion of gastrin (Zollinger-Ellison syndrome).
- **Chronic conditions:** Diseases associated with an increased risk of peptic ulcer disease include cirrhosis, chronic obstructive pulmonary disease, renal failure and organ transplantation.
- **Rare conditions:** Other rare, miscellaneous causes include radiation-induced or chemotherapy-induced ulcers, vascular insufficiency and duodenal obstruction. These factors weaken the normal protective barrier of the mucous membrane of the stomach and small intestine and may cause increased secretion of acid and pepsin, with resulting inflammation and subsequent ulceration [1].

TREATMENT FOR PEPTIC ULCER:

1. Reduction of gastric acid secretion

(a) H2 antihistamines: Cimetidine, Ranitidine, Famotidine, Roxatidine.

(b)Proton pump inhibitors: Omeprazole, Esomeprazole, Lansoprazole, Pantoprazole, Rabeprazole, Dexrabeprazole.

(c) Anticholinergic drugs: Pirenzepine, Propantheline, Oxyphenonium.

(d) Prostaglandin analogue: Misoprostol.

2. Neutralization of gastric acid (Antacids)

(a) Systemic: Sodium bicarbonate, Sodium citrate

(b) Nonsystemic: Magnesium hydroxide, Magnesium trisilicate, Aluminium hydroxide gel, Magaldrate, Calcium carbonate.

3. Ulcer protectives

Sucralfate, Coillidal bismuth subcitrate(CBS).

4. Anti-H.Pylori drugs

Amoxicillin, Clarithromycin, Metronidazole, Tinidazole, Tetracyline[4].

Branded names of drugs

Cimetidine: Cimetin, Tagamet, Aciloc, Azylec.

Ranitidine: Zantac, Intac, Giran.

Famotidine: Pepcid

Nizatidine: Axid

Lansoprazole: Prevacid

Rabeprazole: Aciplexs

Pantoprazole: Protonix

Herbal drugs

Alsarex (Himalaya charak product)

Herbolax

Boswellia

Vara churna

Triphalas[1].

SIDE EFFECTS OF THE DRUGS:

H2 ANTIHISTAMINES

Mechanism of action

Histamine is secreted directly into the interstitial fluid cells within the fundic mucosa and reaches neighbouring parietal cells by diffusion. Histamine is released in response to a number of physiological stimuli, and blockade of histamine receptors inhibits most forms of stimulated acid secretion. H receptors on gastric parietal cells mediate stimulation of acid secretion. H receptors antagonists like cimetidine and ranitidine bind competitively to parietal cell, H receptors, producing a potent but reversible inhibition of acid secretion [5].

Adverse effect

Most of the clinically significant adverse effects result from nonspecific blockade of extra gastric H, receptors. In addition to the effects of nonspecific blockade, the chronic suppression of gastric physiologic functions and predispose to long term complications due to bacterial colonization of the stomach or to disturbances of gastric endocrine regulation. A number of dose dependent neuropsychiatric effects have been reported with the use of cimetidine. Agitation, confusion, lethargy, and mental depression have been most frequency noted in elderly patients and in those with hepatic or renal dysfunction in whom drug metabolism is altered. Histamine receptors have been reported on the surface of subpopulations of suppressor T-lymphocytes and histamine may suppress immunologic function. The decrease in hepatic blood flow caused by these H2 receptors antagonists has been shown to interfere with metabolism of drugs such as propranolol and lidocaine, which are cleared by the liver.

PROTON PUMP INHIBITORS

Mechanism of action

Acid secretion by the parietal cell is due to an enzymatic pump which transports hydrogen ions form the parietal cell cytoplasm into the lumen of the secretory canaliculus in exchange for potassium. This hydrogen-potassium ATPase utilizes energy derived from the hydrolysis of ATP to transport the hydrogen ions against a steep electrochemical gradient. The proton pump is tissue-specific, demonstrated only in gastric parietal cells. Omeprazole is the first of a new class of compounds which selectively blocks this proton pump. Because the proton pump represents the terminal stage of the acid secretory process, omeprazole effectively blocks all forms of stimulated acid secretion-histaminergic, gastrinergic, and cholinergic[6-8].

Adverse effect

Omeprazole inhibits the oxidative metabolism of some drugs by the hepatic microsomal enzyme system. Another concern regarding the long-term use of omeprazole has been bacterial overgrowth in the achlorhydric stomach.

ANTICHOLINERGICS

Mechanism of action

Anticholinergic agents decrease acid secretion by blocking muscarinic receptors for acetylcholine. Pirenzepine is a selective anticholinergic agent, a member of a new class of anti-muscarinic drugs that may again permit the use of anti muscarinic agents in the treatment of peptic ulceration. As a result of this receptor selectively, pirenzepine effectively inhibits vagally stimulated acid secretion while causing almost no undesirable cardiac, visual, or urinary side effects. Pirenzepine neither exhibits muscarinic agonist activity nor H_2 receptor blocking activity [9].

Adverse effect

For no selective anti cholinergic drugs such as atropine and propantheline bromide, unpleasant side effects such as dry mouth, blurred vision, urinary retention, tachycardia, and drying of bronchial secretion are frequent. Side effects seen with pirenzepine is dryness of mouth. Central nervous system effects are unusual and rarely require termination of treatment. Other adverse effects, such as skin reactions, allergy, and nausea, are unusual. Cardiovascular side effects are rare.

CYTOPROTECTIVES

Mechanism of action

Prostaglandins are one of several classes of compounds with cytoprotective action. Two mechanisms of action in healing ulcers are proposed for these drugs.

1. Inhibition of acid secretion (Antisecretory effect)
2. Cytoprotective effect which can occur at doses lower than the antisecretory dose.

