5.1 INTRODUCTION TO ULCER:

Definition of Ulcer

An ulcer is the result of an imbalance between aggressive and defensive factors. On one hand, too much acid and pepsin can damage the stomach lining and cause ulcers. On the other hand, the damage comes from some other causes, making the stomach lining susceptible to even at an ordinary level of gastric acid\(^1\). A peptic ulcer of the stomach is called a gastric ulcer, of the duodenum, a duodenal ulcer; and of the esophagus, an esophageal ulcer. An ulcer occurs when the lining of these organs is corroded by the acidic digestive juices which are secreted by the stomach cells\(^2\). Peptic ulcer disease is common, affecting millions of people yearly.

Fig: 5.01 Peptic ulcer disease
The medical cost of treating peptic ulcer and its complications runs in the billions of dollars annually. Recent medical advances have increased our understanding of ulcer formation. Improved and expanded treatment options are now available.

**Historical Perspective**

Looking back at history, peptic ulcer was a rare and generally unrecognized as a cause of symptoms/ complications or death until the early 19th century. Despite sporadic case reports beginning in late 18th century, peptic ulcer disease did not become widely appreciated until early 20th century. The first 6 decades saw the dominance of surgery in the treatment of peptic ulcer. With the introduction of acid suppressive drugs like H$_2$ blockers in 1970’s the treatment of PUD was revolutionized. By 1980’s the advent of *Helicobacter pylori* (HP) brought about a dramatic twist and possibly cure.

**Physiology of acid secretion**

The parietal cell contains receptors for gastrin, Histamine (H$_2$) and Acetylcholine (M3). When gastrin or acetylcholine or histamines binds to their receptors an increase in cytosolic calcium, which in turn stimulates protein kinases that stimulates acid secretion from a H$^+$/ K$^+$ATPase (proton pump) on the canalicular surface. In close proximity to the parietal cells are gut endocrine cells called Enterochromaffin like cells (ECL cells). ECL cells have receptors for gastrin and acetylcholine are the major source for histamine release. Histamine binds to the H$_2$ receptor on the parietal cells, resulting in activation of adenyl cyclase, which increases intracellular cyclic adenosine monophosphate (CAMP). CAMP activates protein kinase that stimulates acid secretion by the H$^+$/K$^+$ATPase. In human, it is believed that the major effect of gastrin upon acid
secretion is mediated indirectly through the release of histamine from ECL cells rather than through direct parietal cell stimulation\textsuperscript{5}.

**Fig:5.02 Mechanism of secretion of gastric hydrochloric acid**

The oxyntic cells of fundic glands which secretes hydrochloric acid, have canaliculi within them. From various experimental data, it appears that the mechanism of secretion of hydrochloric acid as follows.

1. The parietal cells secrete hydrogen ions (H\(^+\)) and chloride ions (Cl\(^-\)) separately into stomach lumen, the net effect is secretion of hydrochloric acid.

2. Proton pump powered by H\(^+\)/K\(^+\) ATPases actively transport H\(^+\) into the lumen while bringing potassium ions into the cell.
3. At the same time Cl⁻ and K⁺ diffuse out through Cl⁻ and K⁺ channels in the apical membrane.

4. The enzyme *carbonic anhydrase*, which is especially plentiful in parietal cells, catalyzes the formation of carbonic acid (H₂CO₃) from water (H₂O) and carbon dioxide (CO₂).

5. As carbonic acid dissociates, it provides a ready source of H⁺ for the proton pumps but also generates bicarbonate ions (HCO₃⁻).

6. As a result HCO₃⁻ builds up in the cytosol; it exits the parietal cell in exchange for Cl⁻ via Cl⁻/HCO₃⁻ antiporters in the basolateral membrane. HCO₃⁻ diffuses into nearby blood capillaries. “This alkaline tide” of bicarbonate ions entering the blood stream.

7. As a result, one molecule of NaHCO₃ is formed in the blood against one molecule of HCl formed and excreted into the stomach.

8. The H⁺ ions developed as stated in step (5) join with OH⁻ ions as described in step (1) to form water⁶.

**Etiology and Pathogenesis:**

Most peptic ulcers occur in the presence of acid and pepsin when *Helicobacter pylori*, NSAIDs or other possible factors disrupt normal mucosal defense and healing mechanisms. Hypersecretion of acid is the primary pathogenic mechanism, in hypersecretory states such as Zollinger Ellison syndrome.
The pathogenesis of DU and GU is multifactorial and most likely reflects a combination of pathophysiologic abnormalities, environmental, and genetic factors. Ulcer location appears to be related to a number of etiologic factors, most of the DUs occur in the first part of the duodenum. Benign GUs can occur anywhere in the stomach, although most are located on the lesser curvature, just distal to the junction of the antral and acid secreting mucosa.

**Clinical features**

- Abdominal pain, classically epigastric with severity relating to meal times (duodenal ulcers are classically relieved by food, while gastric ulcers are exacerbated by it).
- Bloating and abdominal fullness.
- Water brash (bitter regurgitation).
- Nausea and sometimes vomiting.
- Loss of appetite and weight loss.
- Hematemesis (vomiting of blood).
- Melena (tarry, foul-smelling feces due to oxidized iron from hemoglobin).
• Rarely, an ulcer can lead to a gastric or duodenal perforation. This is extremely painful and requires immediate surgery.

A history of heart burn, Gastroesophageal reflux disease (GERD) and use of certain forms of medication can raise the suspicion for peptic ulcer.7

Screening and diagnosis

Upper gastrointestinal X-ray.

It may begin with this test, with the outlines of esophagus, stomach and duodenum. During the X-ray, after swallowing a white, metallic liquid (containing barium) that coats the digestive tract and makes an ulcer more visible. An upper GI X-ray can detect some ulcers, but not all.

Endoscopy.

This procedure may follow an upper GI X-ray if the X-ray suggests a possible ulcer, this test may perform endoscopy first. In this more sensitive procedure, a long narrow tube with an attached camera is threaded down by throat and esophagus into the stomach and duodenum. With this instrument, doctor can view upper digestive tract and identify an ulcer. It detects an ulcer, he or she may remove small tissue samples (biopsy) near the ulcer. These samples are examined under a microscope to rule out cancer. A biopsy can also identify the presence of H. pylori in your stomach lining. Depending on where the ulcer is found, this may recommend a repeat endoscopy after two to three months to confirm that the ulcer is healing.
Additional tests.

In addition to a biopsy, these other tests can determine if the cause of your ulcer is *H. pylori* infection.

Blood test.

This test checks for the presence of *H. pylori* antibodies. A disadvantage of this test is that it sometimes can't differentiate between past exposure and current infection. After *H. pylori* bacteria have been eradicated, it may still have a positive result for many months.

Breath test:

This procedure uses a radioactive carbon atom to detect *H. pylori*. Person should blow a small plastic bag, which is then sealed. Then, he should drink a small glass of clear, tasteless liquid. The liquid contains radioactive carbon as part of a substance (urea) that will be broken down by *H. pylori*. Thirty minutes later, he should blow into a second bag, which also is sealed. If the person is infected with *H. pylori*, his second breath sample will contain the radioactive carbon in the form of carbon dioxide. The advantage of the breath test is that it can monitor the effectiveness of treatment used to eradicate *H. pylori*, detecting when the bacteria have been killed or eradicated. With the blood test, *H. pylori* antibodies may sometimes still be present a year or more after the infection is gone.

Stool antigen test:

This test checks for *H. pylori* in stool samples. It's useful both for helping to diagnose *H. pylori* infection and in monitoring the success of treatment.

