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# ANTIULCER ACTIVITY OF ETHANOLIC EXTRACT OF *PIPER BETEL* LEAVES ON ALBINO RATS

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**Abstract:**

*The antiulcer activity of ethanolic extract of Piper betel leaves (EEPB) was investigated in pylorus ligation and ethanol induced ulcer models in experimental rats. In both models the common parameter determined was ulcer index. Ethanolic extract of Piper betel at a dose of 150 and 300mg/kg produced significant inhibition of the gastric lesions induced by pylorus ligation induced ulcer and ethanol induced gastric ulcer. The extract (150mg/kg and 300mg/kg) showed significant ($p<0.05$) reduction in gastric volume, free acidity and ulcer index as compared to control. This present study indicates that EEPB have potential anti-ulcer activity in both models. These results may further suggest that the extract was found to possess antiulcerogenic as well as ulcer healing properties, which might be due to its antisecretory activity.*

***Keywords*** *Piper betel, antisecretory, cytoprotective, Gastric ulcer, ethanol induced ulcers and pylorus ligation induced ulcers.*

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## INTRODUCTION

**Stomach[24]:** Stomach is a hollow organ situated just below the diaphragm on the left side in the abdominal cavity. When empty, its volume is 50ml and normally it can expand to accommodate 1 to 1.5 liters of solids and liquids. Gastric juice is the mixture of secretions from different glands of the stomach.
**Properties:** Its volume ranges from 1200 to 1500 ml/day. Gastric juice is highly acidic with pH of 0.9 to 1.2. The acidity of gastric juice is due the HCl. The specific gravity ranges from 1.002 to 1.004.

**Composition**: It contains 99.5% of water and 0.5% solids. The solids are organic and inorganic substances.
**Organic substances:**

**Gastric enzymes**: The enzymes present in gastric juice are pepsin, rennin, lipase and other enzymes.

**Pepsin:** This is the major protein splitting enzyme in the gastric juice. the precursor of pepsin is pepsinogen.

**Rennin:** It is a milk curdling enzyme.

**Gastric lipase**: It is a weak lipid splitting enzyme.

**Other gastric juice:** The other enzymes of gastric juice are the gelatinase and urase.

**Gastric mucus**: It is secreted by mucus neck cells of the gastric glands and surface mucus cells in fundus, body and others parts of stomach. It is like a flexible gel covering the gastric mucus membrane. Mucus is a glycoprotein.

**Intrinsic Factor**: This is necessary for absorption of the extrinsic factor.

**Inorganic substances:** The Inorganic substances present in the gastric juice are HCL, sodium, calcium, potassium, chloride, bicarbonate, phosphate and sulfate.

**DIGESTIVE SYSTEM**
The function of the digestive system is to digest and absorb food. It consists of a tubular gastrointestinal tract and accessory organs that aid in digestion and absorption.
All organisms require food to sustain life. The cells of the body require nutrients for the chemical reactions of enzyme synthesis, cell division, growth and repair and also for the production of heat energy. Most of the food we eat requires considerable processing before it can be used by the cells. It must be broken down mechanically and chemically before it is transported by the blood to the cells [25, 26].
The activities that are performed by the digestive system include the following activities: Ingestion: the taking of food into the mouth.

**Mastication:** chewing food which pulverizes it and mixes it with saliva

**Deglutination**: Swallowing; moving food from the mouth to the pharynx and into the esophagus.

**Digestion:** The mechanical and chemical breakdown of food to prepare it for absorption.

**Absorption:** the passage molecules of food through the mucous membrane of the small intestine and into the blood and lymph for distribution to the cells.

**Peristalsis:** the rhythmic wavelike contractions of the smooth muscle of the intestines that¬ move food through the GI tract.

**Defecation:** the discharge of indigestible wastes (feces) from the GI tract. Anatomically and functionally the digestive system can be divided into a tubular gastrointestinal(GI) tract and accessory digestive organs. The GI tract which extends from the mouth to the anus is a continuous tube approximately 30 feet (9m) long. It goes through the thoracic cavity and enters the abdominal cavity through the diaphragm. The organs of the digestive system include the oral cavity (mouth), pharynx, esophagus, stomach, small intestine and large intestine. The accessory organs include teeth, salivary glands, liver, gall bladder and pancreas. It usually takes about 24-48 hours for food to travel the length of the GI tract. Food travels in an assembly line manner through the tract where it is broken down to the molecular level and transported to the cells. Each region of the GI tract has a specific function in the process [27,28]

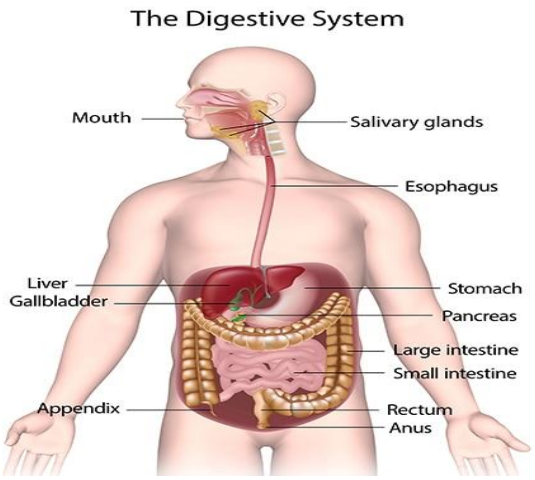


**Fig 1.2: Digestive System**

**Membranes of the Abdominal Cavity**

Most of the digestive organs are located in the abdominal cavity. These organs are covered by serous membranes that line the cavity and cover the organs within. Serous membranes secrete a lubricating serous fluid that continuously moistens the organs. The parietal membrane lines the wall of the abdominal cavity and the visceral membrane covers the internal organs. The membrane that lines the wall of the abdominal cavity is called the parietal peritoneum. It comes together to form a double layered peritoneal fold called the mesentery[29]. The mesentery supports the GI tract and at the same time allows the small intestine freedom for peristaltic contractions. It also provides a structure for the passage of blood vessels and nerves. The peritoneal membrane continues around the intestinal organs as the visceral peritoneum. The peritoneal cavity is the space between the parietal and visceral portions of the peritoneum. Certain organs lie posterior to the peritoneal cavity and are said to be retroperitoneal.

