Evaluation of Anti-ulcerant effect of *Oroxylum indicum* in ulcerative rat model at high dose

Department of Pharmacy
East West University

B. PHARM THESIS

A dissertation submitted to the Department of Pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy

Submitted by
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Declaration by the Candidate

I, **Md. Sohanur Rahman**, hereby declare that the dissertation entitled "**Evaluation of Anti-ulcerant effect of *Oroxylum indicum* in ulcerative rat model at high dose**" *s*ubmitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, work carried out by me during the period 2017 of my research in the Department of Pharmacy, East West University, under the supervision and guidance of **Mst. Marium Begum**, Senior Lecturer, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Supervisor

This is to certify that the thesis entitled **"Evaluation of Anti-ulcerant effect of *Oroxylum indicum* in ulcerative rat model at high dose**" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by **Md. Sohanur Rahman**, (ID# 2014-1-70-034), during the period 2017 of his research in the Department of Pharmacy, East West University, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Endorsement by the Chairperson

This is to certify that the thesis entitled **"Evaluation of Anti-ulcerant effect of *Oroxylum indicum* in ulcerative rat model at high dose**" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by **Md. Sohanur Rahman**, (ID: 2014-1-70-034), during the period 2017 of his research in the Department of Pharmacy, East West University.

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Dr. Chowdhury Faiz Hossain
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Dedication

This research paper is dedicated to my beloved Parents and my family members.

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Abstract

The medicinal plants signify a massive basis of potential phytoconstituents that could be valuable as a substitute to allopathic drugs or considered as an analogue in drug development. *Oroxyllum indicum* is one of the medicinal plants that show anticancer, anti-ulcer, anti-dysenteric, antimicrobial, and anti-inflammatory activity. Various part of this plant is used in medical purpose. Alkaloids, glycocides, terpinoids, steroids, flavanoids, phenolics, tannins, anthraquinone, Coumarins etc. component has been found in this plant extract that show different therapeutic effects. In this project we investigated the methanolic extract of bark of *Oroxylum indicum* for anti-ulcer activity. To identify the plant`s therapuetic effects we conducted anti-ulcer and histopathology tests.

The anti-ulcer activity of methanol extract of O. *indicum* bark was evaluated as doses of 200mg/kg and 400 mg/kg p.o while using omeprazole (20mg/kg,p.o) as the standard drug. Ulcer was induced by feeding 25ml/kg of ethanol (0.3M HCL in 60% ethanol). Gastric mucosa from the different groups were examined for ulcers by magnifying lens and scoring ulcer.
For these experiments long evans rat were used.

O. indicum is highly significant ($P < 0.01$) in protection of ethanol-induced ulcer, it showed protection of 73% and 76% at doses 200mg/kg/day and 400 mg/kg/day, p.o. respectively. These findings were further supported by the histological study.

The investigation suggests that methanolic extract of *O. indicum* bark possess anti-ulcer activity.

Keywords: *Oroxyllum indicum,* Anti-ulcer, Ethanol.

CHAPTER-1

INTRODUCTION

1.1. Ulcer

A peptic ulcer is a sore in the lining of our stomach, the first part of our small intestine. A burning stomach pain is the most common symptom. Peptic ulcers happen when the acid damage the walls of the stomach or duodenum. The most common cause is- infection with a bacterium called Helicobacter pylori. Another cause is the long-term use of nonsteroidal anti-inflammatory medicines (NSAIDs) such as aspirin and ibuprofen. Stress and spicy foods do not cause ulcers, but can make them worse. To see if an individual has an H. pylori infection, doctor will test his blood, breath, or stool. The doctor also may look inside the stomach and duodenum by doing an endoscopy or x-ray. Peptic ulcers will get worse if it is not treated.[1]

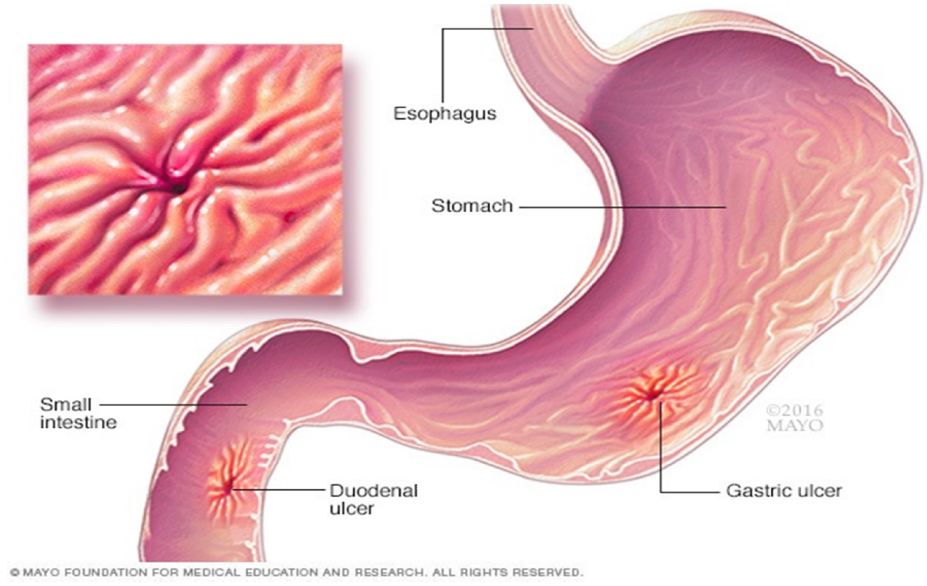


Fig 1.1: Gastric Ulcer[2]

1.2. Complications due to the peptic ulcers

Untreated ulcers can become worse over time. They can lead to other more serious health complications such as:

- **Perforation:** A hole develops in the lining of the stomach or small intestine and causes an infection. A sign of a perforated ulcer is sudden, severe abdominal pain.

- **Internal bleeding:** Bleeding ulcers can result in significant blood loss and thus require hospitalization. Signs of a bleeding ulcer include- lightheadedness, dizziness, and black stools.
- **Scar tissue:** This is thick tissue that develops after an injury. This tissue makes it difficult for food to pass through your digestive tract. Signs of scar tissue include vomiting and weight loss.[3][4]

1.3. Symptoms of peptic ulcers

Symptoms of peptic ulcer may include:

- Abdominal pain with a burning sensation
- Pain 2 to 3 hours after eating
- Heartburn
- Indigestion (dyspepsia)
- Belching
- Nausea
- Vomiting
- Poor appetite
- Weight loss
- Sudden increase in the abdominal pain or sharpness in the quality of the pain
- Vomiting blood or material that looks like coffee grounds
- Blood in stool or black, tarry stools.[5]

1.4. Reasons behind peptic ulcer

Ulcer is due to an imbalance between digestive fluids in the stomach and duodenum. Most happen because of an infection in the lining of the small intestine with a type of bacteria called *Helicobacter pylori (H. pylori)*.

Factors that can increase an ulcer include-

- Use of painkillers called nonsteroidal anti-inflammatory drugs (NSAIDs) like- aspirin, ibuprofen, and naproxen.
- A history of ulcers in family.
- Being age 50 or older.
- Drinking alcohol.
- Smoking.
- Chewing tobacco.
- Stress.
- Spicy foods.
- Certain medications like steroids, anticoagulants, and drugs called selective serotonin reuptake inhibitors (SSRIs).
- Medical problems like liver, kidney, or lung diseases.
- Radiation treatment[6][7][8]

1.4.1. Effect of smoking for the induction of ulcer

Smoking increases risk of developing a peptic ulcer and it can make that ulcer slower to heal. In one study of more than 4,000 smokers and non-smokers 18 to 30 years old, researchers at the University of Minnesota found that the smokers were nearly twice as likely to have numerous ailments, including ulcers, when they were re-examined 7 to 15 years later.[9]

Cigarettes interfere with the body's natural protective mechanisms against stomach acid. The stomach acid isn't absorbed but is neutralized by sodium bicarbonate, a natural antacid. This neutralization occurs in the duodenum, the first part of the intestine. Sodium bicarbonate is made by the pancreas. There's evidence to suggest that smoking increases stomach acid production

over time, and it reduces bicarbonate production. The duodenum, the first part of the small intestine, is also a major ulcer site. Smokers are particularly at risk of developing duodenal ulcers. They can also develop ulcers in the esophagus. Epidemiologic studies have shown that cigarette smoking is closely related to peptic ulcer disease. Experimental findings suggest that cigarette smoking increases xanthine oxidase activity, leukotrienes, and nitric oxide production and also neutrophil infiltration in the gastric mucosa. On the other hand, it reduces blood flow, prostaglandin production, epithelial cell proliferation, and formation of blood vessels in the tissue. These actions are important for ulcer formation and healing. The evidence strengthens the hypothesis that cigarette smoke is indeed harmful to gastric mucosa through defined mechanisms.[10]

Smoking may also interfere with the action of drugs that can decrease stomach acid production. The two most widely recommended classes of medications for this purpose are proton pump inhibitors (PPI) such as omeprazole, and H2 blockers such as ranitidine.