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A. Factors which responsible for peptic ulcer

1. *Helicobacter pylori* (HP)
2. NSAID’s
3. Adrenocorticosteroids
4. Uncommon forms of peptic ulcer

B. Potential risk factors

1. Cigarette smoking
2. Psychological stress
3. Alcohol
4. Genetic factors
5. Dietary factors

A. Factors which responsible for peptic ulcer

1. *Helicobacter pylori* infection (HP)

*Helicobacter pylori* is an acid-labile, spiral shaped, gram-negative bacteria that resides between the mucous layer and surface epithelial cells in the stomach or any location where gastric epithelium is found. The shape and motility of the bacterium permit penetration of the mucus layer where the local pH is less acid. Before HP enters the mucous, it produces large amounts of ureases, which breaks down urea in gastric juice and converts it to ammonia and carbon dioxide. This metabolic process continues after HP reaches the “safe haven” of the mucous. The neutralizing effect of ammonia forms a microenvironment that protects the organism from the lethal effect of acid. HP attaches to epithelial surface by adhesions or pedestals specific for gastric type epithelium.
The specific pathophysiologic mechanisms by which HP causes ulcers is controversial and remains unknown, however several theories have been proposed. The defense by the elaboration of toxins, potentially toxic enzymes and inflammation. Candidates include lipopolysaccharide, vacuolizing cytotoxin, urease and ammonia, as well as macrophage and neutrophil activation. The role of the immune system in HP infection requires further study.

**Fig : 5.04 Schematic representation of pathogenesis of ulcer**

The gastrin theory hypothesizes that HP increases antral gastrin release, which leads to increased acidity and ultimately gastroduodenal damage. Although chronic HP infections have been shown to induce a chronic hypergastrinemia, increased gastrin does not appear to be a critical factor.
2. NSAID’s

There is overwhelming evidence linking chronic NSAID’s use and gastroduodenal ulcers. In patients receiving NSAID’s, ulcers occur more frequently in the stomach than in the duodenum. Hospitalizations Complications and mortality are increased in chronic NSAID’s users and are in part related to ulcer bleeding and perforation. Several factors, including a history of PUD, NSAID dose duration of exposure, and disability may predispose people to ulcer and complications.

Chronic NSAID’s therapy produces gastro duodenal injury by two mechanisms, a direct action on the mucosa and a systemic effect where by endogenous PG synthesis is inhibited. NSAID inhibition of cyclooxygenase not only decreases protective PG’s, but also generates oxygen derived free radicals and makes available of more Arachidonic acid for metabolism via the lipoxygenase pathway. Leukotrines, products of lipoxygenase metabolism, are inflammatory substances that may contribute to mucosal injury.10

3. Adreno corticosteroids

The association between adreno corticosteroids and PUD remains controversial. Although it is likely that adreno corticosteroids induce ulcers because of their ability to increase gastric acid secretion and inhibit PG production, sufficient evidence is lacking to support a causal relationship. Discrepant findings among study participants. A recent study suggests that elderly patients on concurrent oral adreno corticosteroids and NSAID’s are at a much higher risk for PUD than those receiving either of these agents alone, that ulcer risk is related to adreno corticosteroid dose and duration of therapy. It is
possible that adreno corticosteroids either delay or inhibit the healing of ulcers caused by Patients receiving adreno corticostroids, aspirin and other NSAID’s.

4. Uncommon forms of peptic ulcer

DU and GU have been reported in individuals using crack cocaine and in patients with viral infections, receiving radiation or undergoing chemotherapy administration through a hepatic artery pump. The infusion of 5-Fluorouracil, Mitomycin-C, Doxorubicin or Cisplatin probably causes ulcers by direct toxic effect.

B. Potential risk factors

1. Cigarette smoking

There is strong epidemiologic evidence that links cigarette smoking to PUD. Cigarette smoking increases the risk for the development and recurrence of DU and GU and the risk appears to be proportional to the amount smoked. The threshold for measurable risk appears to be about one half pack per day. The adverse relationship of cigarette smoking to PUD is supported by the fact that smokers are more likely than non smokers to develop ulcers and that relapse occurs sooner and more frequently in smokers than in non smokers. The specific reasons why cigarette smoking influences ulcer incidence, recurrence, healing and complications remains unclear. Possible mechanisms include accelerated gastric emptying of liquids, inhibition of pancreatic bicarbonate secretion, promotion of duodenal gastric reflux, reduction in mucosal PG production. It is uncertain whether nicotine is the component of smoke responsible for these physiologic alterations. Although smoking has been reported that increases gastric acid secretion.
2. Psychological stress.

Reports suggest an association between psychological stress and peptic ulcer disease. Stress ulceration of the stomach is associated with clinical conditions like trauma, head injury, burns, shock, sepsis and neurological disorders. It is reported to result from interactions between mucosal vascular and neurohumoral factors and the autonomic nervous system plays an important role. Electric stimulation of different regions of the limbic area modulates gastric acid secretion, motility and mucosal blood flow, all of which are important factors for the stress induced ulcer development. The CNS more importantly, the brain gut axis are important mediators of stress ulcerogenesis and complex neural mechanisms have been proposed. The disruptive and protective mediators of this neural mechanism now recognized include biogenic amines, amino acids, peptides and neurotransmitters like acetylcholine, Gamma-amino butyric acid (GABA) and several neuropeptides. Stress causes ischemic condition in the gastric mucosa by reducing blood flow following activation of parasympathetic and sympathetic nervous system, resulting in the constriction of the smooth muscles of the blood vessels and gastric tissue. This causes \( \text{O}_2 \), which is dismuted by super oxide dismutase to form \( \text{H}_2\text{O}_2 \). Stress produces loss of gastro protection, increased acidity, pepsin and histamine release and aggravates the situation.


A number of genetic factors have been proposed to explain familial aggregation of PUD. However recent data suggest hyper pepsinogenemia-I offers a more plausible explanation for family clustering than inherited autosomal dominance. Whether the gene for blood group ‘O’ is associated with an increased incidence in DU requires studies to
confirm its independence of HP. Conversely genetic syndromes such as multiple endocrine neoplasia type, Systemic mastocytosis, and amyloidosis type-IV maintain their association with peptic ulcers.

4. Alcohol

Ethanol (50-100 %) rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage, leading to increased intracellular membrane permeability to sodium and water. Ethanol induces solubilization of mucus constituents in stomach with concomitant fall in transmucosal potential difference and increases Na⁺, K⁺ flux into the lumen, also depress tissue levels of DNA, RNA and proteins leading to flow stasis in injured areas. Ethanol appears to stimulate gastric secretions by exciting sensory nerves in the buccal and gastric mucosa and promoting the release of gastrin and histamine. Ethanol induced ulcers are inhibited by agents which increases much defensive factors such as PGE₂. The massive intracellular accumulation of Ca²⁺ represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the superficial epithelium. Further, gastric lesions caused by ethanol have been attributed to free radical damage, which results in lipid peroxidation products. Clinically, cirrhosis due to consumption of alcohol is linked to an increased incidence of peptic ulcer.

5. Diet.

The role of diet and nutrition in peptic ulcer disease is uncertain, but many explain regional variations. Coffee, tea, cola beverages, beer, milk and spices may cause dyspepsia, but do not increase the risk for PUD. In addition, beverage restrictions and bland diets do not alter the frequency of ulcer recurrence. Although caffeine is a gastric
acid stimulant, other constituents in decaffeinated coffee/tea, caffeine free carbonated beverages, beer and wine are responsible for increasing gastric acid. Ethanol is high concentrations is associated with acute gastric mucosal damage and upper GI bleeding.

An association between high salt intake and gastric ulcer as well low dietary fiber and duodenal ulcer has been hypothesized but not substantiated.

**Current status of treatment for peptic ulcer**[^13].

Peptic ulcer arises due to an imbalance of acid secretary mechanism and mucosal protective factors and their rational treatment is aimed at restoring the balance.