These organs include most of the pancreas, the kidneys, adrenal glands and portions of the duodenum and colon as well as the abdominal aorta[30]
Peritonitis is an inflammation of the peritoneum usually caused by an infection. This can occur due to trauma, rupture of an organ, an ectopic pregnancy or postoperative infection. It is a serious life threatening situation. Treatment usually involves massive doses of antibiotics as well as insertion of a tube to drain excess fluid which accumulates[31]. Extensions of the parietal peritoneum serve to suspend or anchor organs within the peritoneal cavity. The falciform ligament attaches the diaphragm and the anterior abdominal wall to the liver. The greater omentum extends from the stomach to the transverse colon forming an apron like covering over most of the small intestine. Function of the omentum includes storage of fat, cushioning visceral organs, supporting lymph nodes and protection against infection. in cases of infection such as appendicitis the greater omentum may actually compartmentalize the infection, sealing it off from the rest of the peritoneal cavity. The lesser omentumpasses from the lesser curve of the stomach and the upper duodenum to the inferior surface of the liver[32].

**Layers of the GI tract** [33,34]

The GI tract from the esophagus to the anal canal is comprised of 4 layers or tunics. Each layer performs specific functions in the digestive process.

**These layers are :** Mucosa- the innermost layer lines the lumen of the GI tract. It is both absorptive and secretory in function. It contains lymph nodes as well as goblet cells which secrete mucous. There is also a thin layer of smooth muscle in this tunid.
Submucosa- this is the second layer, much thicker than the mucosa. It is primarily¬ vascular and nerve containing. Absorbed molecules pass through the mucosa to enter blood or lymph vessels here. The submucosa contain glands and a nerve plexus (Meissner's plexus) which provides autonomic innervation to the muscle layer in the mucosa.

Tunica muscularis- This is the primary smooth muscle layer of the GI tract which is¬ responsible for peristalsis. It has an inner circle and an outer longitudinal layer of muscle. Contraction of this layer causes the movement of food as well as helping to pulverize and churn the food with digestive enzymes. There is a large nerve plexus (Aurebach'splexis ) located between the 2 muscle layers. It provides both sympathetic and parasympathetic innervation.
Serosa- is the outermost layer of the GI tract wall. It is binding an protective in function.

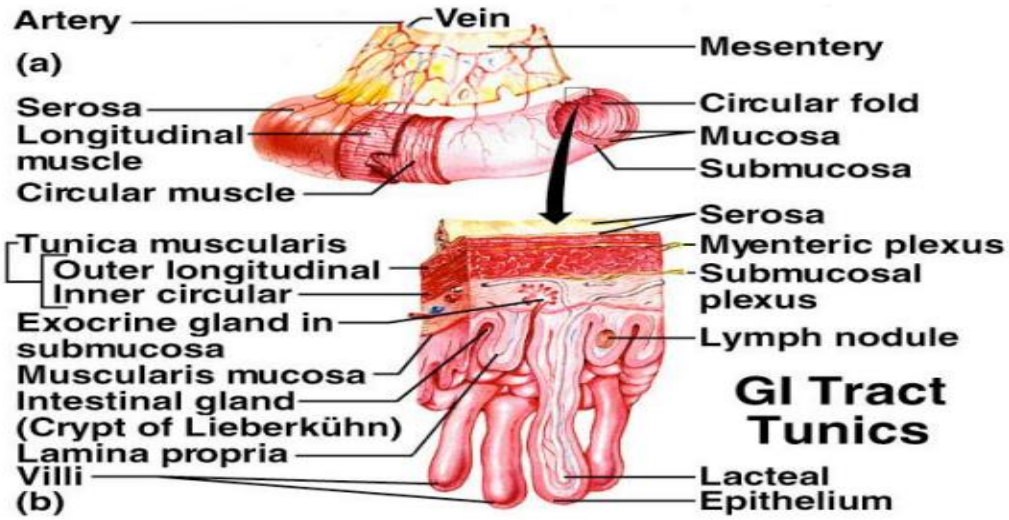


**FIG 1.3: Layers of GI Tract**

**Esophagus**

The esophagusis that part of the GI tract that connects the pharynx to the stomach. It is a collapsible tubular organ about 10 inches long originating at the larynx and lying posterior to the trachea. The esophagus lies within the mediastinum of the thorax and passes through the diaphragm just above the opening to the stomach. This opening through the diaphragm is called the esophageal hiatus. The upper third of the esophagus is made up of skeletal muscle, the middle third is a combination of skeletal and smooth muscle. The terminal portion of the esophagus is smooth muscle only [35,36]. The lower esophageal sphincteris a thickening of circular muscle at the junction of the esophagus and stomach. After food or fluid pass into the stomach this constricts to prevent regurgitation into the esophagus. This occurs normally because thoracic pressure is lower than abdominal pressure.

Because this sphincter is not as large or strong as other sphincters of the GI tract backflow can sometimes occur under some conditions. This is what is referred to as heartburn. The acidic stomach contents are coming up into the esophagus. During vomiting the contents of the stomach are regurgitated completely to rid the stomach of some perceived toxin or irritant. In babies, this sphincter's function is kind of erratic leading them to spit up following a meal. Some mammals have very strong esophageal sphincters. This is the case with rats and mice; which is why they are killed easily by poisoned bait, they cannot regurgitate the poison. The process of swallowing (deglutination) is a three part process which involves both voluntary and involuntary processes. The first stage which is voluntary involves closing the mouth and interruption of breathing. The tongue is elevated against the roof of the mouth due to contraction of the intrinsic muscles of the tongue and the myohyoid and stylohyoid muscles. The second stage is the passage of the bolus (food) through the pharynx. This is involuntary and is elicited by sensory receptors located at the opening of the oropharynx. Pressure of the tongue against the transverse palatine folds seals off the nasopharynx from the oral cavity and creates pressure that forces the bolus into the oropharynx. The soft palate and uvula are elevated to close off the nasopharynx as the bolus passes. The hyoid bone and larynx are elevated.