1.4.2. Effect of alcohol for the induction of ulcer

The association between ulcers and alcohol is complex. Light to moderate drinking is thought to be safe. In fact, some experts believe moderate drinking protects the stomach against *H. pylori*, the bacterium that causes most ulcers. Alcohol in copious quantities irritates the stomach lining, making it red and inflamed. Areas of bleeding may develop. This condition, known as gastritis. [11][12][13]

1.4.3. Ulcer and Spicy foods

The red pepper is popularly known as chili, a common spice. Persons with ulcers are advised either to limit or avoid its use. However, investigations carried out in recent years have revealed that chili or its active principle "capsaicin" is not the cause for ulcer formation but a benefactor. Capsaicin does not stimulate but inhibits acid secretion, stimulates alkali, mucus secretions and particularly gastric mucosal blood flow which help in prevention and healing of ulcers. Capsaicin acts by stimulating afferent neurons in the stomach and signals for protection against injury causing agents. Epidemiologic surveys in Singapore have shown that gastric ulcers are three times more common in the "Chinese" than among Malaysians and Indians who are in the habit of

consuming more chills. Ulcers are common among people who are in the habit of taking NSAIDS and are infected with the organism "Helicobacter Pylori," responsible for excessive acid secretion and erosion of the mucosal layer.[14]

1.4.4. Effect of caffeine for inducing ulcer

The role of caffeine in the production of gastric or duodenal ulcers remains uncertain.[15]

1.4.5. H. pylori bacteria for inducing ulcer

H. pylori is a common type of bacteria that usually infects the stomach. They may be present in more than half of all people in the world, according to the Mayo Clinic.*H. pylori* are adapted to live in the harsh, acidic environment of the stomach. These bacteria can change the environment around them and reduce its acidity. The shape of *H. pylori* allows them to penetrate stomach lining, where they're protected by mucus and body's immune cells are not able to kill them. This can lead to stomach problems.[16]

The bacteria are believed to cause stomach problems when they penetrate the stomach's mucous lining and generate substances that neutralize stomach acids. This makes the stomach cells more vulnerable to the harsh acids. Stomach acid and *H. pylori* together irritate the stomach lining which causes sores or peptic ulcers.

1.4.6. Effect of NSAIDs for inducing ulcer

NSAIDs can cause damage to the gastroduodenal mucosa via several mechanisms, including the topical irritant effect of these drugs on the epithelium, impairment of the barrier properties of the mucosa, suppression of gastric prostaglandin synthesis, reduction of gastric mucosal blood flow and interference with the repair of superficial injury. The presence of acid in the lumen of the stomach also contributes to the pathogenesis of NSAID-induced ulcers and bleeding, by impairing the restitution process, interfering with homeostasis and inactivating several growth factors that are important in mucosal defense and repair. [17]

1.4.7. Effect of stress for inducing ulcer

A stress ulcer is a single or multiple mucosal defect which can become complicated by upper gastrointestinal bleeding during the physiologic stress of serious illness. Ordinary peptic ulcers are found commonly in the gastric antrum and the duodenum whereas stress ulcers are found commonly in fundic mucosa and can be located anywhere within the stomach and proximal duodenum. Stress ulcers, as defined by overt bleeding and hemodynamic instability, decreased hemoglobin, and/or need for transfusion, were seen in 1.5% of patients in the 2252 patients in the Canadian Critical Care Trials group study. People with stress ulcers have a longer ICU length of stay (up to 8 days) and a higher mortality (up to 4 fold) than compared to patients who do not have stress ulceration and bleeding. While the bleeding and transfusions associated with the stress ulcerations contribute to the increased mortality, the contribution of factors like hypotension, sepsis, and respiratory failure to the mortality independently of the stress ulceration cannot be ignored.[18][19]

1.5. Treatment

Most ulcers can be treated and healed through proper treatment. Often, people with ulcers will have to take PPIs for several weeks to heal an ulcer. It is also important to find out the cause of the ulcer. The use of NSAIDs should be restricted. If the person is infected with *H. pylori*, then completing the full dose of antibiotics is very important.

1.5.1. Use of PPI (Proton-pump inhibitors)

These include:

- Esomeprazole
- Lansoprazole
- Omeprazole
- Pantoprazole
- Rabeprazole

1.5.1.1. Mechanism of action of PPI

The proton pump inhibitor is a selective and irreversible inhibitor. It suppresses stomach acid secretion by specific inhibition of the H^+/K^+-ATPase system found at the secretory surface of gastric parietal cells. As this enzyme system is regarded as the acid (proton, or H^+) pump within the gastric mucosa, the PPI inhibits the final step of acid production. The PPI also inhibits both basal and stimulated acid secretion irrespective of the stimulus. The inhibitory effect of PPI occurs within 1 hour after oral administration. The maximum effect occurs within 2 hours. The duration of inhibition is up to 72 hours. When PPI is stopped, baseline stomach acid secretory activity returns after 3 to 5 days. The inhibitory effect of the PPI on acid secretion will plateau after 4 days of repeated daily dosing.[20]

Omeprazole

Omeprazole is indicated for gastroesophageal reflux disease including reflux esophagitis, acid reflux disease, duodenal and benign gastric ulcers, and Helicobacter pylori eradication regimens in peptic ulcer disease, prophylaxis of acid aspiration, Zollinger-Ellison Syndrome (ZES) and for the treatment of NSAID-associated gastric ulcers, duodenal ulcers or gastroduodenal erosions. IV is indicated primarily for the treatment of Zollinger-Ellison syndrome, and may also be used for the treatment of gastric ulcer, duodenal ulcer and reflux esophagitis.[21]

Esomeprazole

Esomeprazole is a proton-pump inhibitor which reduces stomach acid. It is used in the treatment of dyspepsia, peptic ulcer disease, gastroesophageal reflux disease, and Zollinger-Ellison syndrome.It decreases secretion of acid through inhibition of the H^+/K^+-ATPase in the parietal cells of the stomach. By inhibiting the functioning of this transporter, the drug prevents formation of stomach acid. Esomeprazole is the (*S*)-(−)-enantiomer of omeprazole. Esomeprazole is currently sold over the counter in the US, the UK and Australia.[22]

Indication: Treatment of Gastroesophageal Reflux Disease (GERD), Healing of erosive esophagitis, Maintenance of healing of erosive esophagitis, Symptomatic Gastroesophageal Reflux Disease, Risk Reduction of NSAID-associated gastric ulcer, & H. pylori eradication (Triple therapy). [23]

Rabeprazole

Indication: Short-term treatment in healing and symptomatic relief of duodenal ulcers and erosive or ulcerative Gastroesophageal Reflux Disease (GERD). Maintaining healing and reducing relapse rates of heartburn symptoms in patients with GERD. Treatment of daytime and night time heartburn and other symptoms associated with GERD. Long-term treatment of pathological hyper secretory conditions, including Zollinger- Ellison Syndrome. In combination with Amoxicillin and Clarithromycin to eradicate Helicobacter pylori.[24]

Pantoprazole

Pantoprazole belongs to a class of drugs called proton pump inhibitors. It works to shut off the acid-pumping cells in your stomach. It reduces the amount of stomach acid and helps to reduce painful symptoms related to gastroesophageal reflux disease (GERD).[25]

Indication: Benign gastric ulcer, duodenal ulcer, gastroesophageal reflux disease (GERD), NSAID-induced peptic ulcer, acid hypersecretory conditions including Zollinger-Ellison Syndrome, eradication of Helicobacter pylori (in combination with Antibiotics), ulcer resistant to H2 receptor antagonists.[26]

Lansoprazole

Lansoprazole belongs to a group of drugs called proton pump inhibitors. It decreases the amount of acid produced in the stomach. Lansoprazole is used to treat and prevent stomach and intestinal ulcers, erosive esophagitis (damage to the esophagus from stomach acid), and other conditions involving excessive stomach acid such as Zollinger-Ellison syndrome.Over-the-counter lansoprazole (Prevacid OTC) is used to treat frequent heartburn that happens 2 or more days per week.[27]

Indication: Duodenal ulcer, gastric ulcers, erosive esophagitis, Zollinger-Ellison Syndrome, H. pylori eradication.