It appears that antisecretory doses are required to heal ulcers, although cytoprotective doses may protect against injury [10].

Adverse effect

The major side effect of prostaglandin therapy is diarrhoea. The other two side effects of prostaglandins are uterine bleeding and the potential for spontaneous abortions. This potential abortifacient property of prostaglandins is of major concern both in terms of danger to pregnant women and in terms of potential abuse by those wanting to terminate pregnancy.

COLLOIDAL BISMUTH COMPOUNDS

Mechanism of action

Colloidal bismuth compounds promote healing by binding protein and necrotic debris at the ulcer base to form a coating impermeable to acid. An acid medium is presumably required for colloidal bismuth to chelate to the protein compounds of the ulcer bed to create an insoluble coagulum. The influx of these macrophages may expedite healing, and the microvilli of epithelial cells at the duodenal ulcer edge [11-12].

Adverse effect

No serious side effects have been reported with the use of colloidal bismuth compounds. However, Bismuth causes blackening of the stools which may be confused with melena. It also causes the tongue to turn black. Although innocuous, this side effect is cosmetically unappealing.

SUCRALFATE

Mechanism of action

Sucralfate is the basic aluminium salt of sulphated sucrose. In the acid medium of the stomach, it becomes viscous and adheres to defective mucosa to form a protective barrier. Thus, the ulcer bed becomes protected from continuing exposure to acid and pepsin. In addition to this barrier action, sucralfate possesses several potentially beneficial actions:

1. It neutralizes small amounts of acidn (1gm of sucralfate buffers 13mEq of H+ at pH 4.01).
2. It inhibits the action of pepsin.
3. It binds bile-salts, leading to their depletion form the gastric lumen.

4. It stimulates mucus secretion.

Sucralfate is one of the drugs said to have "cytoprotectives" properties [13].

Adverse effect

Side effects are mild and infrequent with the use of sucralfate, occurring in less than 5% of patients. The reported side effects include constipation, dizziness, dry mouth, skin rash, headache, diarrhoea, nausea and abdominal discomfort. This safely factor makes sucralfate attractive to medical practitioners for long-term maintenance use.

ANTACIDS [14]

Antacids are week bases that neutralize gastric acid, raise pH of gastric contents (optimum peptic activity between pH 2-4). The beneficial effect of antacids can also be due to their mucosa-protecting actions such as stimulation of bicarbonate production, enhancement of PG synthesis or reduction of *H.pylori* colonization. The potency of antacids is determined by acid neutralizing capacity (ANC) i.e. number of mEq of HCL that is brought to pH 3.5 in 15 minutes by unit dose of antacid preparation.

Systemic antacids

Sodium bicarbonate (NaHCO3) is water soluble, short acting and acts instantly. It is a potent neutralizer (ANC-12 mEq HCL/g). These can be used in patients of dyspepsia and can give symptomatic relief in peptic ulcer and reflux disease.

Adverse effect

The pH may rise above 7 which can cause dramatic acid rebound phenomenon, alkalosis and CO_2 accumulation in stomach. Increases sodium (Na) load can cause water retention so it is contraindicated in chronic cardiac failure patients.

Non systemic antacids

These are insoluble in water and are poorly absorbed, from chloride salt in stomach and in turn reacts with intestinal HCO_3 so that there is no final acid base disturbance. Mg^{2+} salts absorb water; stimulate cholecystokinin (ANC-10-30 mEq HCL/g). AL^{3+} salt polymerizes in itself and coats the ulcer crater, relaxes intestine, absorbs pepsin at pH>3 and inactivates it (ANC-1-2.5 mEq HCL/g). Calcium carbonate is a potent neutralizer (ANC-20 mEq HCL/g).

Adverse effect

1. Mg^{2+} can promote laxative action.
2. AL^{3+} causes constipation.
3. Ca^{2+} diffuses in GI mucosa which causes direct stimulation of parietal cells for HCL and gastrin secretion and also causes constipation.

There is rebound hyperacidity that increases the antacid requirement. In addition, it causes hypocalcaemia, hypercalciuria, alkalosis and stones. So these are contraindicated in renal failure. Hypophosphatemia can cause osteomalacia and stone formation so it is contraindicated in renal failure.

Milk alkali syndrome

Earlier calcium carbonate was advocated with large doses of milk that led to milk alkali syndrome which comprises of headache, dizziness, anorexia, weakness and abdominal discomfort, Ca deposits and renal stones.

Characteristic of common Antacids

Feature	**Sodium Bicarbonate**	**Calcium**	**Magnesium Hydroxide**	**Aluminium**
Onset of action	Rapid	Intermediate	Rapid	Slow
Duration of action	Short	Modereate	Moderate	Moderate
Systemic alkalosis	Yes	---	No	No
Effect on stool	---	Constipating	Laxative	Constipating

ANIMAL MODELS USED IN THE SCREENING OF ANTI ULCER ACTIVITY

Various screening models are used for the screening of the anti ulcer activity.