Elevation of the larynx against the epiglottis seals off the glottis so that food or fluid are less likely to be aspirated into the trachea. Sequential constriction of the constrictor muscles moves the bolus from pharynx to esophagus. The third and final stage is involuntary as well. The bolus is moved to the stomach by means of peristalsis [37,38] .

Stomach The stomach- the most distensible part of the GI tract- is located in the upper left quadrant, immediately below the diaphragm. It is a J shaped organ that is continuous with the esophagus and empties into the duodenal portion of the small intestine inferiorly.

In the stomach the food is churned mechanically with gastric secretions to form a pasty substance called

chyme. Once formed it is moved to the small intestine.
The stomach is divided into four regions: the cardia, fundus, body and pylorus[39].

The **cardiais** the narrow upper region immediately below the lower esophageal sphincter.

The **fundus** is the dome shaped portion to the left of and in direct contact with the diaphragm. The body is the large central portion and the pylorus is the funnel shaped terminal portion. The pyloric sphincteris the modified circular muscle at the end of the pylorus where it joins the small intestine.

**Pylorus** in Greek means gatekeeper and the pyloric sphincter acts to regulate the flow of chyme into the small intestine. The wall of the stomach is composed of the same 4 tunics found in the other regions of the GI tract, with 2 principal modifications: an extra oblique muscle layer present in the muscularis, and the mucosa has numerous longitudinal folds called gastric folds or gastric rugae. The mucosa also has microscopic gastric pits and gastric glands [40,41]

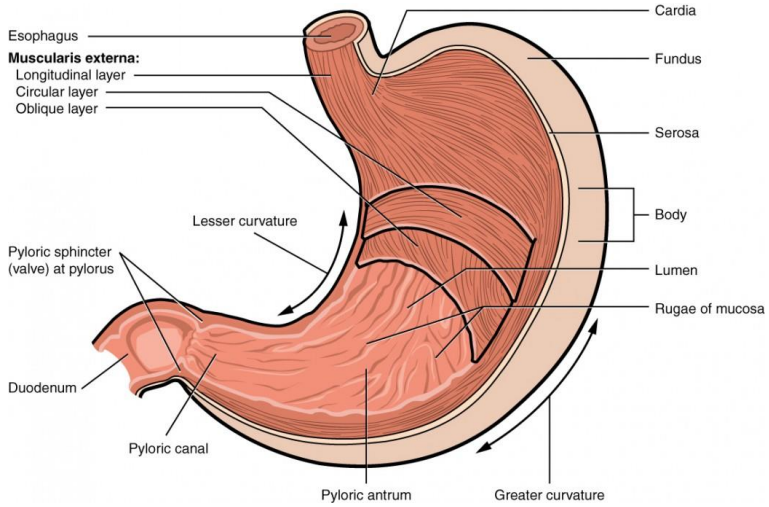


**Fig 1.4: Stomach**

**There are 5 types of cells in the gastric glands that secrete specific products:**

**Goblet cells** secrete protective mucous.

**Parietal cells** secrete hydrochloric acid

**Principal cells** (chief cells) secrete pepsinoge, an inactive form of the protein digesting enzyme pepsin.

**Argentaffin cells** secrete serotonin, histamine and autocrine regulators

**Endocrine cells** (G cells) secrete the hormone gastrin into the blood [42,43].

**Small Intestine**
The small intestine, consisting of the duodenum, jejunum and ileum, is the site where digestion is completed and nutrients are absorbed. The small intestine is the t-portion of the GI tract between the pyloric sphincter of the stomach and the iliocecal valve that opens into the large intestine. It is positioned in the central and lower part of the abdominal cavity and is supported except for the first portion by the mesentary. The fan shaped mesentary permits movement of the small intestine but prevents it from becoming kinked or twisted. Enclosed within the mesentary are blood vessels, nerves and lymphatic vessels that supply the intestinal walls. The small intestine is about 12 feet long (3 m) in a living person but will measure twice that length in a cadaver due to relaxation of the muscular wall. It is called "small" due to its small diameter relative to the large intestine. The small intestine is the body's major digestive organ and the main site of nutrient absorption. It contains digestive enzymes which aid in the final breakdown and absorption of food [44,45] .

**Regions of the small intestine**
1. Duodenum is a fixed C shaped tube measuring 10 inches from the pyloric sphincter of¬ the stomach to the duodeno jejunal flexure. It receives bile secretions from the liver and gall bladder and pancreatic secretions from the pancreatic duct.

2. Jejunum extends from the duodenum to the ileum, is approximately 3 feet long. It has a larger lumen and more internal folds than the ileum[46].

3. Ileum (not to be confused with the ilium of the oscoxae) makes up the remaining 6-7 feet of the small intestine. It empties into the cecum of the large intestine through the ileocecal valve. Lymph nodes called mesentary patches are abundant in the walls of the ileum [47,48].

**Large Intestine**

The large intestine receives food that is undigested or undigestible from the small intestine, absorbs the water and electrolytes from the chyme and passes it as feces out of the GI tract. The large intestine measures about 5 feet in length and 2.5 inches in diameter. The large intestine begins at the end of the ileum in the lower right hand quadrant of the abdomen. From there it leads superiorly on the right side to a point just below the liver; it then crosses to the left, descends into the pelvis and terminates at the anus. A specialized portion of the mesentary, the mesocolon supports the transverse portion of the large intestine along the posterior abdominal wall [49].

The large intestine has little or no digestive function. It absorbs water and electrolytes from the remaining chyme. It also functions to form, store and expel feces from the body.

The large intestine is divided into the cecum, colon rectum and anal canal. The cecum is a dilated pouch positioned slightly below the ileocecal valve. The ileocecal valve is a fold of mucous membrane at the junction of the small and large intestine that prevents back flow of chyme. A finger like projection of the cecum called the appendixis attached to the inferior margin of the cecum. It contains an abundance of lymphatic tissue but it serves no discernible function. It is thought to be a vestigial remnant of an organ that was functional in our ancestors. Because it is a blind pouch and waste material can accumulate within, inflammation and infection can occur. If not treated, rupture will lead to further infection of the peritoneal cavity, resulting in peritonitis.