1.5.2. Use of H2 receptor blocker

Histamine H2-receptor antagonists, also known as H2-blockers, are used to treat duodenal ulcers and prevent their return. They are also used to treat gastric ulcers and for some conditions, such as Zollinger-Ellison disease, in which the stomach produces too much acid. In over-the-counter (OTC) strengths, these medicines are used to relieve and/or prevent heartburn, acid indigestion, and sour stomach. H2-blockers may also be used for other conditions as determined by your doctor.H2-blockers work by decreasing the amount of acid produced by the stomach.H2-blockers are available both over-the-counter (OTC) and with your doctor's prescription.[28]

1.5.2.1. Mechanism of Action of H2 receptor blocker

The H_2 antagonists are competitive antagonists of histamine at the parietal cell's H_2 receptor. They suppress the normal secretion of acid by parietal cells and the meal-stimulated secretion of acid. They accomplish this by two mechanisms: Histamine released by ECL cells in the stomach is blocked from binding on parietal cell H_2 receptors, which stimulate acid secretion; therefore, other substances that promote acid secretion (such as gastrin and acetylcholine) have a reduced effect on parietal cells when the H_2 receptors are blocked.[29]

Ranitidine

Ranitidine is in a group of drugs called histamine-2 blockers. Ranitidine works by reducing the amount of acid your stomach produces. Ranitidine is used to treat and prevent ulcers in the stomach and intestines. It also treats conditions in which the stomach produces too much acid, such as Zollinger-Ellison syndrome. Ranitidine also treats gastroesophageal reflux disease (GERD) and other conditions in which acid backs up from the stomach into the esophagus, causing heartburn.[30]

Cimetidine

Cimetidine is in a group of drugs called histamine receptor antagonists. Cimetidine works by decreasing the amount of acid. Cimetidine is used to treat and prevent certain types of ulcer, and to treat conditions that cause the stomach to produce too much acid. Cimetidine is also used to

treat gastroesophageal reflux disease (GERD), when stomach acid backs up into the esophagus and causes heartburn.[31]

Famotidine

Famotidine a histamine-2 blockers. Famotidine works by decreasing the amount of acid the stomach produces. Famotidine is used to treat and prevent ulcers in the stomach and intestines. It also treats conditions in which the stomach produces too much acid, such as Zollinger-Ellison syndrome. Famotidine also treats gastroesophageal reflux disease(GERD) and other conditions in which acid backs up from the stomach into the esophagus, causing heartburn. Famotidine may also be used for purposes not listed in this medication guide.[32]

Nizatidine

Nizatidine is a histamine-2 blocker that works by decreasing the amount of acid produced by the stomach. Nizatidine is used to treat ulcers in the stomach and intestines. Nizatidine also treats heartburn and erosive esophagitis caused by gastroesophageal reflux disease (GERD), a condition in which acid backs up from the stomach into the esophagus.[33]

1.5.3. Antacids

Antacids are a class of medicines that neutralize acid in the stomach. They contain ingredients such as aluminum, calcium, or magnesium which act as bases (alkalis) to counteract the stomach acid and lower pH. They work quickly and are used to relieve symptoms of acid reflux, heartburn or indigestion (dyspepsia).[34]

Antacids are available as liquids or tablets. Some mixtures contain sodium which may not be suitable for people on a sodium restricted diet. Some products combine antacids with alginates and they are used in the treatment of Gastroesophageal Reflux Disease (GERD). Alginates are gum-like substances that float on top of the stomach contents, forming a raft that acts like a barrier, preventing gastric acid from escaping back up the esophagus. Antacids are usually only used short-term or occasionally.[35]

1.5.3.1. Types of Antacid

- Aluminum hydroxide and Magnesium carbonate
- Aluminum hydroxide and Magnesium hydroxide
- Calcium carbonate

1.5.4. Simethicone

The medication is in the antiflatulent class of drugs. It works by changing the surface tension of gas bubbles in the stomach and intestines. This causes them to combine into larger bubbles that can be passed more easily. Simethicone is used to relieve the symptoms of excessive gas in the gastrointestinal tract, namely bloating, burping, and flatulence.[36]

1.6. Prevention of Ulcer

The best way to cure stomach ulcers is to treat the cause, not the symptoms. Many medicines and remedies are supposedly able to do this, but cannot guarantee any results. There is a possibility that there is a natural treatment is proven to be more effective and safer.

1.6.1. Use of natural products

The following natural products help to cure ulcer.

Flavonoids

Research suggests that flavonoids, also known as bioflavonoids, may be an effective additional treatment for stomach ulcers. Flavonoids are compounds that occur naturally in many fruits and vegetables. Foods and drinks those are rich in flavonoids include:

- Soybeans
- Legumes
- Red grapes
- Kale
- Broccoli

- Apples
- Berries
- Teas, especially green tea

These foods may also help the body fighting against the *H. pylori* bacteria. Flavonoids are referred to as "gastroprotective," which means they defend the lining of the stomach and could allow ulcers to heal. According to the Linus Pauling Institute, there are no side effects of consuming flavonoids in the amount found in a typical diet, but higher amounts of flavonoids may interfere with blood clotting.[37]

Deglycyrrhizinated licorice

Deglycyrrhizinated licorice is just plain old licorice with the sweet flavor extracted. One study showed that deglycyrrhizinated licorice might help ulcers to heal by inhibiting the growth of *H. pylori*.[38]

Deglycyrrhizinated licorice is available as a supplement. Too much licorice candy can be bad for some people. Consuming more than 2 ounces daily for more than two weeks can make existing heart problems or high blood pressure worse.

Probiotics

Probiotics are the living bacteria and yeast that provide healthy and important microorganisms to the digestive tract. They are present in many common foods, particularly fermented foods. These include:

- buttermilk
- yogurt
- miso
- kimchi
- kefir

Studies have shown that probiotics may be helpful in wiping out *H. pylori* and in increasing recovery rate for people with ulcers when added to the traditional regimen of antibiotics.

Honey

Honey shows a significant anti-ulcer activity. Depending on the plant it's derived from, honey can contain up to 200 elements, including polyphenols and other antioxidants. Honey is a powerful antibacterial and has been shown to inhibit *H. pylori* growth.[38]

Garlic

Garlic extract has been shown to inhibit *H. pylori* growth in lab, animal, and human trials. Garlic acts as a blood thinner, so a ulcer patient should ask doctor before taking it if he uses warfarin, other prescription blood thinners, or aspirin.

Cranberry

Cranberry has been shown to be helpful in decreasing urinary tract infections by preventing bacteria from settling on the walls of the bladder. Cranberry and cranberry extract also may help to fight *H. pylori*. No specific amount of consumption is associated with relief. Too much cranberry in any form may cause stomach and intestinal discomfort due to its high sugar content.[39]

Mastic

Mastic is the sap of a tree which is grown in the Mediterranean. Studies of the effectiveness of mastic on *H. pylori* infection are mixed, but at least one small study shows that chewing mastic gum may help fight *H. pylori*, getting rid of the bacteria in about 3 out of 10 people who used it. However, when compared to the traditional combination of antibiotics and acid-blocking medications, the medications were significantly more effective than the gum. The traditional treatment got rid of the bacteria in more than 75 percent of the people studied. In this study, the mastic gum was not associated with any side effects.[40]

Food, Vegetables and Whole grain

A diet centered on fruits, vegetables, and whole grains is not just good for the overall health. According to the scientific research, a vitamin-rich diet can help our body to heal ulcer. Foods containing the antioxidant polyphenols may protect us from ulcers and help ulcers to heal. Polyphenol-rich foods and seasonings include:

- dried rosemary
- flaxseed
- Mexican oregano
- dark chocolate
- blueberries, raspberries, strawberries, elderberries, and blackberries
- black olives[41][42]

1.6.2. Change of lifestyles

- **Eat a balanced diet:** Choosing a variety of foods from each food group, especially fibre-rich fruits, vegetables and whole grains. Eating yogurt is also helpful.
- **Fill-up on fibre:** Fibre may play a role in preventing the formation or recurrence of ulcers. Foods high in soluble fibre, like- oats, apples and legumes, seem to be the most effective. Vitamin A-rich foods such as carrots, leafy greens and butternut may also be protective.
- **Eat small, more frequent meals:** Plan for four to five small meals per day instead of two to three large meals. Plus, avoid eating just before going to sleep as this delays gastric emptying and exacerbates symptoms.
- **Chew more:** Digestion starts in our mouth. Chewing thoroughly helps with the needed breakdown of nutrients.
- **Get some exercise:** Even stretching and walking promote good digestive health.
- **Make lifestyle changes:** Limiting stress aids in decreasing the over-production of stomach acid, while daily exercise and drinking sufficient water (six to eight glasses) can assist with gastric emptying. Quit smoking immediately as it increases ulcer perforation.