**Fig:5.05  Mechanism of action of antiulcer agents**

[^13]: Current status of treatment for peptic ulcer
Approaches for the treatment of peptic ulcer are

A. Reduction of gastric acid secretion

1. H₂ receptor antagonists

They are currently the most popular drugs for peptic ulcer. H₂-receptor antagonists reduce acid stimulation by histamine, gastrin, cholinomimetic drugs and vagal stimulation. All phases (basal, psychic, neurogenic and gastric) of secretion are attenuated. The most prominent action is on acid output but volume pepsin content and intrinsic factor secretion are also reduced. They also prevent occurrence of stress induced ulcers. They include drugs like Ranitidine, Cimetidine, Roxatidine, Famotidine and Nizatidine.

2. H⁺K⁺ ATPase (proton pump) inhibitors

Blockade of the gastric proton pump constitutes a more direct mechanism for acid secretion inhibition compared to blockade of histamine and cholinergic receptors. They are powerful inhibitors of gastric acid. Examples of this class are Omeprazole, Pentoprazole, Rabiprazole and Lansoprazole. They are found to inhibit the growth of \( H. pylori \).

3. Anticholinergics

Anticholinergics or muscarinic cholinergic antagonists can reduce basal secretion of gastric acid by 40 to 50 % without raising the pH. Selective muscarinic M₁ receptor antagonists Pirenzepine and Telenzepine belong to this class. Selective antagonists of M₁ receptors are as effective as Atropine, but are less likely to produce adverse effects that are characteristic of cholinergic blockade (dry mouth and tachycardia).
4. Prostaglandin analogues

Prostaglandins E₂ and I₂ the predominant prostaglandins synthesized by the gastric mucosa inhibit the secretion of acid and stimulate the secretion of mucous and bicarbonate. Their most important action appears to be their ability of reinforce the mucous layer covering gastric and duodenal mucosa, which is buffered by HCO₃⁻ secreted into this layer by underlying epithelial cells. They are known to increase blood flow and are indicated for the prevention of NSAID induced gastric ulceration. Currently available PG’s in the market are Misoprostol, Enprostil, Rioprostil, Arbaprostil and Trimoprostil.

B. Neutralization of gastric acid (antacids):

The function of antacids, which are basic substances, is to neutralize the HCl secreted by gastric parietal cells and raise the pH of gastric contents. Peptic activity is indirectly reduced if the pH rises above 4, because pepsin is secreted as a complex with an inhibitory terminal moiety that dissociates below pH 5.

i) Systemic antacids

They are water soluble, acts instantaneously, but the duration of action is short. E.g: Sodium bicarbonate , Sodium citrate.

ii) Non-systemic antacids

They are insoluble and poorly absorbed basic compounds. Hydroxides of aluminium and magnesium are the most common constituents of antacids preparation. E.g. Aluminium hydroxide, Magnesium hydroxide and Calcium carbonate etc.
C. Ulcer protective:

i) Sucralfate

It is sulfated disaccharide basic aluminum sulfate complex, it forms an adherent coating with pertinacious material at ulcerated mucosal base sites so avidly, that it is difficult to wash the gel from the crater. When pH < 4, there is extensive polymerization and cross linkage of sucralfate forming a sticky, viscid coat and acts as a physical barrier preventing acid, pepsin and bile from coming in contact with the ulcer base.

ii) Bismuth compounds

They have no substantial capacity to neutralize gastric acids. Their beneficial effects have been ascribed to cytoprotection (enhanced secretion of mucous and HCO₃⁻ probably through stimulation of mucosal production, inhibition of pepsin activity and accumulation of bismuth sub citrate preferentially at the craters of the gastric ulcer). Bismuth has been shown to promote healing of both gastric and duodenal ulcers. Bismuth serves as an important component in the “Triple therapy” of *H. pylori* as it detaches *H.pylori* from the surface of the mucosa and directly kills the organism. Chronic use of other bismuth salts has caused encephalopathy and osteodystrophy.

D. Ulcer healing drugs

Carbenoxolone

It is an antiulcer drug obtained from *Glycyrrhiza glabra* (liquorice root) oleandane derivative of glycyrrhizic acid. It was found to promote healing of gastric ulcer without altering the volume or acidity of gastric juice.
E. Anti-*H. pylori* drugs

Examples of drugs under this class include antimicrobials like Amoxicillin, Clarithromycin, Metronidazole, Tinidazole, Tetracycline and Cetraxate (mucosal protective agent).

F. Miscellaneous groups

Proglumide, a cholestokinin and gastrin receptor antagonist is also found to possess antisecretory and antiulcer activity. Meciadanol a new synthetic flavonoid, an inhibitor of histidine decarboxylase has been shown to prevent gross and histological induced gastric ulcers as well as those caused by acidified aspirin probably by inhibiting mast cell degranulation. Dopamine receptors may be involved in gastric cytoprotection since duodenal ulcers are in schizophrenics, who have excess hyperactivity of brain dopamine. Protective effects have been seen with parenterally administered Dopamine agonists like Bromocriptine, Lergotrile and Apomorphine as well as the Dopamine precursor- Levodopa and the MAO-B inhibitor - Deprenyl.

Methods to induce peptic ulcer:

Advances in the discovery of novel and more effective antiulcer drugs have been accompanied by the introduction of a large number of newer experimental methods to evaluate the antiulcer activity of drugs to study their mechanism of action.

1. Pyloric ligation method:

Wistar strain male albino rats are used. The animals are starved for 48 to 72 h depending upon the weight of the animal i.e. less than 48 h for animals weighing 180 g and less than 72 h for animals over 180 g. This variability could be
related to the quantity of food left in the stomach when the pylorus was ligated. Under light ether anesthesia, a midline abdominal is made extending from the xiphoid for a distance of about one inch. The pyloric portion of the stomach is slightly lifted out and ligated, avoiding traction to the pylorus or damage to its blood supply. The stomach is replaced carefully and the abdominal wall is closed with interrupted sutures. The abdominal wound is cleansed thoroughly with normal saline, dried and covered with a solution of collodion. Food and water were deprived after the post operative period. The animals are sacrificed seventeen to nineteen hours after the pylorus ligation. The animal is first anaesthetized and the abdomen is opened, stomach is removed and examined for ulcers, ulcer index, volume of gastric juice, pH, free acidity, and total acidity are used as parameters for assessing antiulcer activity.

2. Stress Ulcers:

Gastrointestinal erosion is one of the consistent findings in the man and experimental animals subjected to different types of stress. The major advantages of stress ulcers over pylorus ligation are that they are technically simple, they bring the CNS into play and the lesions produced are located in the glandular region of the stomach whereas in pylorus ligation they are located mostly in the rumen. The following methods have been commonly used.

i) Restraint ulcers

Restraint ulcers are produced by the method described by Brodie and Hanson. Albino rats are fasted 36 to 48 h and lightly anaesthetized. Each rat is placed in a galvanized steel window screen of appropriate size. The screen is molded around the
animal and held in place with wire staples. The wire cage so formed is molded so that only slight movement is possible. The limbs are held together in pairs and tightened with adhesive tapes to make the animal immovable. The drugs under investigation are administered either orally or subcutaneously 30 min before subjecting the animal to restraint. At the end of 24 h period, the animals are removed from the screen and killed by an overdose of ether. The stomach is opened and the degree of ulceration assessed. Though this technique is a useful one, the lesions however do not penetrate the muscularis mucosa and the technique is somewhat species specific.

ii) Water immersion-induced restraint ulcer:

In this method, Male Wistar rats fasted for 24 h are immobilized in a stress cage and then immersed to the level of the xiphoid process in a water bath (23 °C) for 16 h. Later animals are sacrificed and scorings are done.

iii) Cold and restraint ulcer

In this method wistar rats, are deprived of food for 12 h are immobilized in a stress cage and forced to remain in a cold room (4 °C to 6 °C) for 3 h. The animals are sacrificed by a blow on the head and the ulcer index is calculated. The test drugs are administered 30 min before the animals are immobilized.

iv) Short-term stress and concurrent administration of non steroidal anti-inflammatory drugs (NSAID’s)

Wistar rats are deprived of food for 24-36 h before the experiment. The substances (in 1 % CMC) to be investigated are administered via gastric incubation at the same time as the intraperitoneal injection of a NSAID, Indomethacin (0.2-4 mg/ kg),
Diclofenac (1.25-12.0 mg/ kg) or Aspirin (3.12-25.0 mg/ kg). The rats are placed in a stress cage and immersed to the level of the xiphoid process in a water bath (23 °C) for 7 h. The animals are then sacrificed and evaluated for ulcer index.

v) **Restraint plus aspirin ulcers**

Male wistar rats are deprived of food for 24-36 h. Aspirin (50 mg/ kg, p.o) in 1% CMC is administered 30 min before restraint. The test drugs are given 1 hr before the restraint. The rats are subjected to restraint by placing them individually in a piece of galvanized steel window screen, which is molded tightly around and held with adhesive tapes so that the animals cannot move. After 6 h, the animals are removed from the screen, sacrificed and the intensity of gastric lesions determined.