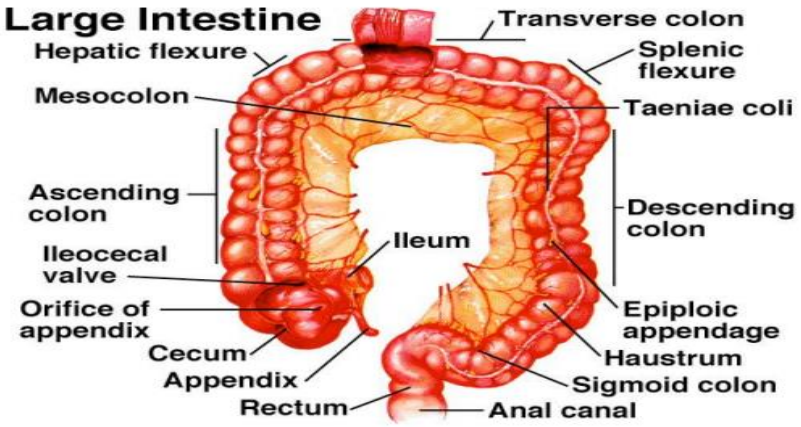


**Fig 1.5: Large Intestine**

The superior portion of the cecum is continuous with the colon, which consists of the ascending, transverse, descending and sigmoid portions. The ascending portion extends superiorly from the cecum along the right abdominal wall to the inferior surface of the liver. The point where the colon bends here is called the hepatic flexure. From this bend it becomes the transverse colon until it reaches another right angle bend on the left side called the splenic flexure. From this point it becomes the descending colon as it transverses inferiorly on the left. At the bottom of the descending colon it angles again in an S shaped bend known as the sigmoid colon. The end of the line, the last 7.5 inches of the tract is the rectum. The final inch (2-3 cm) is the anal canal. The anus is the external opening of the anal canal. Two sphincter muscles are found in this opening: the internal anal sphincter which is smooth muscle and the external anal sphincter which is skeletal muscle [50,51] .

**Mechanical Action of the Large Intestine**

Three types of movements occur throughout the large intestine: peristalsis, haustral churning and mass movement. In Haustral churning, the relaxed haustrum fills with food residue until a point of distension is reached that stimulates contraction of the muscle. This movement churns the food residue and exposes it to the mucosa where the water and

electrolytes are absorbed. As this happens food residue becomes solid or semisolid and becomes feces. Mass movement is a strong peristaltic wave which moves the feces towards the rectum. Mass movement occurs only 2-3 times a day, generally after a meal. In infants this response to eating is called the gastrocolic reflex and results in a bowel movement during or shortly after eating [52,53]

The defecation reflex normally occurs when rectal pressure rises to a particular level that is determined by individual habit. At this point the internal anal sphincter relaxes to admit feces into the anal canal. During defecation, the longitudinal rectal muscles contract to increase rectal pressure and the internal and external anal sphincters relax. This process is aided by contraction of the abdominal muscles which raise intra abdominal pressure and help push the feces through the anal canal and out the anus.

**REGULATION OF ACID SECRETION54**

Parietal cells in the stomach secrete roughly two liters of acid a day in the form of hydrochloric acid. Acid in the stomach functions to kill bacteria and to aid digestion by solubilizing food. The acid is also important to establish the optimal pH (between1.8-3.5) for the function of the digestive enzyme pepsin.

A key protein for acid secretion is the H+ /K + -ATPase (or proton pump). This protein, which is expressed on the apical membrane of parietal cells, uses the energy derived from ATP hydrolysis to pump hydrogen ions into the lumen in exchange for potassium ions.

Stimulation of acid secretion involves the translocation of H +/K +-ATPases to the apical membrane of the parietal cell. When the cell is resting (not stimulated),H+ /K +-ATPases are located in vesiclesin side the cell. When the cell is stimulated, these vesicles fuse with the plasma membrane, thereby increasing the surface area of the plasma membrane and the number of proton pumps in the membrane.

There are three regulatory molecules that stimulate acid secretion (acetylcholine, histamine, gastrin) and one regulatory molecule that inhibits acid secretion(somatostatin ). Acetylcholine is a neurotransmitter that is released by enteric neurons. Histamine is a paracrine that is released from ECL (enterochromaffin-like) cells. Gastrin is a hormone that is released by G cells, endocrine cells that are located in the gastric epithelium. Somatostatin is also secreted by endocrine cells of the gastric epithelium; it can act as either a paracrine or a hormone.

The figure (same as on the handout) shows how the positive and negative regulators interact to stimulate acid secretion. Acetylcholine and histamine directly stimulate parietal cells to increase acid secretion. Gastrinstimulates acid secretion by stimulating histamine release from ECL cells.

(Gastrinalso has a direct effect on parietal cells, which is to stimulate their proliferation).When the pH of the stomach gets to low, somatostatin secretion is stimulated. Somatostatin inhibits acid secretion by direct effects on parietal cells, and also by inhibiting release of the positive regulators histamine and gastrin. The balance of activity of the different regulators changes as food is consumed and passes through different segments of the upper GI tract.