- **Away with alcohol:** Excessive use of alcohol may cause irritation and erosion to the stomach lining.
- **Embrace protective agents:** Studies suggest that green tea, broccoli, blackcurrant oil, cranberries, ginger and kimchi (fermented cabbage) are "protective" foods which may help with the eradication of *H.*
- **Introduce fish oils:** Supplementing our diet with omega-3 fatty acids may help too.[43][44][45]

1.7. Plant profile

1.7.1. *Oroxylum indicum*

Sonapatha or *Oroxylum indicum* belongs to Family Bignoniaceae and it is characterized by brown bark and large pinnate leaves. It is a medium sized, deciduous tree, distributed in India, Sri Lanka, Malaysia, China, Thailand, Philippines, and Indonesia. In India, Oroxylum is found in Eastern and Western Ghats and also in the North-East regions [46]. The existence of *Oroxylum indicum* (L) Vent. in natural population is highly threatened and it has been categorized as endangered medicinal plant by the Government of India. Various parts of *Oroxylum indicum* are utilized for medicinal purposes [47]. It has been used in Ayurveda and other traditional medicinal health systems since centuries [48]. The decoction of the bark is used to cure gastric ulcers and the bark paste is useful in treating mouth cancer, scabies, and other skin diseases. The bark paste is applied to the wounds of animals to kill maggots. Poultice of the bark is topically applied to treat rheumatism, sprains, inflammations, and skin diseases [59]. The bark decoction of Oroxylum is also a useful remedy to deworm cattle [50]. Apart from this, Oroxylum species are reported to have a variety of medicinal properties like anticancer, antiulcer, anti-dysenteric, antimicrobial, and anti-inflammatory [51]. It has been shown to be antibacterial, antioxidant, hepatoprotective, and immune modulatory[52]. From the above it is clear that the systematic evaluation of anti-inflammatory and analgesic activities of *Oroxylum indicum* is lacking, which stimulated us to obtain an insight into the anti-inflammatory and analgesic activities of *Oroxylum indicum* in Wister albino mice.

1.7.2. Scientific name

O. indicum Vent. (L) (Syn. name- *Bignonia indica, Spathodeaindica, Calosanthesindica, Hippoxylonindica, Bignonia quadripinnata*)

1.7.3. Botanical Description

Oroxylum indicum is a small to medium sized deciduous tree measuring upto 12 metres in height with light greyish brown, soft, spongy bark having corky lenticels, large pinnate, bipinnate or tripinnate ovate or elliptic leaves; lurid purple, fleshy, foetid flowers and large, flat, sword shaped capsules full of many flat and papery thin seeds with broad silvery wings. Leaves are large up to 1 – 5 m long, pinnate, bipinnate or tripinnate, leaflets are ovate or elliptic. They form enormous seed pods that hang down from bare branches. Those long fruits curve downward and resemble the wings of a large bird or dangling sickles or swords in the night. The fresh root bark is soft and juicy and ceramic yellow to greyish in colour. The taste is sweet initially later becoming bitter. On drying, the bark shrinks, adheres closely to the wood and becomes faintly fissured9. Flowers are many large, purple and fleshy with perfect five stamens. Fruits are Capsule, large, flat, sword shaped, up to 90 cm x 9 cm valves woody. Seeds are many, flat, thin with broad silvery wing. The seeds are round with papery wings. Flowering starts in the cold season, from January to March and fruits are developed in April to July. The plant is also called as broken bones tree as when the long leaf and flower bearing stalks dry and fall from the tree, their accumulation beneath the tree resembles a pile of broken bones. The tree is a night bloomer and flowers are adapted to natural pollination by bats [53].

1.7.4. Taxonomical Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Lamiales
Family: Bignoniaceae
Genus: *Oroxylum*
Species: *indicum*[54]

Synonyms

*Bignonia indica*L.

Calosanthesindica Blume.

1.7.5. Vernacular Names

English: Broken bones plant, Indian calosanthes, Indian trumpet flower, Midnight horror, Tree of Damocles

Chinese: Handy pinyin, Mud huddle

Bengali: Sona, Khonha, Paharijora, Kani-Dingi, Hanghoal, Aklong-Singh, ThonaGach, NaoriChilana (Chaknma Tribe), Krong-Sa-Bang (Marma Tribe), Tou-Kharung Tripura Tribe), Kaak-Rakung (Tribe Halam), Kanai Dingi (Garo Tribe)

Hindi: Bhut-Vriksha, Dirghavrinta, Kutannat, Manduk (The Flower), Patrorna, Putivriksha, Shallaka, Shuran, Or Son, Vatuk

Kannada: Tattuna

Konkani: Davamadak

Nepalese: Tatel

Malayalam: Palaqapayyani, Vashrppathiri, Vellappathiri

Marathi: Tayitu, Tetu

Sanskrit: Aralu, Shyonaka

Sri Lanka: Totila, Thotila

Tamil: Cori-Konnai, Palai-Y-Utaicci, Puta-Puspam (The Flower)

Telugu: Manduka-Parnamu, Pampena, Suka-Nasamu[55-57].

1.7.6. Distribution

O. indicum is a deciduous tree growing throughout India, South Asia, South East Asia, Sri Lanka, Philippines, Indonesia, China, Bhutan, Malaysia and Mallaca. It is found up to an altitude of 1200 m mainly in ravines, in damp region and moist places in the forests [55]. In India, it is distributed in Himalayan foothills, Eastern and Western Ghats and North East India [58]. It is mostly sighted along the river banks or slopes of the hills. *O. indicum* lives in relationship with the actinomycete *Pseudonocardiaoroxyli* present in the soil surrounding the roots [59].

1.7.7. Ethnobotany

The tree is often grown as an ornamental tree for its strange appearance. The sword like fruit or a branch traditional Indian Ayurvedic medicine, included in famous tonic formulations such as *Chyawanprash*. Bark decoction is taken for curing gastric ulcer and a paste made of the bark powder is applied for mouth cancer, scabies and other skin diseases. The medicated oil of *O. indicum* in sesame oil base instilled into ears mitigates the pain in otitis [55, 56].

1.7.8. Uses

The tree is often grown as an ornamental for its strange appearance. Materials used include the wood, tannins and dyestuffs. It is also a plant with edible leaves and stems. Young shoots and unripe fruits are eaten as vegetables. The tree is also frequently lopped for fodder [60]. Wood of the tree is used to make match boxes. Stem bark and fruits of the tree are used as mordant and yield colour dye [61].

The roots are sweet, astringent, bitter, acrid, refrigerant, expectorant, digestive, carminative, febrifuge, diuretic, antimicrobial, antifungal, anti-inflammatory and tonic. They are useful for vata and kapha, dropsy, flatulence, colic, diarrhea and dysentery. Stem bark paste is applied for the cure of scabies and to treat arthritis. Leaf decoction is given in treating stomachache, ulcers, rheumatism pain and enlarged spleen. Mature fruits are useful in treating cough, bronchitis, piles, jaundice dyspepsia, smallpox, colic, leucoderma, pharngodymia, cardiac disorders, gastropathy, hemorrhoids and cholera. Seeds are used as purgative. Dried seed powder used by women to induce conception. Seeds yield non-drying oil used in perfume industry. Stem bark and fruits are employed as mordent, the stem bark yields a khaki color dye. The decoction of the roots is commonly used for arthritis. Used externally as a paste of its skin of roots, it dries up the discharges and promotes the wound healing.

1.7.9. Ayurvedic preparations

O. indicum is used as one of the important ingredient in most commonly used Ayurvedic preparations such as *Dasamularistha, Syonakaputapaka, Syonakasiddaghrta, Brhatpancamulyadikvatha, Amartarista, Dantyadyarista, NarayanaTaila, DhanawantaraGhrita, Brahma Rasayana* and *Chyavanapra*[62,63].