Vi) **Swimming stress ulcers**

Male wistar rats fasted for 24-36 h are forced to swim inside vertical cylinders (height 30 cm, diameter 15 cm) containing water up to 15 cm ht, maintained at 23 °C. Three hours after the stress, they are removed from the cylinders are sacrificed by a blow on the head. Test drugs are administered 30 min prior to stress.

vii) **Activity stress ulcers**

If young adult rats are individually housed in running wheel activity cage, allowing continuous access to the wheel, and fed only one hour each day, some of these animals will die within 4-16 days. An interesting feature of this-phenomenon is that rat demonstrating high activity levels that die reveal extensive lesions in the glandular stomach. Since these glandular lesions resembled the “**stress ulcer**”, shown to be instrumental in their development, these lesions have been designated as “**activity-stress**
ulcers”. This method is of limited value in the evaluation of antiulcer activity of new drugs as it is time consuming and needs continuous supervision of the animals in the activity cages. Interestingly, animals developing activity stress gastric lesions are hyposecretors of gastric acid and do not respond to histamine H2 blockers. Activity-stress gastric lesions are, however reduced by centrally acting agents such as diazepam and imipramine suggesting that aberration in central neurotransmission play a role in their development.

viii) Hemorrhagic shock induced gastric ulcers

In this model rats were anaesthetized with urethane (125 mg/ 100g i.p.) after 20-30 min of stabilization and baseline measurements, 13 ml/ kg of blood is removed every 1-2 min, from a cannula inserted into the carotid artery, producing hypotension to a mean arterial pressure of 30-40 mm Hg. A transducer is connected via a three-way stopcock to the same arterial line to monitor the arterial blood pressure. Twenty minutes after Shock, the animals are removed and the intensity of the macroscopic lesions is graded by a suitable method.

3. Acetic acid induced chronic gastric ulcer

By injection of acetic acid (1-30 %, 0.05 ml per rat) into the submucosal layer of the stomach, penetrating experimental ulcers, which are confined by adhesion to the contiguous organs (mainly liver), can be induced. Such ulcers in the rat rapidly diminish in size and depth in the early phase of recovery but are present even after 200 days presumably due to repeated healing and reaggrevation. Histologically, the healing process closely resembles that of human peptic ulcer disease. On the other hand application of 100 % acetic acid upon the serosal surface of the rat produces penetrating duodenal ulcers.
as well as gastric ulcers at a low perforations rate. These experimental ulcer models appear to lend themselves to the screening of therapeutic drugs for peptic ulcers of the stomach or duodenum and to the investigation of the mechanism of chronicity of ulceration. Test drugs may be administered twice daily for 10-15 days period in order to assess their gastro protective effect in chronic gastric/duodenal ulcers.

4. Gastric mucosal damage by NSAID’s

NSAID’s induce gastric ulceration in rats and the ability of several agents either to protect against or aggravate this ulceration is observed. The compounds under investigation are administered 30 min to 1 h before the noxious challenge. The animals are sacrificed after a prescribed period of time and are examined for ulceration. The following drugs are routinely used: Aspirin (20 mg/kg in 1% CMC orally), Phenylbutazone (100 mg/kg p.o. or i.p.), Indomethacine (10 mg/kg p.o.), and Ibuprofen (300 mg/kg p.o.). Ibuprofen - it is given in a dose of 300 mg/kg, p.o. (in 1% CMC solution). Two doses are given at the interval of 15 h and 6 h after the second dose, the animals are sacrificed and assessed for gastric mucosal damage.

5. Reserpine induced solitary chronic gastric ulcers

Reserpine produces severe hemorrhagic glandular ulceration of the stomach, which has been attributed to significant degranulation of gastric mast cells, and is, Thought to be cholinergically mediated. Rats are deprived of solid food for 24 h with water *ad libitum* and housed in cages with wide mesh bottoms to prevent coprophagy. Reserpine (5 mg/kg per day) is administered to these animals for 5 days and sacrificed after two weeks. All the rats developed solitary chronic gastric ulceration. Microscopically, the Reserpine-induced ulceration has a narrow surface layer of fibrinous
exudates and consists of full depth mucosal necrosis and loss extending into the submucosa, with chronic inflammatory cell infiltration and fibroblastic reaction particularly at the base.

6. Serotonin induced gastric mucosal lesion:

Male rats of wistar strain are used for these experiments. The animals are fasted for 24 h prior to the experiment, water being provided *ad libitum*. Serotonin creatinine sulphate is dissolved in saline and injected to rats subcutaneously. The 20 mg/ kg dose of serotonin is found to induce a moderate but evident gastric lesion. In gross observation, gastric lesions are scarcely noticed at 30 min after serotonin injection (ulcer index: 1.2), but are obviously distinguishable at 1 h (ulcer index: 7.5) and reach maximum intensity at 4 h (ulcer index: 15.2) the lesions are located mainly at the side of the greater curvature of the corpus. The ulcer index decreases at 8.0 at 8 h and maintained at this level up to 24 h after serotonin injection.

7. Dimaprit induced gastric ulcers:

Dimaprit is administered i.p. or i.v. to 24 h fasted rats and the animals are sacrificed 4 h after the injection. The drugs for studying their gastro protective effects were given 30 min before dimaprit. The procedure is extremely simple and rapid. Its feasibility and specificity are added advantages it is very useful for evaluating not only the absolute potency of the drug given by any route, but also of other pharmacodynamic parameters particularly the duration of action which seems to be an important criterion in selecting new potentially H_2 antagonist drugs.
8. Endotoxin (Lipopolysaccharide B) induced gastric mucosal damage:

Administration of endotoxin (20 mg/ kg, i.p.) produced a moderate degree of gastric mucosal damage in rats. The lesions confined to the glandular mucosa and consisted of small punctiform lesions, erosions and petechial hemorrhages. The characteristic features of these lesions are typical submucosal ecchymosed in the glandular stomach in about 30% of the animals. Pretreatment with Ranitidine, Pirenzepine, Proglumide, Sucralfate and Naloxone provided significant protection.

9. Ethanol induced gastric ulcer:

Male wistar rats weighing 200 g were selected. Food is withdrawn for 14-16 h before experiment. Absolute alcohol (99.5 %) 1 ml/ animal, irrespective of the weight of the animal was administered intra gastrically to control rats and to rats pretreated with test drug one hour before. Animals were killed 4h after alcohol administration and observed for ulceration.

10. Histamine induced gastric ulcers in guinea pigs:

In guinea pig, histamine produces gastric ulceration in 100 % animals along with increase volume of gastric secretion and marked enhancement of free and total acidity. The percentage of area of ulceration and its intensity are reproducible and provide another species for studying the antiulcer and antisecretory activities of novel compounds.

Male guinea pigs weighing 300-400 g are fasted for 36 h (water allowed). Gastric ulceration is induced by injecting 1 ml of histamine acid phosphate (50 mg base i.p.) Promethazine hydrochloride 5 mg is injected i.p. 15 min before and 15 min after histamine to protect the animals against histamine toxicity. The drugs under investigation
are given orally or subcutaneously 30-45 min before histamine injection. The animals are sacrificed four h after histamine administration and the stomach is dissected out. The gastric contents are subjected to analysis and the stomach is cut open and the degree of ulceration is graded\textsuperscript{16}.