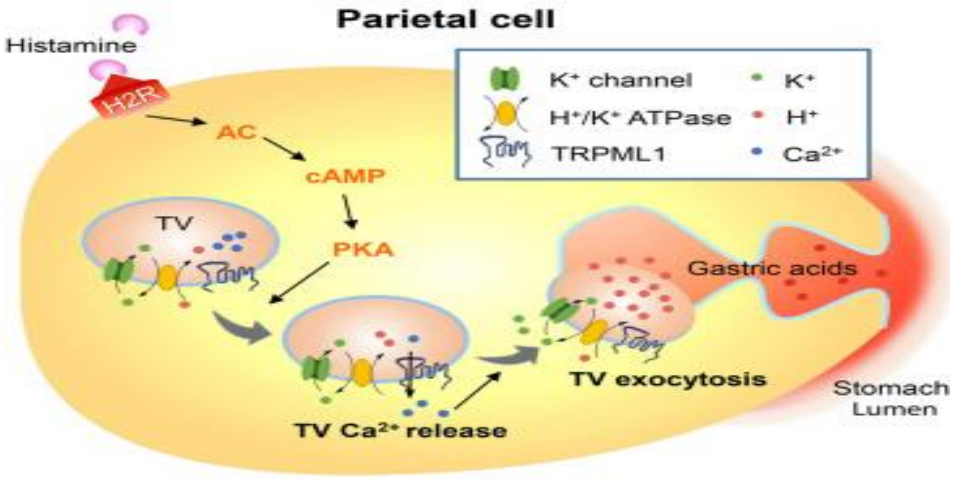


**Fig 1.6: Acid secretion**

Cephalic Phase Cephalic phase stimuli are things like the sight, smell, taste or thought of food. These stimuli, processed by the brain, activate enteric neurons viapara sympathetic preganglionic neurons traveling in the vagus nerve.

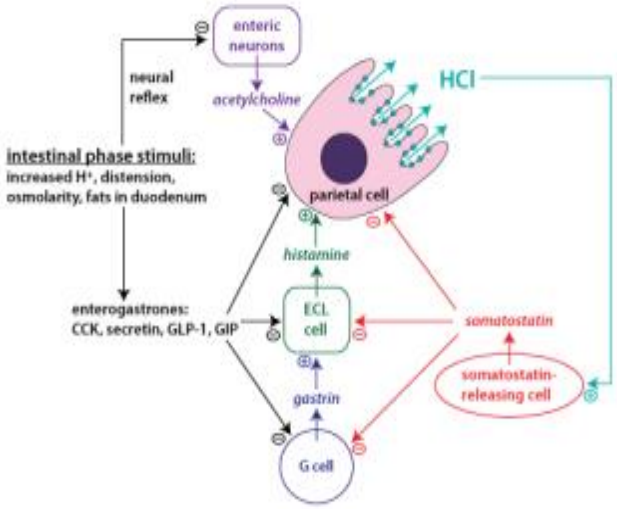


**Fig 1.7: Cephalic Phase**

**Gastric Phase**

The primary factor during the gastric phase is that there is food in the stomach, which stimulates acid secretion. There are three different ways that this occurs. Food will stretch the walls of the stomach; this is sensed by mechanoreceptors, activating a neural reflex to stimulate acid secretion (purple). Peptides and amino acids in food stimulate G cells to release gastrin (blue). Food also acts as a buffer, raising the pH and thus removing the stimulus for so metastatic secretion (light blue-green).

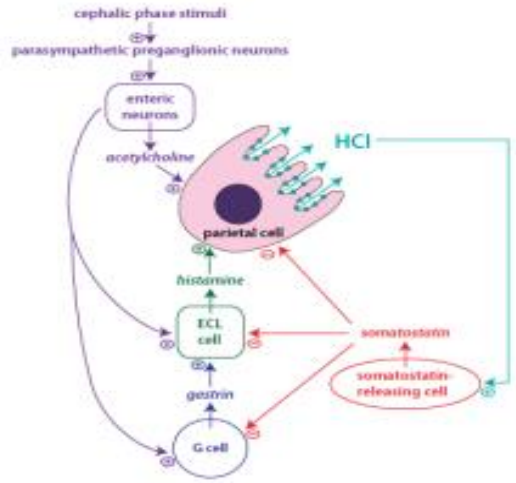


**Fig 1.8: Gastric Phase**

Intestinal Phase Once chymeenters the duodenum, intestinal phase stimuli activate negative feedback mechanisms to reduce acid secretion and prevent the chyme from becoming too acidic. This occurs by neural reflexes and hormonal reflexes. Enterogastrones are hormones that inhibit stomach processes (in this case, acid secretion). In addition to their other actions, CCK ,secreting , GLP-1 , and GIP act as enterogastrones.

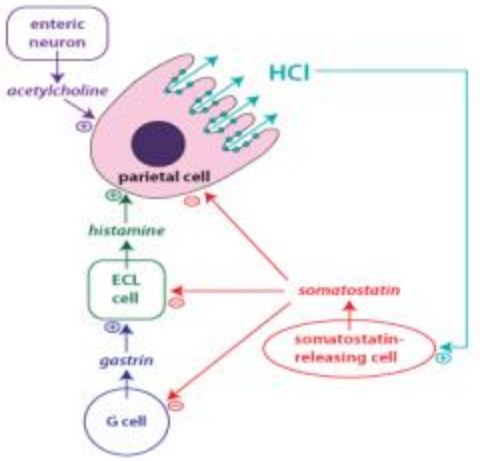


**Fig 1.9: Intestinal Phase**

PEPTIC ULCER

Peptic ulcers are sores that develop in the lining of the stomach, lower esophagus, or small intestine. The most common symptom of a peptic ulcer is burning abdominal pain that extends from the navel to the chest. Untreated ulcers can become worse over time and lead to other health conditions.
Peptic ulcers are sores that develop in the lining of the stomach, lower esophagus, or small intestine (the duodenum) usually as a result of inflammation caused by the bacteria H.pylori, as well as from erosion from stomach acids.

Peptic ulcers are a fairly common health problem There are three types of peptic ulcers:

Gastric ulcers: ulcers that develop inside the stomach.

Esophageal ulcers: ulcers that develop inside the esophagus.

Duodenal ulcers: ulcers that develop in the upper section of the small intestines, called the duodenum.