I) In VITRO Methods:

1) [^{125}I] Gastrin Binding Assay
2) [^{3}H] Tiotidine Binding Assay for Histamine H2 Receptors
3) H^+/K^+-ATPase Inhibition Assay

II) In VIVO Methods:

1) Pylorus Ligation in Rats
2) Stress Ulcer Models

a) Restraint – induced Ulcers

b) Cold Water Immersion – Induced Ulcers

c) Stress and NSAIDs Induced Ulcers

d) Swimming Stress Ulcers

3) Histamine Induced Gastric Ulcers
4) Methylene Blue – Induced Ulcers

5) Serotonin – Induced Gastric Ulcers
6) Ethanol – Induced Mucosal Damage
7) Acetic acid – Induced Gastric Ulcers
8) Indomethacin – Induced Gastric Ulcers
9) Reserpine – Induced Chronic Ulcers
10) Cysteamine – Induced Duodenal Ulcers
11) Dimaprit – Induced Duodenal Ulcers
12) Mepirizole – Induced Duodenal Ulcers
13) Gastric Mucosal Injury by Local Ischemia – Reperfusion in Rats

I) In VITRO Methods:

1) [^{125}I] Gastrin Binding Assay

Gastrin is one of the major stimuli for gastric acid secretion. Its release is also triggered by partially digested proteins, peptides, blood borne factors and by vagal stimulation. After release from gastric antrum, it stimulates acid secretion by binding to its receptors on parietal cells as well as by releasing histamine from enterochromaffin-like cells. Compounds with gastrin receptors antagonistic activity can prove to be useful antiulcer drugs. [^{125}I] Gastrin is used for radioligand binding assay for gastrin receptors. Proglumide like drugs, which are a CCKR antagonists, may also be screened using this assay.

Procedure:

The assay is done using fundic gland suspension obtained from guinea pig stomach. For the binding and competition assays, the gland suspension is incubated with 50 µl of [^{125}I] gastrin in the presence of either buffer alone (for total binding) or in the presence of unlabeled gastrin (for non-specific binding) or in the presence of test compound for 90 mm at 37°C. Subsequently, ice cold buffer, in microcentrifuge tubes, is layered with incubated mixture and centrifuged for 5 min at 10,000g. Radioactivity is quantified in pellet after discarding the supernatant.

Evaluation:

Total binding non-specific binding and specific binding are determined. Percentage of specifically bound [^{125}I] gastrin displayed by a given concentration of the test compound is calculated and its IC_{50} and dissociation constant (Ki) values are calculated.

2) [^{3}H] Tiotidine Binding Assay for Histamine H2 Receptors

Histamine H_2 receptors blockers have been the mainstay of anti-ulcer therapy since the early 1970s due to their potent acid-suppressing properties. Tiotidine is an H_2 receptor blocker that is used for H_2 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 [^{3}H] tiotidine for 90min at 4°C in the presence of Na_2HPO_4/KH_2PO_4 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. 5ml 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 5ml of ice-cold buffer twice and radioactivity measured by liquid scintillation counting.

Evaluation:

Specific binding, i.e. total binding minus non-specific binding and IC_{50} are determined. In another study, the [^{3}H] – tiotidine has also been used as a specific ligand for H_2 receptors in dispersed mucosal cells from guinea pig stomach and it was found to have limited binding to H_2 receptors and as such [^{3}H] – tiotidine was not a suitable ligand for labeling the H_2-receptor on gastric mucosal cells.

3) H^+/K^+-ATPase Inhibition Assay

H^+/K^+-ATPase or proton pump is the final step in the synthesis of acid by parietal cells. It exchanges intracellular H^+ with extracellular K^+ in the canaliculi of parietal cells in response to stimulation by all gastric acid secretagogues, i.e. histamine, acetylcholine and gastrin. Proton pump inhibitors like omeprazole, lansoprazole, etc. are now established antiulcer drugs.

Procedure:

The assay is carried out using homogenates of microsomal gastric H^+/K^+-ATPase obtained from pig gastric mucosa. For inhibition assay, 80 ng microsomal H^+/K^+-ATPase is incubated with 100 µl buffer (pH 7.4), 1 mM ATP and test compound in microtitre plate for 30 min at 37°C. After 30 min of incubation, the reaction is stopped by adding malachite green colorimetric reagent and then after 10 sec 15% sodium citrate is added for 45 min. Release of orthophosphate from ATP is quantified by colorimeter at570 nm. For study of omeprazole and other proton pump inhibitors available at present, which produce active metabolite at acidic pH, the microsomal homogenate is initially suspended in buffer at pH 6.1 along with the drug and incubated for 30 min. This homogenate is then transferred to buffer at 7.4 and the produce described above is followed. Percentage inhibition of H^+/K^+-ATPase is calculated.

Percentage of enzyme inhibition is calculated using the formula:

$$\%\text{inhibition} = \frac{[\text{Activity (control)} - \text{Activity (test)}}{\text{Activity (control)}} \times 100$$ [15]

II) In VIVO Methods:

1) Pylorus ligation induced rats:

Principle:

In Pylorus ligation pylorus part of the stomach was ligated it helpful to produce the ulcers in rats by stopping passage of gastric contents from stomach it creates the acidic medium in stomach for longer time and produces the ulcer.

Procedure:

Albino Wistar rats of either sex weighing between (150-200 gms) are divided into groups of a animal. sIn this method albino rats are fasted in individual cages for 24 hours. Test drug or standard drug or control vehicle is administered 30 minute prior to pyloric ligation. Under light ether anaesthesia, the abdomen is opened and the pylorus was ligated. The abdomen is then sutured. At the end of 4 hours after ligation the animals are sacrificed with excess of anaesthetic ether, and the stomach is dissected out gastric juice is collected were drained into tubes and were centrifuged at 1000 rpm for 10 minutes and the volume is noted. The pH of gastric juice is recorded by pH meter. Then the contents are subjected to analysis for free and total acidity. The stomachs are then washed with running water to see for ulcers in the glandular portion of the stomach. The numbers of ulcers per stomach are noted and severity of the ulcers scored microscopically with the help of 10x lens. Histopathological studies were conducted by fixing stomach tissues in 10% formalin for 24 h. The formalin fixed specimens are embedded in paraffin and section (3-5µm) and stained with haematoxylin and eosin dye. The histochemical sections are evaluated by light microscopy [16].