Table: 5.01 List of some plants, which are screened for antiulcer activity and their parts used\textsuperscript{17}.

<table>
<thead>
<tr>
<th>Name of the plant</th>
<th>Part’s used</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Withania somnifera</em></td>
<td>Roots</td>
</tr>
<tr>
<td><em>Wellia calendulacea</em></td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Pongamia pinnata</em></td>
<td>Seeds, roots</td>
</tr>
<tr>
<td><em>Abies pindrow</em></td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Asparagus recemosus</em></td>
<td>Roots</td>
</tr>
<tr>
<td><em>Glycyrrhiza glabra</em></td>
<td>Roots</td>
</tr>
<tr>
<td><em>Holarrhens antidysentrica</em></td>
<td>Barks</td>
</tr>
<tr>
<td><em>Ficus religiosa</em></td>
<td>Barks</td>
</tr>
<tr>
<td><em>Bacopa monniera</em></td>
<td>Whole plant</td>
</tr>
<tr>
<td><em>Convolvulus pluricaulis</em></td>
<td>Whole plant</td>
</tr>
<tr>
<td><em>Centella asiatica</em></td>
<td>Whole plant</td>
</tr>
<tr>
<td><em>Embilica officinalis</em></td>
<td>Fruits</td>
</tr>
<tr>
<td><em>Cissampelous muronata</em></td>
<td>Leaf</td>
</tr>
<tr>
<td><em>Cuminum cyminum</em></td>
<td>Fruit</td>
</tr>
<tr>
<td><em>Trema orientalis</em></td>
<td>Root</td>
</tr>
<tr>
<td><em>Aegle marmelos</em></td>
<td>Root</td>
</tr>
<tr>
<td><em>Zingiber officinale</em></td>
<td>Rhizome</td>
</tr>
<tr>
<td><em>Citrus limon</em></td>
<td>Fruit</td>
</tr>
<tr>
<td><em>Carumcarvi</em></td>
<td>Fruit</td>
</tr>
<tr>
<td><em>Eclipta alba</em></td>
<td>Whole plant</td>
</tr>
</tbody>
</table>
5.2. MATERIALS AND METHODS:

5.2.1.1 Sources of chemicals and drugs

Omeprazole- standard drug-gift sample from Microlabs, Banglore, Karnataka

0.01N NaOH

Topfer’s Reagent

Phenolphthalein indicator

Plant extracts:

A.S.H.E - *Annona squamosa* Hexane extract (100, 200mg/ml)

A.S.E.A.E - *Annona squamosa* ethyl acetate extract (100, 200mg/ml)

A.S.E.E - *Annona squamosa* ethanol extract (100, 200mg/ml)

A.R.H.E - *Annona reticulata* hexane extract (100, 200mg/ml)

A.R.E.A.E - *Annona reticulata* ethyl acetate extract (100, 200mg/ml)

A.R.E.E - *Annona reticulata* ethanolic extract (100, 200mg/ml)

A.M.H.E - *Annona muricata* hexane extract (100, 200mg/ml)

A.M.E.A.E - *Annona muricata* ethyl acetate extract (100, 200mg/ml)

A.M.E.E - *Annona muricata* ethanolic extract. (100-500µg/ml)

All the other chemicals were obtained from local sources and were of analytical grade.
5.2.1.2 Instruments:

Centrifuge

Microscope

Microtome

5.2.1.3 Animals used

Adult wistar albino rats weighing 150-200g of either sex were obtained from M/S Mahavir Enterprises, Hyderabad. The animals were fed with balanced diet and tap water *ad libitum*. The animals were maintained at a temperature of $22\pm1^\circ$C and 40-70% RH with 12h light period (6:00-18:00), one week before the start and also during the experiment as per the rules and regulation of institutional ethical committee and by animal regulatory body of the government (Regd:No: 516/01/A/CPCSEA).

5.3 ACUTE TOXICITY STUDIES:

Acute toxicity studies were performed for extracts according to the toxic classic method as per guidelines 423 prescribed by OECD$^{19}$. Adult swiss albino mice were used for acute toxicity study. The animals were kept fasting for overnight and provided with water only. These were divided into groups each containing three animals. Each of these groups was then administered separately with hexane, ethyl acetate and ethanol leaf extracts of *Annona squamosa* Linn, *Annona reticulata* Linn, *Annona muricata* Linn at a dose of 300mg/kg p.o. the animals were observed continuously after administration of the first dose for 30 min and then periodically for first 24 h with special attention during the first 4 h and there after daily for a total of 14 days. The observations like sedation, convulsions, tremors, salivation, lethargy, death etc., are systematically recorded with
individually records of each animal. Since no mortality was seen at the dose level of 300mg/kg, the procedure was repeated with higher dose of 600mg/kg p.o, 1200 mg/kg, p.o, 200mg/kg p.o, in fresh animals (OECD 1996).

5.4 EXPERIMENTAL WORK:

Evaluation of Gastroprotective activity:

Among the various methods available pyloric ligation method\(^{20}\) has been widely used in the screening model for gastroprotective activity. In the present work gastroprotective activity of three selected Annonaceae family plants, hexane, ethyl acetate and ethanol leaf extracts of *Annona squamosa* Linn, *Annona reticulata* Linn, *Annona muricata* Linn were tested against pyloric ligation rat model. An decrease in the pH, increase of gastric juice content, lesions formation in the GI tract are the indices of ulcer formation. The ability of above mentioned extracts to increase the pH, decrease in the volume of gastric juice and less ulcer formation near to standard values are indication of their gastroprotective potential.

5.4.1 Protocol for gastroprotective Activity\(^{21}\)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Toxic)</td>
<td>2% gum acacia solution</td>
</tr>
<tr>
<td>Standard</td>
<td>Omeprazole (20mg/kg b.w) in 2% gum acacia</td>
</tr>
<tr>
<td>Test (plant extract)</td>
<td>Plant extracts, 100 and 200 mg/kg b.w in 2% gum acacia</td>
</tr>
</tbody>
</table>
5.4.2 Estimation of following parameters will be done

1. pH of gastric juice
2. Volume of gastric secretion
3. Free acidity
4. Total acidity
5. Ulcer index
6. % protection

5.4.2.1 pH of Gastric Juice: It has been determined by using pH meter

5.4.2.2 Volume of gastric secretion: It was measured by emptying gastric contents into a measuring cylinder.

5.4.2.3 Free acidity and total acidity determination: One ml of gastric juice was pipetted out in to 100ml conical flask, add 2 to 3 drops of Topfer’s reagent and titrated with 0.01N NaOH until all traces of red colour disappears and the colour of the solution turns to yellowish orange. The volume of the alkali added was noted, this volume corresponds to free acidity. Then 2 to 3 drops of phenolphalein solution was added and titration was continued until a define red tinge reappears. Again the total volume of alkali added was noted. The volume corresponds to total acidity.

Acidity was calculated by following formula

\[
\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1 \times 100 \text{ g}} \text{ meq/L}
\]
5.4.2.4: **Ulcer index**: The number of ulcers per stomach were noted and severity of the ulcers and scoring of ulcers was done microscopically.\(^{22}\)

**Scoring of ulcer will be made as follows**

Normal stomach.......(0)

Red coloration........(0.5)

Spot ulcer.............(1)

Hemorrhagic streak..(1.5)

Ulcers....................(2)

Perforation.............(3)

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:

\[
\text{Control mean ulcer index - Test mean ulcer index} \times 100
\]

\[
\%\text{protection} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100
\]

5.4.3: **Statistical Analysis:**

Results were expressed as mean± SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Dunnett’s ‘t’ test\(^{23}\). p values less than <0.05 was considered to be statistically significant , *p<0.05,**p<0.01 and ***p<0.001, when compared with control and toxicant group as applicable.
5.4.4: Experimental procedures:

The wistar albino rats of either sex were divided into 19 groups of six animals (n=6) each.