Causes of Peptic Ulcers [55]

- Different factors can cause the lining of the stomach, the esophagus, and the small intestine to break down. These Include:
- Helicobacter pylori (H. pylori): bacteria that can cause a stomach infection and inflammation.
- Frequent use of aspirin, ibuprofen, and other anti-inflammatory drugs(risk associated with this behavior increases in women and people over the age of 60)
- Smoking
- Drinking too much alcohol
- Radiation therapy
- Stomach cancer

Symptoms of Peptic Ulcers

The most common symptom of a peptic ulcer is burning abdominal pain that extends from the navel to the chest, which can range from mild to severe. In some cases, the pain may wake you up at night. Small peptic ulcers may not produce any symptoms in the earlyphases[56]

Other common signs of a peptic ulcer include:

- ✓ Changes in appetite
- ✓ Nausea
- ✓ Bloody or dark stools (melena)
- ✓ Unexplained weight loss
- ✓ Indigestion
- ✓ Vomiting
- ✓ Chest pain

SCREENING METHODS FOR ANTIULCER ACTIVITY [57]

1) Pylorus ligation in rat (SHAY rat):
This model is a simple and convention method for induction of gastric ulceration in the rat through ligature in the pylorus region, the ulceration is affected by accumulation of acidic juice inside the stomach. Ulcer index & pH of gastric content of treated animals are compared with control groups. Different cumulative group administration followed by dose - response curves establishment for ulcer formation can be measured in these method.
Procedure: Female Wistar rats weighing 150-170 g are starved for 48 hours having access to drinking

water ad libitum. During this time they are housed single in cages in prevent coprophagy. Six animals are used per dose and as control groups. Under mild ether anaesthesia an incision is made at the abdominal midline. The pylorus is closed by using small nylon the higher supervision is required to avoid he damage of blood vessels inside the pylorus region. Grasping the stomach with instruments is to be meticulously avoided; else ulceration will invariably develop at such points. The abdominal wall are sutured through surgical procedure. The test sample are administrated through oral ingestion or injected subcutaneous route. The animals are placed for 19 hours in a suitable plastic container. Afterwards, these animals are sacrificed in CO2 anaesthesia. The abdomen is re ligated and a ligature is placed above the esophagus region and closer to the diaphragm area. The stomach is replaced to a watch glass and the materials are collected in to a centrifugal tube. Above the longer curvature the stomach fully opened and pinned between a cork plates. The mucosal layer is observed with the help of a stereomicroscope.

2) Stress ulcer through immobilization stress:
Psychogenic factors, such as stress, produce a major role in etiology of gastric ulcers in human beings and animals. Hence not only antacids ingestion, anticholinergics, H2– antagonists, proton pump inhibitors treatment, and also psychotropic agentssuch as neuroleptics have also effective for the treatment.

Procedure: Groups of 6 female Wister rats per dose of test drug and for controls weighing 150-170 g are used. Food and water are removed 24 hours before the experimental procedure. After oral or subcutaneous ingestion of the test substance or the placebo drug in animal's extremities are fixed combine and the animals are tied in wire gauze. These animals are horizontally suspended in a dark room at 20oC for one day and last animals are sacrificed in CO2 anaesthesia method. The stomach has been cut, fixed in a cork plate and the count and score the severity of ulcers by the help of video recorded stereo-microscope.

3) Stress ulcers by cold water immersion:
Cold water treatment of rats in during the restraint duration boost the Procedure: Groups of 8-10 Wistar rats weighing 150-200 g are used. After oral administration of the test compound, the rats are placed vertically individual restraint cages in water at 22oC for 1 hour. These are removed, allowed to dry and injectevans blue (30mg/Kg) intravenously through tail vein. After ten minutes, these animals are sacrificed by CO2 anesthesia, stomachs was collected in Formol - saline (2%v/v) overnight storage about 24 hours. After that the stomachs are opened the greater curvature, washed via warm water and examined through 3-fold magnifier.

4) Indomethacin induced ulcers in rats:
Nonsteroidal anti- inflammatory agents, like indomethacin and acetyl-salicylic acid, produce a gastric lesions in human beings and in rodents by the inhibition of gastric cyclo-oxygenase leading for the formation of prostacyclin. Procedure: Groups of 5-6 Wistar rats weighing 150 - 200 g are used. The test drugs are administered orally in 0.1 % tween 80 solution 10 minutes before the oral indomethacin at a dose about 20 mg/kg (4 mg/ml dissolved in 0.1 % tween 80 solution).

## MATERIALS AND METHODS:

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.[91-92]

### 7.1 Drugs and Chemicals

Drugs and Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

**Table No: 7.1 Drugs and Chemicals**

| *S.No* | *Materials* | *Company Name* |
|---|---|---|
| 1. | Ranitidine | Cipla |
| 2. | Omeprazole | Cipla |
| 3. | Alcohol | Merck |

**7.2. Instruments**

Following instruments were required for the study:

**Table No: 7.2- List of Instruments used for study**

| *Name of the instrument* | *Source* |
|---|---|
| Centrifuge | Dolphin |
| Digital weighing balance | Horizon |
| Heating mantle | ASGI® |
| Disection box | Camel |
| Refrigerator | Videocon |
| Actophotometer | Dolphin |
| Glass cylinder | ASGI® |
| Adhesive tape | YVR medivision Pvt Ltd |
| Thread | YVR medivision Pvt Ltd |
| Stop watch | ASGI® |
| Syringes | YVR medivision Pvt Ltd |
| Needles | YVR medivision Pvt Ltd |
| Soxhlet extractor | ASGI® |
| Condenser | ASGI® |
| Burette stand | Dolphin |
| Round bottom flask | ASGI® , Amar |
| Mixer | Videocon |
| Oven | ASGI® |
| Water bath | ASGI® |
| Stirrer/glass rod | ASGI® |
| Watch glass | ASGI® |
| Whatmann filter paper | Manipore microproducts, Ghaizabad. |
| Butter paper | ASGI® |
| Spatula | ASGI® |
| Rubber pipes | ASGI® |

**PRELIMINARY QUALITATIVE TEST**

**7.2. Preliminary Phytochemical Screening [93-96]**
Preliminary phytochemical screening of the plant extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, Flavonoids .as per the standard methods.

**1. Detection of Alkaloids:** Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

**a) Mayer's Test:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).Formation of a yellow coloured precipitate indicates the presence of alkaloids.

**b).Wagner's Test:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

**c). Dragendroff's Test:** Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

**d). Hager's Test:** Filtrates were treated with Hager's reagent (saturated picric acid solution).Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

**2. Detection of Carbohydrates:** Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.[97]

**a). Molisch's Test:** Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of
the violet ring at the junction indicates the presence of Carbohydrates.