2) Stress Ulcer Models:

Stress plays a significant role in the pathogenesis of gastric ulcers in human being. However, the recent studies suggest no harmful effect of chronic stress on gastric ulceration. Selye (1936), for the first time, described the use of restraint for the production of gastric ulcers. Various models involving various types of stress have since been developed. The involvement of psychogenic factors and the ease of production of gastric ulcers using these models is a real advantage over the pyloric ligation method of gastric ulcer production.

i) Restraint ulcers:

Procedure:

Albino rats, of either sex, weighing 150 to 200 g, are used for the study. After 36 hours of fasting, the test drug is administered. Thirty minutes later, the animals are subjected to restraint by molding a special

galvanized steel window screen around the animal and tying the limbs of the animal in pair so that the animal cannot move. The animals are kept under restraint for 24hours. The animals are then sacrificed and their stomachs dissected out. The stomachs are opened along grater curvature and fixed to cork plate. Ulcer index and ulcer severity are determined as described in the pyloric ligation method.

ii) 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.

Procedure:

Wister 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 hour. Azovan blue (Even's blue), in a dose of 30 mg/kg is injected intravenously via the tail vein after removal of the rats from the cage. They are sacrificed 10 min later. The stomach is removed and ligated at both ends. It is filled with formal saline and kept overnight. On the next day, the stomach is opened along the grater curvature, washed in warm water and examined for ulcerative lesions. Even's blue helps in evaluation of the lesion score, which is calculated by adding the lengths of the longest diameters of the lesions. Gastric mucosal lesions can be induced by 2 hours of cold restraint stress in rats.

Abdel-Sater KA et al, 2012 have used acute cold restraint stress by fixing the four limbs of the rat and placing it in a refrigerator at 4°C for 3 hours to study the gender difference of selective serotonin reuptake inhibitors, fluoxetine. Stressed male were found to be more responsive to the antiulcer effect of fluoxetine more than stressed femals.

iii) Stress and NSAIDs Induced Ulcers

Procedure:

Wistar rats (150 to 200 g) are used for the experiment. The animals are fasted for 24-36 hours and then given the test agent (in 1% carboxymethyl cellulose) via gastric intubation and an NSAID such as aspirin, indomethacin or diclofenac intraperitoneally. After placing the rats in stress cages, they are immersed in water up to the level of xiphoid process at 23°C for 7 hours. The animals are then sacrificed, their stomach removed and evaluated for ulcer index. The dose of NSAID required to increase gastric erosion by 100% relative to immobilization is compared with that of NSAID required to produce 100% increase in gastric erosion under the protective effect of test drug.

iv) Swimming Stress Ulcers

Procedure:

Albino rats of either sex are fasted for 24 hours with free access to water. The rats are forced to swim in a deep concrete tube filled with water at 23°C for 5 hours. The animals are removed from the tube after five hours, sacrificed and their stomachs removed. The stomachs are opened along the greater curvature and severity grading is done and ulcer index calculated.

Severity of the ulcerative lesions is graded as follow:

0 – No lesions, 1 – Lesions with diameter less than 1mm, 2 – Lesions with diameter 1-2mm, 3 – Lesions with diameter 2-4 mm, and 4 - Lesions with diameter more than 4 mm.

Ulcer index is calculated by summation of scores for individual erosions and ulcers.

3) Histamine Induced Gastric Ulcers

Histamine has been used widely for the production of gastric ulcers. Enhanced gastric acid secretion has been implicated in the production of ulcers due to histamine.

Procedure:

Male guinea pigs weighing 300 to 400 g, are used for the experiment. The animals are fasted for 36 hours with water available *ad libitum.* Histamine acid sulphate is injected in a dose of 50 mg intraperitoneally. To prevent histamine toxicity, promethazine hydrochloride is injected intraperitoneally 15 min after the histamine injection in a dose of 5 mg. The test drug is administered 30-45 min before the histamine injection. Four hours after the histamine injection, the animals are sacrificed and their stomachs dissected out. The degree of ulceration grading is done as follows.

- Type 0: No visible ulceration on gross examination
- Type 1: 1-3 mm ulcers, ten or less in number
- Type 2: 1-3 mm ulcers, eleven or more in number
- Type 3: 4-6 mm ulcers, one or more in number
- Type 4: 7 mm or bigger ulcers, one or more in number
- Type 5: Gastric or esophageal wall perforation

Other routes of administration of histamine such as intraperitoneal/intramuscular have also been found useful. Repeated administration of histamine by intraperitoneal route (0.09 mg/kg) or intramuscular route (0.09 mg/kg × 8 doses) has been found to selectively induce gastric or duodenal ulcers demonstrating differential of histamine in ulcer induction.

Modification:

Duodenal ulcers were also induced by repeated ip administration of histamine acid phosphate at a dose of 0.25 mg/kg at every 30 min interval for 4 hour after 45 min of test drug administration.

Apart from guinea pig, other animals such as rats can also be used to induce histamine gastric ulcers. Histamine, intraperitoneal injection at a dose of 300 mg/kg induces gastric lesions.

4) Methylene Blue – Induced Ulcers

Methylene blue (MB), a synthetic drug has been used for the induction of duodenal and gastric ulcers in rats. Methylene blue uncouples the ATPase enzyme and also possesses the affinity for muscarinic receptors. The model is used for the screening of antiulcer agents involving H^+/K^+-ATPase system or via anticholinergic action exerted via muscarinic receptors.

Procedure:

Methylene blue produces ulceration of gastric mucosa by reduction in blood supply to gastric mucosal region that causes oxidative stress. Rats are fasted for 24hour before the administration of MB. Animals are administered MB at a dosage of 5-125 mg/kg body weight p.o. followed by the administration of the test drug. After 4 hours the animals are sacrificed and ulcer index is determined.