1. Group –I served as control and received 2% gum acacia suspension 1ml/kg b.w p.o
2. Group-II served as standard and received Omeprazole (20mg/kg b.w, p.o) 2% gum acacia suspension
3. Groups-III-IV were treated with A.S.H.E at a dose of 100, 200mg/kg b.w p.o suspended in 2% gum acacia suspension.
4. Groups-V-VI were treated with A.S.E.A at a dose of 100, 200mg/kg b.w p.o suspended in 2% gum acacia suspension.
5. Groups-VI-VII were treated with A.S.E.E at a dose of 100, 200mg/kg b.w p.o suspended in 2% gum acacia suspension.
6. Groups-VIII-IX were treated with A.R.H.E at a dose of 100, 200mg/kg b.w p.o suspended in 2% gum acacia suspension.
7. Groups-X-XI were treated with A.R.E.A.E at a dose of 100, 200mg/kg b.w p.o suspended in 2% gum acacia suspension.
8. Groups-XII-XIII were treated with A.R.E.E at a dose of 100, 200mg/kg b.w p.o suspended in 2% gum acacia suspension.
9. Groups-XIV-XV were treated with A.M.H.E at a dose of 100, 200mg/kg b.w p.o suspended in 2% gum acacia suspension.
10. Groups-XVI-XVII were treated with A.M.E.A.E at a dose of 100, 200mg/kg b.w p.o suspended in 2% gum acacia suspension.
Gastroprotective Studies

11. Groups-XVIII-XIX were treated with A.M.E.E at a dose of 100, 200mg/kg b.w p.o suspended in 2% gum acacia suspension.

After 45 min of extracts and Omeprazole treatment, pyloric ligation was done by ligating the pyloric end of stomach of rats of respective groups under light ether anaesthesia at a dose of 35 mg/kg of body weight. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during postoperative period. After 4 h of surgery, rats were sacrificed with excess of anaesthetic ether. Abdominal end was opened, esophageal end of the stomach was opened and entire stomach from the body of the animal was removed. Gastric juice present in each stomach of the respective group was measured by collecting into a graduated centrifugation tube and was centrifuged at 1000rpm for 10 min and gastric volume was noted. The pH of the gastric juice was recorded by pH meter. Then the centrifuged supernatant contents were subjected to analysis for free and total acidity. Open the stomach along the greater curvature and washed with running water to see for ulcers in glandular portion of the stomach.

The number of ulcers per stomach was noted and severity of the ulcers of the ulcer scored microscopically with the help of hand lens (10x) and scoring was done. Mean ulcer score for each animal is expressed as ulcer index. Percentage protection was calculated\(^2^4\).
5.4.5 Histological studies\textsuperscript{25} 

The stomachs were immersed in 10\% formalin solution for histopathological examination. These tissues were processed and embedded in paraffin wax. The central part of damaged or ulcerated tissue (if present) was cut on half along the long diameter. If the stomach was protected from the damage then the section was taken from basal part using a rotary microtome sections of thickness of about 5µm were cut and stained with Haemotoxylin and Eosin. These were examined under microscope for histopathological changes such as congestion, haemorrhage, necrosis, inflammation, infiltration, erosion and ulcer and photographs were taken.

5.5 RESULTS AND DISCUSSION:

Acute toxicity studies were performed for extracts of selected three plants according to the toxic classic method as per guidelines 423 prescribed by OECD. None of these extracts showed any mortality even at the dose of 2000mg/kg. From the results of acute toxicity studies 1/10\textsuperscript{th}, 1/20\textsuperscript{th} doses were selected for the experimental study.

Hexane, ethyl acetate and ethanol leaf extracts of \textit{Annona squamosa} Linn, \textit{Annona reticulata} Linn, \textit{Annona muricata} Linn at a dose of 100, 200mg/kg b.w., were tested for gastroprotective activity using pyloric ligation rat model. The results were recorded in Table: 5.02, Table: 5.03 and 5.04 for \textit{Annona squamosa} Linn, \textit{Annona reticulata} Linn, \textit{Annona muricata} Linn respectively and the results are prescribed as mean±SEM. The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms\textsuperscript{26}. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal
defense mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid\textsuperscript{27}.

The comparative efficacy of the extracts tested for their gastroprotective activity, and the relationship between dose and other gastric parameters were depicted in the form of a bar diagram as shown in (Graphs 5.01-5.12):

As pH increases acidity decreases and protection increases towards the ulcer. The standard group treated with Omeprazole (20mg/kg b.w) drug significantly increased the pH, from $1.1\pm0.07$ to $5.1\pm0.09$ when compare with the control group showed regeneration and prevents formation of hemorrhagic condition of stomach.

pH shown by the A.S.H.E treated group (100, 200mg/kg b.w) was found to be $(1.9\pm0.23, 2.07\pm0.54)$ respectively.

pH shown by the A.S.E.A.E treated group (100,200mg/kg b.w) was found to be $(4.1\pm0.65, 4.8\pm0.54)$ respectively.

pH shown by the A.S.E.E treated group (100,200mg/kg b.w) was found to be $(2.8\pm0.21, 3.9\pm0.11)$ respectively.

A.R.H.E treated group (100,200mg/kg b.w) exhibited pH of $(2.02\pm0.56, 2.9\pm0.02)$ respectively.

A.R.E.A.E treated group (100,200mg/kg b.w) exhibited pH of $(3.8\pm0.71, 4.6\pm0.06)$ respectively.
A.R.E.E treated group (100, 200mg/kg b.w) exhibited pH of (3.21±0.16, 4.0±0.08) respectively.

A.M.H.E treated group at a dose of 100, 200mg/kg b.w exhibited pH of (1.9±0.23, 2.7±0.04) respectively.

A.M.E.A.E treated group at a dose of 100, 200mg/kg b.w exhibited pH of (4.1±0.01, 4.7±0.09) respectively.

A.M.H.E treated group at a dose of 100, 200mg/kg b.w exhibited pH of (3.3±0.52, 3.9±0.06) respectively.

As there is increase in the total acidity and free acidity content the chances of ulcer formation will also be high.

Total acidity exhibited by the standard group was significantly less 30.4±2.6 when compared to the total acidity exhibited by the control group 96.7±2.5.

Total acidity shown by the A.S.H.E treated group (100, 200mg/kg b.w) was found to be (91.2±0.51, 85.2±4.6) respectively.

Total acidity shown by the A.S.E.A.E treated group (100, 200mg/kg b.w) was found to be (45.2±0.55, 39.1±0.23) respectively.

Total acidity shown by the A.S.E.E treated group (100, 200mg/kg b.w) was found to be (65.9±0.45, 59.5±0.12) respectively.

Total acidity shown by the A.R.H.E treated group (100, 200mg/kg b.w) was found to be (79.4±3.6, 49.6±3.4) respectively.

Total acidity shown by the A.R.E.A.E treated group (100, 200mg/kg b.w) was found to be (39.6±1.92, 31.4±2.1) respectively.
Gastroprotective Studies

Total acidity shown by the A.R.E.E treated group (100, 200mg/kg b.w) was found to be (61.4±4.5, 39.5±2.9 ) respectively.

Total acidity shown by the A.M.H.E treated group (100, 200mg/kg b.w) was found to be (82.1±1.4, 50.2±3.3 ) respectively.

Total acidity shown by the A.M.E.A.E treated group (100, 200mg/kg b.w) was found to be (43.4±1.8, 33.3±2.1) respectively.

Total acidity shown by the A.M.E.E treated group (100, 200mg/kg b.w) was found to be (63.2±3.8, 41.1±3.1) respectively.

Ulcer index is the index which indicates the severity of ulcers. Increase in the ulcer index more is the severe condition of ulcer.