1.8. Literature review

1.8.1. Chemistry

O. indicum leaves are known to contain flavones and their glycosides, baicalein (5,6,7-trihydroxy flavone) and its 6 and 7-glucuronides, chrysin (5,7-dihydroxy flavone) [64], scutellarein and its 7-glucuronides,anthraquinone and aloe-emodin[58,65,66] chrysin-7-Oglucuronide, chrysin-diglucoside and irridoids[67]. Ethyl acetate extract of leaves of *O. indicum* was separated using high speed counter-current chromatography to get chrysin (160.9 mg, 97.3% purity), baicalein (130.4 mg, 97.6% purity), baicalein 7-O-glucoside (314.0 mg, 98.3% purity), baicalein-7-O-diglucoside (179.1 mg, 99.2% purity) [68]. From methanol extract of the leaves of *O. indicum*, chrysin-7-O-glucuronide, chrysindiglucoside and baicalein were separated. Structure of the chrysin-diglucoside has yet to be obtained [67]. Chloroform extraction of defatted leaves gives gummy solid yielding anthraquinone and aloe-emodine[68].Stem bark contain flavones oroxylin A (5,7-dihydroxy- 6-methoxy flavone), chrysin, baicalein and its 6 and 7-glucuronide, scutellarin-7-rutinoside, traces of alkaloid [58,69], tannic acid, sitosterol and galactose, baicalein, biochanin-A, ellagic acid [59] .Ethyl acetate extract of root of *O. indicum* is reported to contain two flavonoids- i) 2,5-dihydroxy-6,7-dimethoxy flavone and ii) 3,7,3′,5′,-tetramethoxy-2-hydroxy flavone. Flavonoid (i) has R_f value 0.621 in solvent system (petroleum ether: ethyl acetate, 3:1). This flavonoid was separated as fine crystals with M.P. 195-198o. Flavonoid (ii) has R_f value 0.721 in solvent system (petroleum ether:ethyl acetate, 3:1) and this flavonoid was separated as needle shaped crystals with M.P. 210-211o [70]. Root bark contain chrysin, scutellarin-7-rutinoside, weak acids, traces of alkaloids [69], sitosterol, galactose, baicalein, biochanin-A, ellagic acid, oroxylin-A [58] and a yellow crystalline coloring matter 5,7-dihydroxy-6-methoxy flavone [62]. Heartwood contains prunetin, sitosterol. Methanol extract of the fruits pods is reported to contain oroxylinA, chrysin, baicalein, a triterpene carboxylic acid and ursolicacid [71]. Seeds contain oils and flavonoids such as chrysin, oroxylinA, baicalein, baicalein-7-O-diglucoside (Oroxylin B), baicalein-7-Oglucoside, apigenin[72],terpenes, alkaloids, saponins[73],tetuin, the 6-glucoside of baicalein, benzoic acid and fatty acids [74]. A new flavone glucuronide-oroxindin and chrysin-7-O-diglucoside were also isolated. The seed oil contains caprylic, lauric, myristic, palmitic, palmotoleic, stearic, oleic and linoleic acids. Seeds also contain twenty percent shiny oil. Ether fraction of *O. indicum* gave scutellarein[75]. Aqueous

mother liquor gave scutellarein and baicalein[75].Baicalein was found to be major flavonoid present in petroleum ether extract [62].

1.8.2. Toxicological studies

A research work conducted to investigate the toxicological activities of the medicinal plant *Oroxylum indicum* has shown that the root and stem extracts of the plant are toxic for the brine shrimp nauplii[90].

Bioactivity studies of the *Oroxylum indicum* revealed that *Oroxylum indicum* exhibited moderate toxicity to the growth of brine shrimp (BST) naupli and wheat rootlet growth (WRG), but was not toxic to the lettuce seed germination (LSG) [90].

Shrikant V. Joshi, et al, 2011, while evaluating the protective effect of aqueous extract of *Oroxylum indicum* root bark against DNBS-induced colitis in rats, performed the acute oral toxicity study on long evans rats by feeding the overnight fasting rats with doses ranging from 175 mg/kg bw to 5000 mg/kg BW and found that the aqueous extract did not cause any mortality in the rats [90].

Similarly, Ashpak M. Tamboli, et al., determined the hypoglycemic activity of extracts of *Oroxylum indicum* Vent roots in animal models. They, while performing the acute toxicity tests, found that both the ethanolic and aqueous extracts were safe upto a dose of 5 g/kg BW a day [140].

Bichitra Nandy Tripathi, et al., 2011 studied the toxicity to find out the lethal dose of ethanolic extract of *Oroxylum indicum*in adult mice. *Oroxylum indicum* stem aqueous and ethanolic extract were administered orally in mice with graded doses (5 mg-3000 mg/kg BW) and mortality was observed for a period of 72 hours. The administration of aqueous extract did not produce any acute toxic symptoms (100% survival) at doses upto 2000 mg/kg BW .

Oroxylum indicum is being used as medicinal herb for thousands of years without any known adverse effects. There have been number of scientific studies conducted to evaluate the toxic effects of the plant. Almost all the studies conducted on *Oroxylum indicum* have shown that *Oroxylum indicum* is not toxic to humans and experimental animals even upto high doses.

1.9. Pharmacological Properties

Several workers have reported different biochemical activities of *O. indicum* in various *in vivo* and *in vitro* test models. Different part of this plant have been found to exhibit anti-

inflammatory, antimicrobial, antioxidant, anticancer, antimutagenic, photocytotoxic, antiarthritic, immunostimulant, hepatoprotective, antiproliferative and hepatoprotective activities.

1.9.1. Anti-inflammatory activity

The anti-inflammatory activity was evaluated by carageenan induced rat paw edema model in rats using diclofenac sodium as standard drug. Two doses 150 mg/kg and 300 mg/kg of aqueous extract of *O. indicum* were used. Result showed that paw volume was significantly reduced in dose dependent manner as compared to control. Extract at a dose of 300 mg/kg showed maximum anti-inflammatory activity. However, the activity produced by both the doses was less than the reference standard. Extract at both doses showed significant ($P<0.05$) anti-inflammatory activity at 5 h suggesting that the extract predominantly inhibit the release of prostaglandin like substances [76].

1.9.2. Anti-ulcer activity

The 50% alcohol extract of root bark of *O. indicum* and its petroleum ether, chloroform, ethyl acetate and n-butanol fractions were studied against ethanol induced gastric mucosal damage. The alcohol extract (300 mg/kg, p.o.) and its different fractions (100 and 300 mg/kg, p.o.) showed reduction in gastric ulceration. The petroleum ether and n-butanol fractions showed maximum inhibition of gastric lesions against ethanol-induced gastric mucosal damage. The results were comparable with omeprazole (reference standard). In the ethanol induced gastric ulcer model, treatment with both the active fractions and omeprazole showed significant antioxidant activity as evident from the reduction in the extent of lipid peroxidation. The effect of active fraction of root bark on the ulcer index, total acidity, total acid output, pepsin activity, pepsin output and total carbohydrate to protein ratio in pyloric-ligated rat was studied. The active fraction of root bark at a dose level of 100 mg/kg p.o. showed significant reduction ($P<0.05$) in the ulcer index, total acidity, total acid output, pepsin activity and pepsin output along with a significant rise in total carbohydrate to protein ratio. The mechanism of antiulcer activity could be attributed to a decrease in gastric acid secretory and antioxidant activities leading to gastric cytoprotection. This activity could be linked to the presence of baicalein in the root bark of the plant [77].

1.9.3. Antimicrobial activity

The antimicrobial activity of crude extract of *O. indicum* (petroleum ether, ethyl acetate and methanol), compound 1 (2,5-dihydroxy-6,7-dimethoxy flavone) and compound 2 (3,7,3′,5′-tetramethoxy- 2-hydroxy flavone) was tested against fourteen pathogenic bacteria (5 Gram-positive and 9 Gramnegative) and seven pathogenic fungi. Nutrient agar and nutrient broth were used as bacteriological media and potato dextrose agar (PDA) was used for fungal growth. In antibacterial screening, each sample was dissolved in methanol at a concentration of 200 µg/10 µl. The activity of these samples was compared with standard kanamycin disc (K-30 µg/ disc) using the standard disc diffusion method. Similarly antifungal screening was done at a concentration of 300 µg/disc for each sample and the activity was compared with the standard clotrimazole disc (K-30 µg/disc). From the antibacterial and antifungal experimental results, it was evident that the crude extracts (petroleum ether, ethyl acetate) and the compound 1 and 2 showed significant antibacterial and antifungal activity but were less potent than that of standard kanamycin and clotrimazole, where as methanol extract showed little activity. The findings support the use of *O. indicum* in traditional medicine for the treatment of bacterial and fungal infection [70,78].

1.9.4. Antioxidant activity

The antioxidant activity of ethanol and aqueous extract of *O. indicum* leaves was studied in two *in vitro* models viz. radical scavenging activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) reduction and nitric oxide radical scavenging activity in Griess reagent system. Ethanol extract possessed significant antioxidant activity in both the models. In scavenging DPPH radical, extracts activity was IC_{50}=24.22 µg/ ml while in scavenging nitric oxide (NO) radical, the activity was IC_{50}=129.81 µg/ml. The result showed that ethanol extract of *O. indicum* leaves possesses free radical scavenging activity [79].

1.9.5. Anticancer activity

Methanol extract of the fruits of *O. indicum* inhibited *in vitro* proliferation of HL-60 cells. The flavonoid baicalein was found as an active component in the extract. The *in vitro* effects of baicalein on the viability and induction of apoptosis in the HL-60 cell line was further

investigated. The cell viability after treating with baicalein for 24 h was quantified by counting viable cells using tryptan blue staining. The result showed that baicalein caused a 50% inhibition of HL-60 cells at concentration of 25-30 μM. The inhibition of proliferation of HL-60 cells due to 36-48 h exposure with 10 or 20 μM baicalein was associated with the accumulation of cells at S or G2M phases. The results indicated that baicalein has antitumors effects on human cancer cells [71].