**b).Benedict's Test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

**c).Fehling's Test:** Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicates the presence of reducing sugars.[98]

**3. Detection of saponins**

**a). Froth Test:** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for15 minutes. Formation of 1cm layer off a am indicates the presence of saponins.

**b). FoamTest:** 0.5gm of extract was shaken with 2ml of water. If foam produced persists forten minutes it indicates the presence of saponins.[99]

**4. Detection of steroids.**

**a). Salkowski's Test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

**b). Libermann Burchard's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.[100]

**5. Detection of Phenols**

**Ferric Chloride Test:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

**6. Detection of Tannins**
**Gelatin Test:** To the extract,1 %gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

**7. Detection of Flavonoids**

**Alkaline Reagent Test:** Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

**Leadacetate Test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

**7.3. Experimental animals [101]**
Wistar rats (150-200 g) and were procured from Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. All the animals were maintained under standard conditions, that is room temperature 26 ± 1°C, relative humidity 45 - 55% and 12:12 h light – dark cycle. The animals were housed in large spacious hygienic cages during the course of the

experimental period. Animal studies had approval of IAEC.

**7.4. Plant Material Collection**

The leaves of *Piper betel* were collected from the Botanical garden and was identified and authenticated from Botanical Department. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

**7.5. Preparation of plant extracts [102]**

**7.5.1 Preparation of ethanolic Extract:**

Shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. Defatted powdered of *Piper betel* has been extracted with ethanol solvent using maceration process for 48 hrs., filtered and dried using vacuum evaporator at 40°C (yield of extract was 9.40% with respect to dry material). Just prior to use, the substance was dissolved in physiological saline solution.

**7.6. Selection of dose for animal study [103]**

The dose considered for the experiment on rats was obtained from conversion of human dose of *Piper betel* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats. Hence the calculated dose for the rats (considering human dose 3 and 5 g/kg) is 200 mg/kg. Acute toxicity was done at dose of 2000mg/kg body weight as per OECD guidelines No 423.

**7.7. PHARMACOLOGICAL EVALUATION**

**ACUTE ORAL TOXICITY: [104]**

The acute oral toxicity of ethanolic extracts of *Piper betel* was determined by using rats which were maintained under standard conditions. The animals were fasted 12 hours prior to the experiment, up and down procedure OECD guideline no. 423 were adopted for toxicity studies. Animals were administered with single dose of individual extract up to 2000mg/kg and observed for its mortality during 14days and 21days study period (long term) toxicity and observed up to 14days for their mortality, behavioral and neurological profiles.

**7.9 SCREENING FOR ANTI-ULCER ACTIVITY**

The Ethanolic extracts of *Piper betel* were tested for antiulcer activity using various methods like pyloric ligation induced gastric ulcer and Ethanol-induced gastric ulcer.

**7.9.1 PYLORIC LIGATION IN RATS[105]**

Animals were divided into four (I-V) groups.

**Group I** - Normal (Distilled water)

**Group II**- Control (Pyloric ligation)

**Group III** - EEPB (150mg/kg) Single dose

**Group IV** - EEPB (300mg/kg) Double dose

**Group V** - Ranitidine (20mg/kg)

The animals were divided into 5 groups, each consisting of six rats. Control group received distilled water only. Second group of rats are pyloric Ligated. Third and fourth groups received EEPB in a dose of 150 and 300 mg/kg. The fifth group of animals received Ranitidine in the dose of 20mg/kg as a reference drug for ulcer protective studies. After 45 min of the treatment, pyloric ligation was done by legating the pyloric end of stomach of rats of respective groups under ether anesthesia at a dose of 35mg/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 post-operative period. Rats were sacrificed after 4hr of surgery and ulcer scoring was done. Gastric juice was collected and gastric secretion studies were performed according to the standard procedure.

**Ethanol induced ulcer model**

**Group I** - Normal (Distilled water)

**Group II**- Control (Ethanol)

**Group III** - EEPB (150mg/kg)

**Group IV** - EEPB (300mg/kg)

**Group V** - Omeprazole (20mg/kg)

The ulcer was induced by administering absolute ethanol (1ml/200g). All the animals were fasted for 36 hours and then ethanol was administered to induce ulcer. The animals were divided into five groups, each consisting of six rats. The Group I - Normal group received Distilled water, second group received Ethanol. Third and fourth groups received EEPB in a dose of 150 and 300 mg/kg. The fifth group of animals received Omeprazole in the dose of 20 mg/kg as a reference drug. They were kept in

specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 hr later with anaesthetic ether and stomach was incised along the greater curvature and ulceration was scored. A score for the ulcer was studied to pyloric ligation induced ulcer model.

**Scoring of ulcers**
Normal stomach - 0
Red coloration - 0.5
Spot ulcer - 1
Hemorrhagic streak - 1.5
Ulcers (< 2mm) - 2
Ulcers (>2 < 4 mm) perforation -3 Ulcers (< 4mm) -4
Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer protection was determined by

% of ulcer protection= Control mean ulcer index – Test mean ulcer index/ Control mean ulcer indexx100

Acidity = Determination of free acidity/ Volume of sodium hydroxide x Normality x 100mEq/L/100g

0.1

## STATISTICAL ANALYSIS

The values are represented as mean ± S.E.M, and Statistical significance between treated and control groups was analyzed using of one-way ANOVA, followed by Dennett's test where $P<0.05$ was considered statically significant.

## CONCLUSION

Peptic ulcer disease is one of the most common gastrointestinal disorders. It mainly involves an imbalance between the offensive and defensive factors. Many synthetic drugs such as H2 blockers, gastro protective and proton pump inhibitors are available in the market but they are showing many side effects. Medicinal plants and their products are considered to less side effects and more efficacy when compared to synthetic drugs. Many medicinal plants and natural analogues showed prominent anti-ulcer and gastro-protective activities. Based on this literature review the plant *Piper betel was* selected for screening antiulcer activity.

NSAID's like aspirin and paracetamol causes gastric mucosal damage by decreasing prostaglandin levels through inhibition of PG synthesis. ethanolic extract of the plant of *Piper betel* was significantly effective in protecting gastric mucosa against paracetamol induced ulcers at all the dose level studied.