Standard drug Omeprazole treated group showed ulcer index of 0.18±0.04 which is significantly less as compare to the ulcer index of control group 0.65±0.06.

Ulcer index shown by the A.S.H.E treated group (100, 200mg/kg b.w) was found to be (0.31±0.54, 0.28±0.01) respectively.

Ulcer index shown by the A.S.E.A.E treated group (100, 200mg/kg b.w) was found to be (0.25±0.11, 0.19±0.06) respectively.

Ulcer index shown by the A.S.E.E treated group (100, 200mg/kg b.w) was found to be (0.26±0.06, 0.23±0.05) respectively.

Ulcer index shown by the A.R.H.E treated group (100, 200mg/kg b.w) was found to be (0.45±0.54, 0.36±0.01) respectively.

Ulcer index shown by the A.R.E.A.E treated group (100, 200mg/kg b.w) was found to be (0.24±0.15, 0.21±0.45) respectively.
Ulcer index shown by the A.R.E.E treated group (100, 200mg/kg b.w) was found to be (0.36±0.15, 0.30±0.05) respectively.

Ulcer index shown by the A.M.H.E treated group (100, 200mg/kg b.w) was found to be (0.35±0.09, 0.29±0.05) respectively.

Ulcer index shown by the A.M.E.A.E treated group (100, 200mg/kg b.w) was found to be (0.26±0.12, 0.20±0.05) respectively.

Ulcer index shown by the A.M.E.E treated group (100, 200mg/kg b.w) was found to be (0.28±0.03, 0.25±0.04) respectively.

Standard drug Omeprazole treated group has shown the percentage protection of 72%.

Whereas, A.S.H.E at a dose of 100, 200mg/kg b.w has shown percentage protection of 52%, 56% respectively.

A.S.E.A.E at a dose of 100, 200mg/kg b.w has shown percentage protection of 61%, 70% respectively.

A.S.E.E at a dose of 100, 200mg/kg b.w has shown protection of 60%, 64% respectively.

A.R.H.E at a dose of 100, 200mg/kg b.w has shown protection of 30%, 44% respectively.

A.R.E.A.E at a dose of 100, 200mg/kg b.w has shown protection of 63%, 67% respectively.

Whereas, A.R.E.E at a dose of 100, 200mg/kg b.w has shown percentage protection of 44%, 53% respectively.
A.M.H.E at a dose of 100, 200mg/kg b.w has shown protection of 46%, 55% respectively.

A.M.E.A.E at a dose of 100, 200mg/kg b.w has shown protection of 60%, 69% respectively.

Whereas, A.M.E.E at dose of 100, 200mg/kg b.w has shown protection of 56%, 61% respectively.

Photographs of rats stomach treated with selected plant extracts were given in the (Fig:5.06-5.08)

Histopathological changes on pylorus ligation model showed the degeneration, hemorrhage, edematous appearance of the gastric tissue in control group, whereas ethyl acetate at a dose of 200mg, 100mg/kg b.w and Omeprazole (20 mg/kg) treated groups showed regeneration and prevents the formation of hemorrhage and edema and it was shown in (Fig: 5a,5b,5c).

The gastric tissue of Ethyl acetate, ethanol treated group in all three selected plants showed regains of cellular structure, less hemorrhage condition, and with less edema which is almost equal to that of the standard group, exhibiting high significant action. whereas, in hexane treated groups it was not showing significant effect and it exhibits high degeneration of tissue, red patches, lesions, hemorrhage, edema condition was observed at a higher dose of hexane extract treated group shows less hemorrhagic condition.
Table: 5.02 Gastro protective results of *A. squamosa* Linn leaf extracts.

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume of HCl</th>
<th>pH</th>
<th>Total acidity mEq/L</th>
<th>Free acidity mEq/L</th>
<th>Ulcer index</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control 2% gum acacia solution</td>
<td>6.59±0.12</td>
<td>1.1±0.07</td>
<td>96.7±2.5</td>
<td>62.2±4.6</td>
<td>0.65±0.06</td>
<td>-</td>
</tr>
<tr>
<td>II Standard Omeprazole 20mg/kg</td>
<td>3.41±0.24***</td>
<td>5.1±0.09***</td>
<td>30.4±2.6***</td>
<td>19.3±1.4</td>
<td>0.18±0.04</td>
<td>72%</td>
</tr>
<tr>
<td>III A.S.H.E 100mg/kg</td>
<td>6.69 ± 0.27*</td>
<td>1.9±0.23*</td>
<td>91.2±0.51*</td>
<td>60.0±2.1</td>
<td>0.31±0.54</td>
<td>52%</td>
</tr>
<tr>
<td>IV A.S.H.E 200mg/kg</td>
<td>5.34±0.35**</td>
<td>2.07±0.54**</td>
<td>85.2±4.6*</td>
<td>54.2±5.4</td>
<td>0.28±0.01</td>
<td>56%</td>
</tr>
<tr>
<td>V A.S.E.A.E 100mg/kg</td>
<td>4.25±0.31***</td>
<td>4.1±0.65***</td>
<td>45.2±0.55***</td>
<td>30.2±5.6</td>
<td>0.25±0.11</td>
<td>61%</td>
</tr>
<tr>
<td>VI A.S.E.A.E 200mg/kg</td>
<td>3.6±2.41***</td>
<td>4.8±0.54***</td>
<td>39.1±0.23***</td>
<td>21.3±4.5</td>
<td>0.19±0.06</td>
<td>70%</td>
</tr>
<tr>
<td>VII A.S.E.E 100mg/kg</td>
<td>4.62±0.11**</td>
<td>2.8±0.21**</td>
<td>65.9±0.45**</td>
<td>49.2±0.5</td>
<td>0.26±0.06</td>
<td>60%</td>
</tr>
<tr>
<td>VIII A.S.E.E 200mg/kg</td>
<td>3.89±2.90***</td>
<td>3.9±0.11**</td>
<td>59.5±0.12**</td>
<td>40.1±0.6</td>
<td>0.23±0.05</td>
<td>64%</td>
</tr>
</tbody>
</table>

Values are mean±SEM of 6 rats

Significant *p<0.05, **p<0.01, ***p<0.0001 compared with control
Table 5.03 The gastro protective results of *A. reticulata* Linn leaf extracts

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume of HCl</th>
<th>pH</th>
<th>Total acidity meq/L</th>
<th>Free acidity meq/L</th>
<th>Ulcer index</th>
<th>%Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control 2% gum acacia solution</td>
<td>6.59±0.12</td>
<td>1.1±0.07</td>
<td>96.7±2.5</td>
<td>62.2±4.6</td>
<td>0.65±0.06</td>
<td>-</td>
</tr>
<tr>
<td>II Standard Omeprazole 20mg/kg</td>
<td>3.41±0.24</td>
<td>5.1±0.09*</td>
<td>30.4±2.6*</td>
<td>19.3±1.4</td>
<td>0.18±0.04</td>
<td>72%</td>
</tr>
<tr>
<td>III A.R.H.E 100mg/kg</td>
<td>6.89±2.6*</td>
<td>2.02±0.56**</td>
<td>79.4±3.6**</td>
<td>43.2±1.86</td>
<td>0.45±0.54</td>
<td>30%</td>
</tr>
<tr>
<td>IV A.R.H.E 200mg/kg</td>
<td>5.63±0.35**</td>
<td>2.9±0.02**</td>
<td>49.6±3.4**</td>
<td>35.11±1.7</td>
<td>0.36±0.01</td>
<td>44%</td>
</tr>
<tr>
<td>V A.R.E.A.E 100mg/kg</td>
<td>4.52±0.44***</td>
<td>3.8±0.71***</td>
<td>39.6±1.92***</td>
<td>24.3±1.64</td>
<td>0.24±0.15</td>
<td>63%</td>
</tr>
<tr>
<td>VIA.R.E.A.E 200mg/kg</td>
<td>4.12±0.62***</td>
<td>4.6±0.06***</td>
<td>31.4±2.1***</td>
<td>21.27±1.9</td>
<td>0.21±0.45</td>
<td>67%</td>
</tr>
<tr>
<td>VII A.R.E.E 100mg/kg</td>
<td>5.04±2.21**</td>
<td>3.21±0.16***</td>
<td>61.4±4.5***</td>
<td>35.11±2.5</td>
<td>0.36±0.15</td>
<td>44%</td>
</tr>
<tr>
<td>VIII A.R.E.E 200mg/kg</td>
<td>4.96±0.37**</td>
<td>4.0±0.08***</td>
<td>39.5±2.9***</td>
<td>24.54±1.6</td>
<td>0.30±0.05</td>
<td>53%</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M of 6 rats