1.9.6. Anti-mutagenic activity

Methanol extract of *O. indicum* strongly inhibited the mutagenicity of Trp-P-1 in an Ames pre-incubation method in the presence of S9 mix using *Salmonella typhimurium*. Only 5 μl of the crude extract inhibited 91±5% of the mutagenesis induced by 50 ng of Trp-P-1. The major anti-mutagenic constituent was identified as baicalein with an IC_{50} value of 2.78±0.15 μM. The potent anti-mutagenicity of the extract was correlated with the high content (3.95±0.43%, dry weight) of baicalein. Baicalein act as desmutagen since it inhibits N-hydroxylation of Trp-P-2. The anti-mutagenic effect of baicalein was found mainly due to the inhibition of N-hydroxylation catalyzed by P450 monooxygenases in S-9 [80].

1.9.7. Photocyotoxic activity

The photocytotoxic activity of methanol extract of leaves of *O. indicum* was studied against promyelocytic-leukemia cell line, HL60. The HL60 was incubated with 21 μg/ml of crude extracts for 2 h and irradiated with 9.6 J/cm^2 of a broad spectrum light source in four replicates. Survival of cells was assessed 24 h later following the colorimetric MTT protocol. Pheophorbide-a, a commercially available and well-characterized photosensitizer was used as the positive control. To determine samples that have general cytotoxicity, a parallel assay without irradiation was also carried out. The result showed that methanol extract of leaves of *O. indicum* have photocytotoxic activity at concentration 21 μg/ml [81].

1.9.8. Anti-arthritic activity

Aqueous and ethanol extract of *O. indicum* were tested for *in vitro* release of myeloperoxidase (MPO) from rat peritoneal leukocytes. The results indicated that aqueous extract had a significant effect i.e. 64% inhibition of release of MPO [82].

1.9.9. Immuno-stimulant activity

n-Butanol extract of root bark of *O. indicum* (100 mg/kg, once daily for 22 days) was studied for immunomodulatory activity in rats using measures of immune responses to sheep red blood cells (SRBC haemagglutinating antibody [HA] titer) and delayedtype hypersensitivity (DTH) reactions. In response to SRBC, treatment with the *n*-butanol fraction caused a significant rise in circulating HA titers during secondary antibody responses, indicating a potentiation of certain aspects of the humoral response. The treatment also resulted in a significant rise in paw edema formation, indicating increased host DTH response. Histopathologic analysis of lymphoid tissues in the treatment group showed an increase in cellularity, e.g., T-lymphocytes and sinusoids. In contrast, dexamethasone treatment caused significant reduction in the HA titer, DTH responses, and antioxidant activity. In a triple antigen-mediated immunological edema model, the extent of edema raised in drug-treated rats was greater compared to that in control rats, thus confirming enhanced DTH reactions in response to the drug treatment. Activity of the *O. indicum* might be attributed to its ability to enhance specific immune response (both humoral and cell-mediated) [83].

1.9.10. Anti-proliferative activity

The anti-prolifirative activity of *O. indicum* was studied on human breast tumor cell lines. Results indicated that *O. indicum* have anti-proliferative activity against MCF7 and MDA-MB-231 breast cancer cell lines [83].

1.9.11. Hepato-protective activity

The hepatoprotective activity of *O. indicum* was studied against carbon tetrachloride (CCl_4)-induced hepatotoxicity in mice and rats. Biochemical study indicated that alcoholic (300 mg/kg), petroleum ether (300 mg/kg) and n-butanol (100 and 300 mg/kg) extracts significantly ($P<0.05$) lowered the elevated serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin (TB) levels as compared to the control group. The increased lipid peroxide (LPO) formation, reduced glutathione (GSH) and decreased antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT) in the tissues of CCl_4-treated animals were significantly normalized by *O. indicum* treatment. Histopathological study also revealed that pretreatment with *O. indicum* restored CCl_4-induced alteration in antioxidant status of the tissues. It is suggested that root bark showed significant antioxidant activity, which might be in turn responsible for its hepatoprotective activity [84].

Chapter- 2

Methods and Materials

2.1. Raw materials

The standard drug omeprazole was collected from Eskayef Bangladesh Limited and the bark of *Oroxylum indicum* was collected from Kapashia, Gazipur, Dhaka, Bangladesh which was after authenticated from Bangladesh National Herbarium, Dhaka.

2.2. Selection of animal

A total number of thity male long evans rats weighing between 80 to 92gm, age 3weeks were purchased from ICDDRB (International Center for Diarrheal Disease Research Center). Prior to commencement of the experiments, all the rats were acclimatized to the new environmental condition for a period of three days. They were housed in polypropylene cages in groups of 4 rats per cage and were kept in a room maintained at 25 ± 2◦C.They were supplied with standard pellets from ICDDRB (International Center for Diarrheal Disease Research Center) and fresh drinking water. All the rats were kept in cages and maintained with natural 12 hour light and dark cycle. They were fasted over night before the experiment. All surgery was performed under chloroform anesthesia, and all efforts were made to minimize suffering. Animal care and research protocols were based on principles and guidelines approved by the Guide for the Care and Use of Laboratory Animals (NIH publication No: 85-23, revised in 1985).

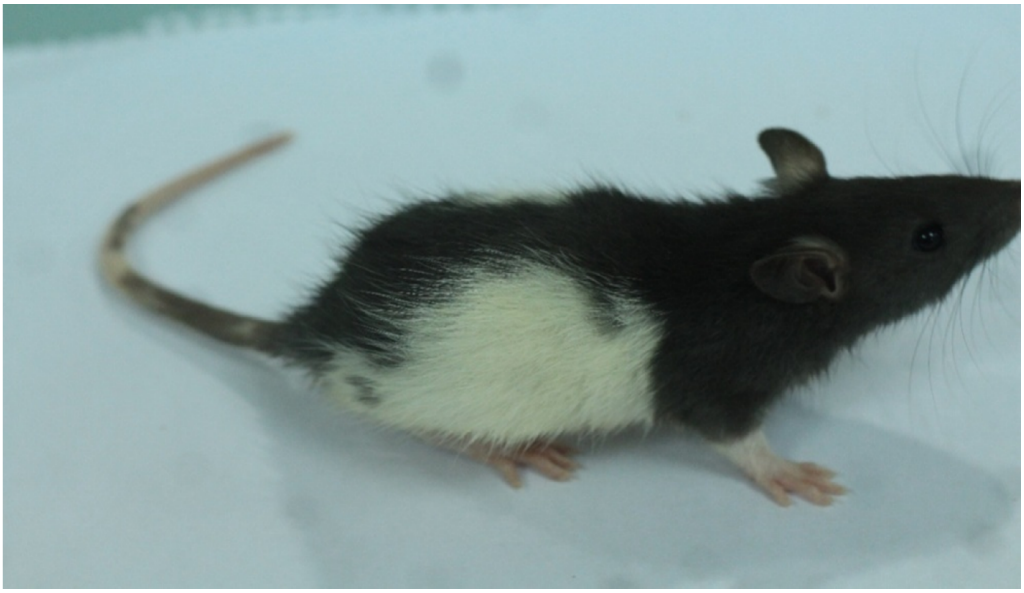


Fig 2.1: Long Evans Rat

Table 2.1: Apparatus used in the extraction of *O.indicum*

Extraction
Electronic balance
Beaker
Conical flask
Funnel
Filter paper
Aluminium foil
Glass rod

Table 2.2: Apparatus used in the phytoscreening of *O.indicum*

Phytochemical Screening
Test tube
Dropper
Pipette
Water Bath
Electric balance
Spatula
Pipette filler

Table 2.3: Apparatus used in Anti-Ulcer test

Anti-Ulcer test
Beaker
Electronic balance
Slides
Feeding needle
Gloves
5ml disposable syringe
Dissection box
Measuring cylinder
Pipette
Pipette filler
Volumetric flask
Dissection board

Table 2.4: Chemicals used in the phytoscreening of *O.indicum*

Chemicals
Ethanolic alpha-naphthalene
Concentrated sulphuric acid
Benedict's solution
Olive oil
Distilled water
Mayer's reagent
Hager's reagent
Chloroform
Acetic acid
Lead acetate
Ferric chloride
Benzene