5) Serotonin-induced Gastric Ulcers

Adinortey et al. 2013 have reviewed the serotonin (5-HT)-induced gastric ulcer model. Serotonin produced local vasoconstriction and thus reduced the gastric mucosal blood supply, resulting in local mucosal injury.

Procedure:

Rats were administered with a single dose of serotonin creatinine sulfate (0.5 ml of 50 mg/kg subcutaneous injection) for the induction of glandular lesions after fasting for 24-36 hours and water deprivation for 2 hours before the experiments. They are housed in wide mesh wire bottom to prevent coprophagy. Serotonin was administered by intragastric intubation with the aid of an orogastric cannula. 6 hours later, the animals are sacrificed by cervical dislocation for examination.

Endogenous serotonin in also reported to play a dual role in the pathogenesis of indomethacin induced small intestinal ulceration in mice – proulcerogenic action via 5-HT_3 receptor and antiulcerogenic action via 5-HT_4 receptors.

6) Ethanol-induced Mucosal Damage

Ethanol (absolute)-induced gastric lesion is a reproducible method in experimental animal. Using a transmission densitometer, it is possible to quantify the extent of gastric lesions induced by ethanol, by measuring the optical density of photographic negatives of gastric mucosa.

Procedure:

Wistar rats, weighing 250 to 300 g, are used for the experiment. The animals are placed individually in cylindrical stainless steel cages with flat bottoms to limit their mobility and prevent coprophagy. They are fasted for 18 hours but given water ad libitum. Now the test drug or the vehicle is given to the animals orally. Thirty minutes later, 1 ml of absolute ethanol is given orally. The animals are sacrificed 1 hour later and their stomachs dissected out. The stomachs are opened along the greater curvature, washed with tap water and ulcer severity grading done. Using a cork borer, 13 mm, full thickness circular patches are cut form each lobe of fundus below the ridge dividing glandular from non-glandular potion of the stomach and placed into holes of a special template. Photographs of the tissues are taken and the negatives examined under light transmission densitometer. Damaged areas have lower optical density values.

Absolute ethanol has also been used at higher dose of 5 ml/kg to induce gastric lesions in rats. Animals were sacrificed after 1 hour and stomach opened to observe gastric lesions.

Khazaei and Salehi, 2006 have induced gastric ulcers in male albino rats by administering ethanol 50% (in distilled water) at a dose of 10ml/kg. 1 hour after ethanol administration, rats were killed and lesions produced in glandular part of test drug observed. Ethanol induced severe gastric hemorrhagic erosions. It induced both long ulcers and petechial lesions. Decrease in gastric mucus and increased lipid peroxidation are the mechanisms suggested for ethanol induced gastric ulcers.

7) Acetic Acid-induced Gastric Ulcers

This model produces chronic ulcers that resemble human ulcers and is used to screen drugs for their gastroprotective effect in chronic gastric and duodenal ulcers. Spontaneous relapse of healed ulcers >100 days after ulceration is the characteristic feature of this model.

Procedure:

Albino rats are used for the experiment. A volume of 0.05 ml of acetic acid (1-30%) is injected into the submucosal layer of the stomach, which results in the formation of penetrating peptic ulcers that are confined by adhesions to contiguous organs like liver. They are typically chronic ulcers with repeated healing and re-aggravation. 100% acetic acid can also be applied to the serosal surface of stomach or duodenum for production of ulcers. Effect of test drug given twice daily for 10-15 days is noted.

Rabbit gastric ulcer models namely acetic acid induced and mucosal resection-induced models are more clinically relevant models in terms of round, deep ulcers with clear-cut margin and well-defined healing stages that are difficult to define in rat models

Qin and Chen, 2005 have used modified method for induction of gastric ulcers. Gastric ulcers were produced in male Sprague-Dawley rats by application of round filter paper (diameter 5 mm) immersed

in a 100% acetic acid on the serosal surface of the anterior wall of the stomach approximately at the center of the corpus for 30 sec and the process was repeated twice. Immediate production of necrosis of the entire mucosa and submucosa (but not serosa) within the area ($20mm^2$) where the acetic acid applied was observed. Excess of acetic acid was then removed and serosa was gently washed with saline. The abdomen was then sutured and the animals were allowed to recover and returned to their cages with free access to food and water. The above method was found useful to study the synergistic action of famotidine and chlorpheniramine on acetic aicd-induced gastric ulcer in rats.

8) Indomethacin-induced Gastric Ulcers

Gastric ulcers may be induced by oral administration of indomethacin (25 mg/kg) after 24 hours fasting male albino rats. Indomethacin is dissolved in sodium bicarbonate to form a clear solution. Gastric ulcer can be examined after 4 hours of indomethacin administration by opening the stomach along the grater curvature, washed in normal saline to remove debris and pinned on a cork mat for ulcer screening. This can be done by locating the wounds in the glandular region under a simple microscope. The length (mm) of all the elongated black-red lines parallel to long axis of the stomach in the mucosa is measured. Ulcer index is calculated by adding the lengths of all the lesions in the glandular region of the stomach. NSAIDs are believed to cause inhibition of COX and thereby inhibiting the production of cytoprotective prostaglandins and causing gastrointestinal side effects. Indomethacin has shown to cause oxidative stress also leading to formation of gastric ulcers.

9) Reserpine-induced Chronic Ulcers

Histamine liberation from the mast cells in stomach wall with subsequent increase in gastric acid secretion has been implicated in reserpine-induced gastric glandular ulcer formation in rats.