Significant *p<0.05, **p<0.01, ***p<0.0001 compared with control
Table: 5.04 The gastro protective results of *A. muricata* Linn leaf extracts

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume of Hcl</th>
<th>pH</th>
<th>Total acidity mEq/L</th>
<th>Free acidity mEq/L</th>
<th>Ulcer index</th>
<th>%protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>6.59±0.12</td>
<td>1.1±0.07</td>
<td>96.7±2.5</td>
<td>62.2±4.6</td>
<td>0.65±0.06</td>
<td>-</td>
</tr>
<tr>
<td>II Standard</td>
<td>3.41±0.24</td>
<td>5.1±0.09*</td>
<td>30.4±2.6*</td>
<td>19.3±1.4</td>
<td>0.18±0.04</td>
<td>72%</td>
</tr>
<tr>
<td>III A.M.H.E 100mg/kg</td>
<td>6.08±0.21*</td>
<td>1.9±0.23**</td>
<td>82.1±1.4**</td>
<td>46.6±0.12</td>
<td>0.35±0.09</td>
<td>46%</td>
</tr>
<tr>
<td>IV A.M.H.E 200mg/kg</td>
<td>5.32±0.11*</td>
<td>2.7±0.04**</td>
<td>50.2±3.3**</td>
<td>37.5±1.3</td>
<td>0.29±0.05</td>
<td>55%</td>
</tr>
<tr>
<td>V A.M.E.A.E 100mg/kg</td>
<td>3.71±0.21***</td>
<td>4.1±0.01***</td>
<td>43.4±1.8***</td>
<td>25.8±0.15</td>
<td>0.26±0.12</td>
<td>60%</td>
</tr>
<tr>
<td>VIA M.E.A.E 200mg/kg</td>
<td>3.12±0.14***</td>
<td>4.7±0.09***</td>
<td>33.3±2.1***</td>
<td>22.7±1.1</td>
<td>0.20±0.05</td>
<td>69%</td>
</tr>
<tr>
<td>VII A.M.E.E 100mg/kg</td>
<td>4.61±0.11**</td>
<td>3.3±0.52***</td>
<td>63.2±3.8***</td>
<td>37.2±1.4</td>
<td>0.28±0.03</td>
<td>56%</td>
</tr>
<tr>
<td>VIII A.M.E.E 200mg/kg</td>
<td>4.01±0.12**</td>
<td>3.9±0.06***</td>
<td>41.1±3.1***</td>
<td>26.5±1.4</td>
<td>0.25±0.04</td>
<td>61%</td>
</tr>
</tbody>
</table>

Values are mean±SEM of 6 rats

Significant *p<0.05, **p<0.01, ***p<0.0001 compared with control
**Graph: 5.01:** Graphical representation of *Annona squamosa* Linn leaf extracts effect on pH

![Graph 5.01](image1.png)

**Graph: 5.02:** Graphical representation of *Annona squamosa* Linn leaf extracts effect on total acidity

![Graph 5.02](image2.png)
Graph: 5.03: Graphical representation of *Annona squamosa* Linn leaf extracts effect on ulcer index

Graph: 5.04: Graphical representation of *Annona squamosa* Linn leaf extracts effect on percentage protection
Graph: 5.05 Graphical representation of *Annona reticulata* Linn leaf extracts on pH

![Graphical representation of *Annona reticulata* Linn leaf extracts on pH](image)

Graph : 5.06 : Graphical representation of *Annona reticulata* Linn leaf extracts on Total Acidity

![Graphical representation of *Annona reticulata* Linn leaf extracts on Total Acidity](image)
Graph: 5.07 Graphical representation of *Annona reticulata* Linn leaf extracts on Ulcer Index

Graph: 5.08 Graphical representation of *Annona reticulata* Linn on percentage Protection
**Graph: 5.09** Graphical representation of *Annona muricata* Linn leaf extracts on pH

**Graph: 5.10** Graphical representation of *Annona muricata* Linn leaf extracts on Total Acidity
Graph: 5.11 Graphical representation of *Annona muricata* Linn leaf extracts on Ulcer Index

Graph: 5.12 Graphical representation of *Annona muricata* Linn leaf extracts on percentage Protection
Fig: 5.06 Picture of rat stomach which are treated with leaf extracts of *Annona squamosa* Linn

Toxic control

Standard group

A.S.H.E 100mg/kg

A.S.H.E 200mg/kg

A.S. E.A.E-100mg/kg

A.S.E.A.E- 200mg/kg

A.S.E.E-100mg/kg

A.S.E.E- 200mg/kg
Fig: 5.07 Pictures of rat stomach which are treated with leaf extracts of *Annona reticulata* Linn

- Toxic control
- Standard group
- A.R.H.E-200mg/kg
- A.R.H.E 100mg/kg
- A.R.E.A.E-200mg/kg
- A.R.E.A.E-100mg/kg
- A.R.E.E-200mg/kg
- A.R.E.E-100mg/kg
Gastroprotective Studies

Fig: 5.08 Pictures of rat stomach which are treated with leaf extracts of *Annona muricata* Linn

- Toxic control
- Standard group
- A.M.H.E-200 mg/kg
- A.M.H.E-100mg/kg
- A.M.E.A.E-200 mg/kg
- A.M.E.A.E-100 mg/kg
- A.M.E.E-200 mg/kg
- A.M.E.E-100 mg/kg
Fig: 5a Histopathological section of rat stomach region in *Annona squamosa* Linn extracts treated group

- **Toxic control**
- **Standard**
- A.S.H.E-100mg/kg
- A.S.H.E-200mg/kg
- A.S.E.A.E-100mg/kg
- A.S.E.A.E-200mg/kg
- A.S.E.E-100mg/kg
- A.S.E.E-200mg/kg
Fig. 5b: Histopathological section of rat stomach region in *Annona reticulata* Linn extracts treated group.
Fig:5c Histopathological section of rat stomach region in *Annona muricata* Linn extracts treated group

Toxic control

standard

A.M.H.E-100mg/kg

A.M.E.A.E-100mg/kg

A.M.E.E-100mg/kg

A.M.H.E-200mg/kg

A.M.E.A.E-200mg/kg

A.M.E.E-200mg/kg
5.6: CONCLUSION:

1. All the extracts of three selected plants have exhibited significant gastroprotective activity.

2. The ethylacetate extract of *Annona squamosa* Linn at a dose of 100, 200mg/kg b.w has showed highly significant gastroprotective activity than ethanol and hexane extracts.

3. The ethylacetate extract of *Annona reticulata* Linn at a dose of 100, 200mg/kg b.w has showed highly significant gastroprotective activity than ethanol and hexane extracts.

4. The ethylacetate extract of *Annona muricata* linn leaf at a dose of 200mg/kg b.w has showed highly significant gastroprotective activity than ethanol and hexane extracts.

5. Gastroprotective action of certain phytoconstituents like flavonoids\textsuperscript{28}, alkaloids\textsuperscript{29}, Tannins\textsuperscript{30}. Have been well documented in the literature.

6. The above mentioned phytoconstituents alone or in combination may be responsible for the gastroprotective acitivity of the selected plants.
5.7 REFERENCES