2.3. Methods

2.3.1. Collection and identification of plant

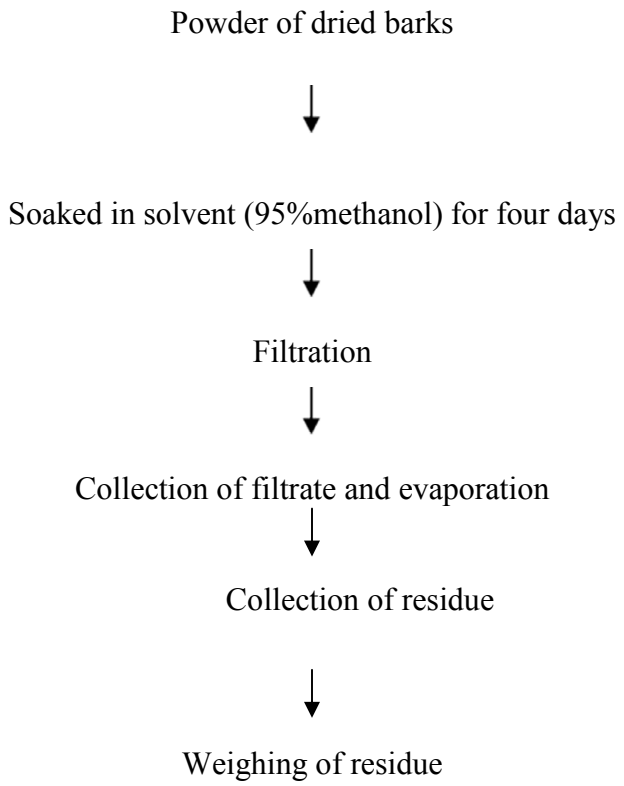
The fresh bark of *Oroxylum indicum* were collected in the month of January-February 2016 from Gazipur Dhaka and its identity was authenticated from the Bangladesh National Herbarium, Dhaka where a voucher specimen was deposited having different accession number which is given here in parenthesis: *Oroxylum indicum(43471).*Bark of *Oroxylum indicum* were used for the experiments.

2.3.2. Preparation of plant material for extraction

The not infected and matured stem bark of *Oroxylum indicum* (Family: Bignoniaceae) was collected from Kapashia, Gazipur,Dhakaduring the month of January. The plant was identified by the Bangladesh National Herbarium, Dhaka where a voucher specimen was deposited having different accession number which is given here in parenthesis: *Oroxylum indicum.* The bark of *O. indicum* was thoroughly rinsed with clean water and shade dried at room temperature in the dark in clean and hygienic conditions. The dried bark was powdered in an electrical grinder at room temperature. The stem bark powder of *O. indicum* was extracted in methanol. The methanol extract was concentrated using rotary evaporator and stored at −70°C until further use. Henceforth the methanol extract of *O. indicum* will be referred to as OIM throughout the paper. All the procedures are performed in Pharmacognosy & Phytochemistry Lab of East West University.

2.3.3. Extraction procedure

About 150gm of coarse powder is soaked by 95%methanol (900ml) in a conical flask and plugged with cotton and then covered with aluminium foil for twenty days with constant stirring with glass rod. After twenty days the preparation was filtered and filtrate was collected for the purpose of preparing extract. The filtrate was evaporated by rotary evaporator and kept in normal air for few days so that the remaining parts of solvent get evaporated. The residue was then collected and weighed (30gm). The residue was stored in a close container.



2.2 Figure: Preparing steps of extract of *Oroylum inicum* bark.

2.4. Preparation of standard solution (Omeprazole)

20 mg/kg omeprazole solution was prepared by dissolving it in the saline solution. As we took pellets of omeprazole, we weighed it before crushing and then crushed in mortar pestle and then again weighed. Then the powder was dissolved in saline. Omeprazole was purchased from Eskayef Bangladesh Limited.

2.5. Phytochemical analysis

Phytochemistry is the branch of chemistry deals with the chemical nature of the plant or plant products (chemistry of natural products). Plants contain many chemical constituents which are therapeutically active or inactive like carbohydrates, triterpenoids, alkaloids, glycosides, tannins, flavonoids, essential oils and other similar secondary metabolites. The qualitative chemical tests are to be performed for establishing profile of a given extract for its nature of chemical composition.

2.5.1. Test for carbohydrates:

- **Molisch's Test**: 2ml of extract 2-3 drops of alpha naphthalene solution in alcohol was added, shaken for 2 min and 1 ml of concentrated sulphuric acid was added slowly from the sides of the test tube. A deep violet colour at the junction of two layers indicates the presence of carbohydrates.
- **Benedict's Test**: Equal volume (2ml each) of Benedict's solution and extracts were mixed in a test tube and heated in boiling water bath for 10min the changes in colour to yellow, green and red indicates the presence of reducing sugars.

2.5.2. Test For saponins

- **Foam Test**:

a) 5 ml extract was shaken vigorously with 5 ml of water and observed for persistent foam, which is stable for 15 minutes indicates the presence of saponins.

b) In 5ml extract few drops of olive oil was added which formed a soluble emulsion, confirming the presence of saponins.

2.5.3. Test for alkaloids:

- **Mayer's Test**: To 3 ml of the extract, 1ml of Mayer's reagent (potassium mercuric iodide) was added. The appearance of white precipitate indicates the presence of alkaloids.

- **Hager's Test**: To 2 ml of extract, 1m few drops of Hager's reagent (saturated picric acid solution) was added. The appearance of yellow precipitate indicates the presence of alkaloids

2.5.4. Test for glycosides

- ***Liebermann's Test*** : 2ml extract , 2ml chloroform and 2ml acetic acid were added. A color change from violet to blue to green confirming the presence of glycosides

2.5.5. Test for Terpenoids

- **Salkowski Test:** 2 ml of extract was added to 2 ml of chloroform. Concentrated sulphuric acid 2-3 drops was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids

2.5.6. Test for Steroids:

- **Salkowski's Test:** 2 ml of extract was treated with 2 ml of chloroform and equal amount of concentrated sulphuric acid was added. Reddish ring at the junction indicates the presence of the steroids.

2.5.7. Test for Flavonoids:

- **Lead acetate solution Test:** Test solution when treated with few drops of lead acetate (10%) solution would result in formation of yellow precipitate.

- **Ferric chloride test :**Test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids

2.5.8. Test for Tannins:

- **Braymer's Test:** 2 ml of extract, 2 ml of distilled water and 5% of 2-3 drops of ferric chloride solution were added. The green colour precipitate indicates the presence of tannins.

2.5.9. Test for Anthraquinone:

- **Borntrager Test:** 3 ml of extract, 3 ml Benzene and 5 ml 10% ammonia solution were added. Red, pink or violet colour produced indicates the presence of anthraquinone.

2.5.10. Test for Coumarins:

In a test tube 2 ml of extract and 10% 3 ml NaOH were added. Yellow colour indicates the presence of coumarins.

2.6. Induction of ulcer

The animals were barred from access to any nutrients for a day and were only allowed access to drinking water for two hours before the experiment commenced. During the fasting period, the rats were placed individually in separate cages to prevent coprophagy. Thirty minutes after pre-treatment with standard (omeprazole at the dose of 20 mg/kg, p.o.) and test samples (MEPN at the doses of 200mg/kg and 400 mg/kg p.o., gastric ulcers were induced with ethanol-acid in these groups of rats (25 ml per kg of 0.3 M HCl in 60% ethanol [91]. These rats were sacrificed 90 min after induction and their stomachs were immediately excised. Each stomach was opened along the larger curvature, washed with distilled water. The gastric mucosa was examined for ulcers by magnifying lens and scoring of ulcer was made as follows [92].

Scoring of Ulcer					
Normal stomach	Red coloration	Spot ulcer	Hemorrhagic streak	Ulcer	Perforation
0	0.5	1	1.5	2	3

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

$$\% \text{ protection} = \frac{\text{control mean ulcer index} - \text{test mean ulcer index} \times 100}{\text{control mean ulcer index}}$$

Groups	Treatment
Group 1	5 ml/kg/day distilled water, p.o.; (Normal control)
Group 2	5 ml/kg/day distilled water, p.o. + 25ml per kg of 0.3M HCl in 60 percent ethanol; (Ethanol control)
Group 3	20 mg/kg/day omeprazole, p.o + 25ml per kg of 0.3M HCl in 60 percent ethanol;
Group 4	200 mg/kg/day methanolic extract of *O. indicum*,, p.o + 25ml per kg of 0.3M HCl in 60 percent ethanol;
Group 5	400 mg/kg/day methanolic extract of *O. indicum*,, p.o + 25ml per kg of 0.3M HCl in 60 percent ethanol;

Chapter- 3

Results and Discussion

3.1. Preliminary phytochemical analysis

The traditional use of the species was scientifically validated through the identification of the phytochemicals responsible for their use in indigenous systems of health care. The result of qualitative chemical analysis of the methanolic extract of *O. indicum* is tabulated in Table 1.