Procedure:

Female Sprague-Dawley rats, weighing 130 to 180g, are used. The animals are fasted for 48 hours with free access to 0.8% sucrose in 0.2% NaCl w/v. They are housed in wide mesh wire bottom cages to prevent coprophagy. One hour before starting the experiment, the liquid diet is also withdrawn. Now the animals are injected with the test drug intraperitoneally and half an hour later reserpine (5mg/kg) or vehicle is injected intraperitoneally. Four hours later, the animal is sacrificed and stomach removed and examined for mucosal lesions. Reserpine dose (5mg/kg intraperitoneally, 18 hours before sacrifice) has also been used by other investigators, and it induced marked glandular ulceration with release of free β-glucuronidase.

10) Cysteamine-induced Duodenal Ulcers

Selye and Szabo first described the production of duodenal ulcers in rats by cysteamine HCl (β-mercaptoethylamine HCl). The pathogenesis of cysteamine-induced duodenal ulcers involves: Inhibition of alkaline mucus production, increased gastric acid secretion, increased serum gastrin levels and delayed gastric emptying. This model is widely used to evaluate the anti-ulcer activity of anticholinergics, antacids, prostaglandins and H_2 receptor antagonists.

Procedure:

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

Acute duodenal ulcers can be induced in rats by administration of a single dose of cysteamine hydrochloride (400 mg/kg p.o.). For induction of chronic type duodenal ulcers, rats are administered with cysteamine (400 mg/kg p.o) twice at an interval of 4 hours followed by addition of cysteamine hydrochloride to drinking water.

11) Dimaprit-induced Duodenal Ulcers

Dimaprit, an H_2 receptor agonist, has been shown to induce gastric erosions in rats after a single intravenous dose and duodenal ulcers in guinea pigs after repeated subcutaneous doses. This model is especially useful for screening of H_2-blockers.

Procedure:

Female Sprague-Dawley rats (150 to 180 g) or female guinea pigs (250 to 300 g) are used for the experiments. The animals are fasted for 24 hours before the experiment but allowed free access to water. In rats, dimaprit is given in a dose of 100 mg/kg intravenously, single dose. The animal is sacrificed one hour later and the stomach dissected out and examined for gastric erosions. The test drug or vehicle is given orally 60 min before injecting dimaprit.

In case of guinea/pigs, the animals are given multiple subcutaneous injections of dimaprit (2mg/kg every hour for 6 hours). The test drug is given either 30 min before the first dose of dimaprit or 30 min before and then hourly along with dimaprit. The animals are sacrificed one hour after the last dimapirt injection and stomach and duodenum are examined for lesions.

Other routes of administration of dimaprit such as intramuscular injection at a dose of 0.09 or 0.18 mg/kg body weight and intraperitoneal injection at a dose of 1.81 or 3.62 mg/kg have also been used in guinea pigs for induction of duodenal ulcers. The intramuscular injection produced particularly severe ulcers.

12) Mepirizole-induced Duodenal Ulcers

Okabe et al, (1982) have described the production of duodenal ulcers in rats by mepirizole, a non-steroidal anti-inflammatory drug. This model is a useful model for screening of antiulcer drugs like antacids, anticholinergics and H_2 receptor antagonists as mepirizole-induced gastric secretions are inhibited by these drugs thereby important in study of pathogenesis of duodenal ulcers.

Proceudre:

Male Sprague-Dawley rats, 200-220 g in weight, are administered 200 mg/kg of mepirizole suspended in 1% carboxymethyl cellulose solution via gastric intubation. Subsequently rats are kept in cages with raised mesh bottom and deprived of food and water for 24 hours. This leads to ulceration in proximal duodenum and erosions in antrum. Antiulcer drug therapy is started after 24 hours of mepirizole administration. On the 11^{th} day, the animals are sacrificed and their duodenum and stomach evaluated for ulcer area under microscope. Ulcer or erosion indices are calculated from the sum of area of ulcers and erosions, respectively.

Duodenal ulcers can also be induced by subcutaneous administration of mepirizole at doses of 60 and 200 mg/kg to increase acid secretion in a dose dependent manner within 8 hours.

13) Gastric Mucosal Injury by Local Ischemia-Reperfusion in Rats

Gastric ischemia-reperfusion is an important model for studying acute gastric mucosal injury**.**

Procedure:

Wistar rats, 180-200 g weight, are deprived of food for 24 hours before experiments, but allowed free access to tap water. The animals are anesthetized and their abdomen opened by a midline incision. Celiac artery is identified and clamped by a microvascular clamp, 0.5 cm from its origin, for 30 min to induce ischemia and then reperfusion for 60 min is performed by removal of clamp. After completion of reperfusion, the animals are sacrificed by exsanguination and stomach removed and lesions examined macroscopically and microscopically. Ulcer index is then calculated.

Modifications:

Hassan et al. (1997) produced ischemia by clamping left gastric artery for 15 min and then reperfusion for 30 min before sacrificing the animals by cervical dislocation. The stomachs were removed and opened along the greater curvature and photographed for assessed of macroscopic mucosal injury

planimetrically. Microscopic injury was also assessed by staining a sample of corpus with hematoxylin and eosin, and examining under a microscope.

Mojzis et al. 2000 induced gastric ischemia in male Wistar rats by 30 min clamping of the coelic artery followed by 30 min of reperfusion and the mucus content, extent of gastric lesions and length of lesions was determined at the end of ischemia/reperfusion. The method has been used to evaluate the effects of sucralfate, malotilate, 0.5% methylcellulose and N-acetylcysteine.

In Silico Model Based Approach for the Development of Antiulcer Antibiotics

Mandal and Das, 2014 have developed an *in silico* based approach for the development of antiulcer antibiotics. They targeted bacterial lysine biosynthetic pathway for antibacterial drug development using inhibitors of *H.pylori* DapE-encoded N-succinyl-L,L-diaminopimetic acid desuccinylase, an essential enzyme responsible for lysine synthesis in bacterial cell wall.