Pyloric ligation induced gastric ulcer and Ethanol-induced gastric ulcer model ethanolic extract of *Piper betel* at a dose of 150 and 300mg/kg body weight p.o was found to exhibit significant cytoprotective action when compared to control (pyloric ligation) group using Omeprazole 20mg/kg p.o as a standard drug

On the basis of the present results and available reports, it can be concluded that the anti-ulcer activity elucidated by *Piper betel* could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition.

**Recommendations:**
The Research work can be extended:

- ✓ This study confirmed the claim in Ethiopian traditional medicine that the plant has therapeutic value in stomach ulcers. So, it needs further in-depth investigation by using other models, isolation, and characterization of the active principles responsible for the anti-ulcer activity and to elucidate the exact mechanism action of this plant. Evaluation of its anti-ulcer efficacy in case of co-morbid illness in those previously reported.

## FUTURE SCOPE OF RESEARCH WORK:

- ✓ Present study mainly focused on using natural resources in greater amount both from toxicity as well as cost-oriented issues.
- ✓ Natural components are easily obtainable. Hence, in future it can effectively replace synthetic derivatives.
- ✓ Benefits are like free from toxicity, needed in little quantity plus effortlessly obtainable at fewer prices in contrast to synthetic component to achieve higher yield, optimization as well as novel processes serve such purpose by providing optimal criteria to conduct experiments. Such issues should be focus in near future.
- ✓ The plant was found having activity against GI Ulcers as evident from this study.
- ✓ Pharmacologic activities which may be a hint to investigate use of herbal as therapeutic agents.
- ✓ Hence, this may be useful to discover safer substitute for Ulcer management for numerous ailments.
- ✓ However, Future work can be done for isolating its main constituents who are

responsible for this activity and for elucidating its mechanism of action of Anti-ulcer activity of these plant extracts.

**REFERENCES:**


1. Burks TF. Principles of Pharmacology. United States of America: International Thomson publishing Inc; 1995. p. 1063.
2. Kumar V, Abbas KA, Fausto N. Robbins and Cotran Pathologic Basis of Disease. 7th ed. New Delhi: Elsevier Inc; 2004. p. 817.
3. Peptic ulcer diagram. Available from:www.wanderiner.blogspot.com.
4. Statistics. Available from:http://www.cureresearch.com/p/peptic_ulcer/statscountry.htm
5. Danger of ulcer. Available from:http://www.murrasaca.com/Gastriculcer.htm.
6. Allison MC, Howatson AG, Caroline MG.Gastrointestinal damage associated with the use of nonsteriodal anti-inflammatory drugs. N Engl J Med 1992; 327: 749–54.
7. Lenz HJ, Ferrari-Taylor J, Isenberg JI. Wine and five percent ethanol are potent stimulants of gastric acid secretion in humans. Gastroenterology 1983; 85: 1082-7.
8. Cohen S, Booth GH Jr. Gastric acid secretion and lower-esophageal - sphincter pressure in response to coffee and caffeine. N Engl J Med 1975; 293: 897–9.
9. Feldman EJ, Isenberg JI, Grossman MI. Gastric acid and gastrin response to decaffeinated coffee and a peptone meal. JAMA 1981; 246: 248–50.
10. Dubey P, Sundram KR, Nundy S. Effect of tea on gastric acid secretion. Dig Dis Sci 1984; 29: 202–6.
11. Korman MG, Hansky J, Eaves ER, Schmidt GT. Influence of cigarette smoking on healing and relapse in duodenal ulcer disease. Gastroenterology 1983;85: 871– 4.
12. Katchinski BD, Logan RFA, Edmond M, Langman MJS. Duodenal ulcer and refined carbohydrate intake, a case-control study assessing dietary fiber and refined sugar intake. Gut 1990; 31: 993–6.
13. Suadicani P, Hein HO, Gyntelberg F. Genetic and life-style determinants of peptic ulcer, a study of 3387 men aged 54 to 74 years, the copenhagen male study. Scand J Gastroenterology 1999; 34: 12 – 7.
14. Yudkin J. Eating and ulcers. BMJ 1980; Feb. 16: 483 [letter]
15. Sonnenberg A. Dietary salt and gastric ulcer. Gut 1986; 27: 1138 – 42.
16. Pfeiffer CJ, Cho CH, Cheema A, Saltman D. Reserpine-induced gastric ulcers: protection by lysosomal stabilization due to zinc. Eur J Pharmacology, 1980; 61: 347– 53.
17. Shive W, Snider RN, DuBilier B. Glutamine in treatment of peptic ulcer. TexasState J Med. 1957; 11: 840.
18. Chaturvedi A, Kumar MM, Bhawani G, Chaturvedi H, Kumar M, Goel KR. Effect of ethanolic extract of Eugenia Jambolana seeds on gastric ulceration and secretion in rats. Indian J Physiol Pharmacol 2007; 51(2): 131-140.
19. Nadkarni KM. Indian Materia Medica. 2 nd ed. Bombay: Popular Prakashan; 1976.vol 2 p. 394-95.
20. Hisham A, Pieters L A C, Claeys M, Esmansa E, Dommisse R and Vlietinck A J (1991). Squamocin-28-one and panalicin, two acetogenins from Uvaria narum. Phytochemistry 30(2): 545-548.
21. Peptic Ulcer definition. Available from:http://www.medicinenet.com/peptic_ulcer/article.htm
22. Gary AT, Kevin TP. Anthony's Textbook of Anatomy And Physiology. 14th ed. St.Louis: Mosby Year Book, Inc; 1994. p. 667.
23. Rubin E, Gorstein F, Rubin R, Schwarting R, Strayer D. Clinicopathologic Foundations of Medicine. 4th ed. United State of America: Lippincott Williams & Wilkins; 2005. p. 680-3.
24. Mohan H. Text Book of Pathology. 4rth ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2003: 564-66.
25. Sembulingam K, Sembulingam P. Essentials of Medical Physiology. 3rd ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2004. p. 176-79.