Table 3.1: Preliminary phytochemical analysis of *O. indicum* barks extract

Phytoconstituents	**MEOI**
Carbohydrates	-
Saponins	-
Alkaloids	+
Glycosides	+
Terpenoids	+
Steroids	
Flavonoids	+
Phenolics and Tannins	+
Anthraquinone	+
Coumarins	+
Reducimg sugar	-
Proteins	
Vitamin C	-
	-

MEOI:methanolic extract of leaves of *O. indicum,* + = Present,− = Absent

3.2. Effect of *O. Indicum* on ethanol-induced gastric ulcer

In ethanol control animal, oral administration of ethanol produced characteristic lesions in the glandular portion of rat`s stomach which appeared as elongated bands of thick, black & dark red lesions. MEOI showed significant protection index of 73% and 76% with the dose of 200mg/kg/day and 400 mg/kg/day, p.o. respectively in comparison to ethanol control. Whereas omeprazole (standard drug) reduced ulcer by 77% (Results are tabulated in Table-3).It showed that MEOI have similar potency as Omeprazole.

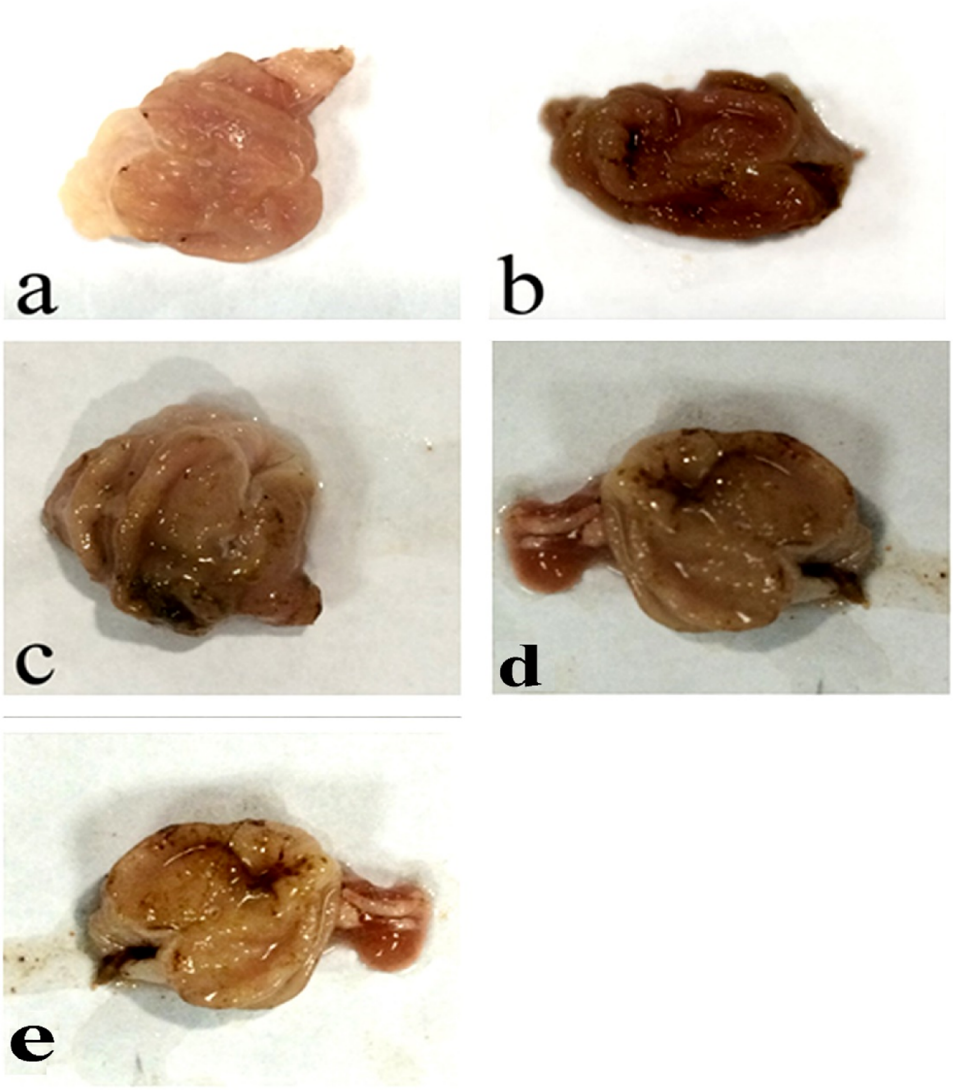


Figure 3.1: Ulcer induced stomach sample

Table 3.2: Effect of *O. indicum* extract on various parameters in ethanol induced gastric ulcer:

Groups	Ulcer Index	% of protection
Normal control	1.24 ± 0.03^{a}	-
Ethanol Control	$13.8\pm1.01^{b**}$	-
Omeprazole 20 mg/kg	$3.2\pm0.04^{c**}$	77%
MEOI 200 mg/kg BW	$3.8\pm0.04^{d*}$	73%
MEOI 400 mg/kf BW	$3.\ 3.\pm0.04^{e**}$	76%

MEOI-**methanolic extract of *O. Indicum.***

Each value is Mean ± S.E.M (n=6). (*) indicates statistically significant alteration from respective group using one way analysis of variance followed by Dunnett's multiple comparison test ($^{**}p< 0.01, ^{*}p< 0.05$). a = when compared with normal control, b = when compared with ethanol control, c = when treated with omeprazole 20mg/kg, d = when treated with MEOI 200mg/kg, e= when treated with MEOI 400 mg/kg.

3.3. Gross evaluations of gastric lesions:

Ethanol controlled rats exhibited severe mucosal injury whereas, the rats that were treated with *O. indicum* bark extract before ethanolic induction had significantly reduced areas of gastric ulceration revealing flattening of gastric mucosal folds compared to rats treated with only distilled water. There were no significant differences between doses of 200mg/kg and 400 mg/kg methanolic extract in terms of area of ulceration. It was also observed that protection of gastric mucosa was more prominent in rats treated with 400 mg/kg methanolic extract (Figure 3.1).

3.4. Discussion

Beginning with phytochemical screening of the methanolic extract of *O. indicum* in veil the presence of alkaloids, phenols, steroids, triterpinoids, flavonoids and coumarins. In different studies have proclaimed that certain terpenoids, steroids and phenolic compounds (tannins, coumarins and flavonoids) have protective effects in view of their antioxidant properties. [93] Latterly, a number of natural products of traditional medicines and ingredients of healthy foods have been competently explored and withstand to clinical trials to lay foundation as anti-

ulcerative agents. Existence of major Phytoconstituents in the methanolic extract of leaves of *O. indicum* makes it a conceivable contender for further exploration.

The anti-ulcer effect of the methanolic extract was evaluated using ethanol induced gastric ulcer model. Ethanol induced gastric lesions formed due to interference in gastric blood flow which contributes to the development of the hemorrhage and necrotic aspects of tissue injury. Alcohol swiftly penetrates the gastric mucosa superficially causing cell and plasma membrane damage leading to augmented intracellular membrane permeability to sodium and water. The mammoth buildup of calcium describes a chief step in the pathogenesis of gastric mucosal injury. This sequence leads to the demise of cells and erosion of epithelium`s surface.

The results revealed that the ethanol administration in the control group resulted in immense ulceration in comparison with the normal group. However, treatment with omeprazole at the dose of 20 mg/kg and methanolic extract of *O. indicum* at the doses of 200mg/kg and 400 mg/kg prior to ethanol administration exhibited significant inhibition. Among the test samples, the best result was obtained with *O. indicum* at an optimum dose of 400 mg/kg which was potentially effective as compared to the standard drug, omeprazole. Edema, cellular debris and damaged mucosal epithelium were found in ulcerated stomach membranes. However, the findings observed in the current studies support and extend previous results that reported anti-ulcer activities of *Oroxylum indicum* bark extract.

Conclusion

In our study the extract exhibited protection against characteristic lesions produced by ethanol administration. This antiulcer effect of methanolic extract of *O. indicum* may be due to both reductions in gastric acid secretion and gastric cytoprotection. Further studies are needed for their exact mechanism of action on gastric acid secretion and gastric cytoprotection. However, the present investigation concluded that the treatment of extracts reduced the ethanol induced ulcer in a dose-dependent manner and at the higher dose (400 mg/kg) the effect was similar to that of reference drug.

In conclusion, MEOI exhibited antiulcerogenic activity. MEIO at the dose of 400 mg/kg showed higher level of cytoprotection. The depletion in ulcer may have occurred due to high flavonoid, triterpenoids, steroids, saponins and tannin content. However, the mechanisms behind these events are still vague. Therefore, further experiments should be undertaken to identify which of the phytoconstituents and mechanisms are involved in the actions illustrated by the results.

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