Procedure:

The study used 3D structural model of DapE-encoded *H.pylori* having two domains, one catalytic domain and other dimerization domain developed by MODELLER software. After conformation of the stability of the model by GROMACS, the identification of inhibitors of DapE, drug-like small molecule screening library was developed by Tanimoto based Pubchem Database with DapE substrate L, L-SDAP as a query molecule followed by docking approach by using GLIDEXP to identify the potential inhibitors of DapE which can be used further in the development of novel antibacterial antiulcer drugs [17].

HERBAL REMEDIES (TRADITIONAL MEDICINES)

Traditional system of medicine has been in use by *Homosapiens* since years, man started learning from the nature and depending upon the nature for his mundane actions. Gradually and slowly a fundamental knowledge (in other words traditional system of medicines) got developed via accidental experiments or chance discoveries. The first record of practice of traditional medicine by humans comes from the tablet around 2600 B.C. old. The tablet mentions the significance and use of oils from *Cedrus specie* (cedar), *Cupressus sempervirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora species* (myrrh) and *Papaver somniferum* (poppy juice). No matter how much advance in allopathic medicine or chemotherapy is there, the changes of adverse effects or side-effects cannot be ruled out. Therefore there is needed to find an alternative therapy for the cure of disease which has negligible adverse impact on the patient. The answer lies in the use of traditional medicines and herbal remedies citing their low-incidence of side-effects.

MEDICINAL PLANTS HAVING ANTI-ULCER PROPERTY

Common Name	Botanical Name	Parts Used	Effects
Tulsi	Ocimum sanctum	All parts	Anti-ulcer, Antibacterial
Tippani	Allophylus serratus	Leaves	Antiulcer
Shaparni	Desmodium gangeticum	Root Extract	Inflammation, typhoid, Anti-ulcer
Neem	Azadirachta indica	Dried Bark	GIT disorders
Indian Sarsaparilla	Hemidesmus indicus	Extract of Leaves	Antidiarrheal, Anti-ulcer
Satavari	Asparagus racemosus	Root extract	Anti-diarrheal, Anti-ulcer
Triphala	Terminalia pallida	Plant extract	Anti-ulcer
Aamla	Embilica officinalis	Fruit extract	Anti-ulcer
Gotu Kola	Centella asiatica	Fresh juice	Anti-ulcer
Brahmi	Bacopa monniera	Fresh juice	Anti-ulcer
Apple Bananas	Musa sapientum	Fruits	Anti-ulcer
Pappeta	Carica papaya	Seeds	Anti-amoebic, Anti-ulcer
Pausanto	Kielmeyera coriacea	Stem	Anxiolytic, Anti-ulcer
Brindle Berry	Garcinia cambogia	Fruit extract	Anti-ulcer
Winter melon	Banincasa hispida	Fruits	Anti-ulcer, epilepsy
Wild papal	Ficus arnottiana	Fruits	Anti-ulcer, Demulcent
Indian devil tree	Alstonia scholaris	Whole plant	Anti-ulcer
Indian mulberry	Morinda citrifolia	Fruit	Anti-ulcer, Anti-diabetic
Indian borage	Plectranthus amboinicus	Whole plant	Diuretic, Anti-ulcer
Babul	Acacia Arabica	Leaves, Gums	Ulcer, Wound
Garlic	Allium sativum	Bulb juice	Antiulcer
Boabab	Adansonia digitate	Leaves and Bark	Antiulcer, syphilitic ulcer, irritable inflammatory ulcer disease
Bael tree	Aegle marmelos	Fruits	Antiulcer
Kattalai	Aloe vera	Leaves and powder	Antiulcer
Custard apple	Annona squamosal	Leaves	Antiulcer

Kanchanara/ Orchid tree	Bauhinia variegate	Bark and roots	Antiulcer
India or Nepal barberry	Berberis aristata	Root and wood	Antiulcer
Beetroot	Beta vulgaris	Roots	Antiulcer
Slow match tree	Careya arborea	Leaves, stem and bark	Antiulcer
Arasha-maram	Ficus religiosa	Bark and Leaves	Antiulcer

NEWER ANTIULCER DRUGS

AN5; A 4-Phenyl-tetrahydroisoquinoline derivate has been found through research as an effective Anti-ulcer agent. Upon comparison studies with cimetidine and ranitidine it was found that AN5 had higher antiulcer activity with maximal antiulcer activity shown at the dose of 1mg/kg. A new parasympatholytic agent, Ba-5473 has been researched upon to find an effective anti-ulcer agent. Through the clinical studies it was found to have potent Antiulcerant property in the cases [18].

DIAGNOSTIC TEST FOR ULCER

Some tests will be ordered so that diagnosis can be confirmed, such as

Blood test

A blood test can determine whether *H.pylori* bacteria are present. However, a blood test cannot determine whether the patient had past exposure or is currently infected. Also, if the individual has been taking antibiotic or proton pump inhibitors a blood test can give a false-negative result.

Endoscopic Biopsy

During the endoscopy, a piece of stomach tissue is removed, so that it can later be analysed. This type of test is typically used on older people, or those that have experienced weight loss or bleeding.

Breath test

A radioactive carbon atom is used to detect *H.pylori.* The patient drinks a glass of clear liquid containing radioactive carbon as part of a substance (urea) that the *H.pylori* will break down. If is infected with *H.pylori* the breath sample will contain radioactive carbon in carbon dioxide. The breath test is also useful in checking to see how effective treatment has been in eliminating *H.pylori.*

Stool antigen test

This test determines the presence of *H.pylori* in the feces (stools). This test is also useful in determining how effective treatment has been in getting rid of the bacteria.

Upper gastrointestinal X-ray (Upper GI X-rays)

The test outlines the esophagus, stomach and duodenum. The patient swallows a liquid which contains barium. The barium coats the digestive tract and shows up on the X-ray, making the ulcer easier to see. Upon GI X-rays are only useful in detecting some ulcers.

Endoscopy

A long-narrow tube with a camera attached to the end is threaded down the patient's throat and esophagus into the stomach and duodenum [19].

GLOBAL MARKET FOR ANTI-ULCER DISEASES

During literature survey it was found that anti-ulcer drugs have huge market in the global drug industry. Gastrointestinal tract drugs contribute 11% of the global pharmaceutical market. In Indian scenario, the Indian pharmaceutical market is at present $ 20 billion dollar market. 11% of $ 20 billion drug market accounts for $ 2.2 billion dollar market. Another study projects that Anti-ulcer market which falls under Gastro-intestinal drug market is around Rs. 8413 crore as per IMS 2015 report. It is estimated that Indian pharmaceutical market would reach $ 55 billion dollar market by 2035, which, makes the total GIT (Gastro-intestinal tract) market to $ 6.05 billion dollar market by 2035.

CONCLUSION

Ulcer has a known history for morbidity and mortality in humans. Ever since the age of Hippocrates and till present times, drastic changes were seen in disease pathophysiology and treatment goals. Chemotherapy has evolved to great extent and continues to do so with newer findings by the scientist across globe. No matter how much research done to find an effective molecule, the chances of side effects or adverse effects cannot be ruled out. Thus, the prime focus on developing traditional medicines or herbal cure shall be carried out. There are lots of clinical developments happening across the globe. The author feels the times will change the whole or complete management of ulcer and other disease and we will become more advance in treatment strategic terms [20].

REFERENCE:

1. Thomas S, Femeesh M, Nafia K, Siyad M, Shrikumar S. Pharmacological review of Anti ulcer screening. World journal of pharmaceutical sciences 2005; 6(5):1369-1387.2
2. Kulkarni K. Evaluation of Antiulcer Activity of DGL (Deglycyrrhizinated liquorice). Journal of Pharmaceutical Sciences and Research. 2017 Nov 1;9(11):2019-22.
3. Raju D, Ilango K, Chitra V, Ashish K. Evaluation of Anti-ulcer activity of methanolic extract of Terminalia chebula fruits in experimental rats. Journal of Pharmaceutical Sciences and research. 2009 Sep 1;1(3):101.
4. Tripathi KD. Essentials of medical pharmacology. "Gastrointestinal drugs" 2013; 7:649.

5. McGuigan JE. Side effects of histamine – receptor antagonists. *Clin Gastroenterol* 1981; 12:819-838.
6. Fellenius E, Elander B, Wallmark B, Helander HF, Berglindh T. Inhibition of acid secretion in isolated gastric glands by substituted benzimidazoles. American Journal of Physiology-Gastrointestinal and Liver Physiology. 1982 Dec 1;243(6):G505-10.
7. Lind T, Cederberg CH, Ekenved GU, Haglund U, Olbe L. Effect of omeprazole--a gastric proton pump inhibitor--on pentagastrin stimulated acid secretion in man. Gut. 1983 Apr 1;24(4):270-6.
8. Londong W, Londong V, Cederberg C, Steffen H. Dose-response study of omeprazole on meal-stimulated gastric acid secretion and gastrin release. Gastroenterology. 1983 Dec 1;85(6):1373-8.
9. Hammer R, Berrie CP, Birdsali NJM. Pirenzepine distinguishes between different subclasses of muscarinic receptors. Nature 1982;2:900-902.
10. Ito S, Lacy ER. Morphology of rat gastric mucosal damage, defense, and restitution in the presence of luminal ethanol. Gastroenterology. 1985 Jan 1;88(1):250-60.
11. Brogden RN, Pinder RM, Sawyer PR, Speight TM, Avery GS. Tri-potassium di-citrato bismuthate: a report of its pharmacological properties and therapeutic efficacy in peptic ulcer. Drugs. 1976 Dec 1;12(6):401-11.
12. Wilson TR. The pharmacology of tri-potassium di-citrato bismuthate (TDB). Postgraduate medical journal. 1975;5:18-21.
13. Nagashima R. Mechanisms of action of sucralfate. Journal of clinical gastroenterology. 1981;3(Suppl 2):117-27.
14. Goel RK, Bhawani G. Drugs acting on gastrointestinal system. 2008; 11-12.
15. Gupta SK. Drug screening methods. Jaypee Brothers 2016; 3rd edition:527-529.
16. Thomas S, Femeesh M, Nafia K, Siyad M, Shrikumar S. Pharmacological review of Anti Ulcer Screening. 2017;6(5): 1378-1379.
17. Gupta SK. Drug screening methods. Jaypee Brothers 2004; 3rd edition:529-536.
18. Devansh Mehta. Ulcer -Review on Types, Anti-ulcer Drugs, Anti-ulcer Medicinal Plants, Anti-ulcer Drug Market, Diagnostics and Current Global Clinical Trials Status. Inventi Journals (P) Ltd 2016; 2016(2):5-6.
19. Schubert ML, Peura DA. Control of gastric acid secretion in health and disease. Gastroenterology. 2008 Jun 1;134(7):1842-60.

20. Devansh Mehta. Ulcer -Review on Types, Anti-ulcer Drugs, Anti-ulcer Medicinal Plants, Anti-ulcer Drug Market, Diagnostics and Current Global Clinical Trials Status. Inventi Journals (P) Ltd 2016; 2016(2):6-7.