PHARMACOLOGICAL EVALUATION OF ETHANOLIC EXTRACT OF LEAVES OF *CANTHIUM PARVIFLORUM*

A Dissertation submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI - 600 032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

DEPARTMENT OF PHARMACOLOGY

Submitted by

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Under the guidance of

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ARULMIGU KALASALINGAM COLLEGE OF PHARMACY

ANAND NAGAR, KRISHNANKOIL-626126

May 2019

CERTIFICATE

This is to certify that the investigation described in this dissertation entiled **"Pharmacological evaluation of ethanolic extract of leaves of *Canthium parviflorum*"** submitted by **Reg. No:261725351** to The Tamil Nadu Dr. M.G.R. Medical University, Chennai for the partial fulfillment of the requirement for the Degree of Master of Pharmacy in Pharmacology. This research work was carried out in the Department of Pharmacology under the guidance and supervision of **Dr.V.Lavakumar, M.Pharm., Ph.D.,** Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil-626126.

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EVALUATION CERTIFICATE

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S.L.Shanu

DECLARATION

I **S.L.SHANU (Reg no : 261725351),** hereby declare that the dissertation work entitled **"PHARMACOLOGICAL EVALUATION OF ETHANOLIC EXTRACT OF LEAVES OF *CANTHIUM PARVIFLORUM*"** submitted by me, in partial fulfillment of the requirement for the degree of **MASTER OF PHARMACY** IN **PHARMACOLOGY** to **The TamilnaduDr.M.G.R Medical University, Chennai** is the result of my original and independent research work carried out under the guidance and supervision of **Dr.N.VENKATESHAN., M.Pharm.,Ph.D.,** during the academic year 2018-2019 and this has not formed the basis for the award of any Degree/ Diploma/ Fellowship or similar title to any candidate of any university.

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A.K.C.P.

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CHAPTER-I

INTRODUCTION

1.1Ulcers

Ulcers are sores or open wounds that occur on the skin or along the lining of the digestive tract due to loss of tissue. They are also defined histologically as a breach in the mucosa of the alimentary tract that extends through the muscularis mucosa in to the submucosa layer[1].

1.2Types of ulcers

There are three types of ulcers. They are

- Peptic ulcers
- Gastric ulcers
- Duodenal ulcers

i.Peptic ulcers

A peptic ulcer is an excoriated area of stomach or intestinal mucosa caused by the digestive action of gastric juice or upper small intestinal secretions. The usual cause of peptic ulceration is an imbalance between the rate of secretion of gastric juice and the degree of protection afforded by the gastro duodenal mucosal barrier and the neutralization of the gastric acid by duodenal juices peptic ulcers frequently occur along the lesser curvature of the antral end of the stomach or, more rarely, in the lower end of the oesophagus where stomach juices frequently reflux. Peptic ulcers are chronic, most often solitary, lesions that occur any portion of the gastro intestinal tract exposed to the aggressive action of acid and peptic juices.

Symptoms

Abdominal pain with a burning or gnawing sensation, pain 2-3 hours after eating, pain is often made worse by an empty stomach; night time pain is common, heartburn, indigestion (dyspepsia), belching, nausea, vomiting, poor appetite and weight loss[2].

ii.Gastric ulcers

Ulceration of the gastric mucosa due to contact with gastric juice. It is often associated with Helicobacter pylori infection or consumption of nonsteroidal anti-inflammatory drugs (NSAIDS).It is usually more common in males than in females and occurs mostly in the people aged above 60 years.

Symptoms

Recurrent abdominal pain, dull and burning type pain usually located in epigastric area, abdominal pain after meals, no abdominal pain at night, blood in vomit, nausea, anorexia, black stools, fatigue, breathlessness, deep tenderness in the midline of epigastrium, perforation, hemorrhage and obstruction[3].

iii.Duodenal ulcers

A peptic ulcer located in the duodenum. Some of the possible causes of duodenal ulcer include excess stomach acid and helicobacter pylori infection.It usually occurs in the males at the age of 25-50 years.

Symptoms

Abdominal pain after meals, pain below the ribs, gastrointestinal bleeding, no loss of weight, no vomiting, melaena, deep tenderness in the right hypochondria[4].

1.3Historical Perspective

Looking back at history, peptic ulcer was a rare and generally unrecognized as a cause of symptoms/ complications or death until the early 19^{th} century.Despite sporadic case reports beginning in late 18^{th} century. Peptic ulcer disease did not become widely appreciated until early 20^{th} century. The first 6 decades saw the dominance of surgery in the treatment of peptic ulcer. With the introduction of acid suppressive drugs like H_2 blockers in 1970's the treatment of treatment of PUD was revolutionized. By 1980's the advent of Helicobacter pylori (HP) brought about a dramatic twist and possibly cure[5].

1.4Physiology of acid secretion

The parietal cell contains receptors for gastrin, Histamine (H_2) and Acetylcholine (M_3). When gastrin or acetylcholine or histamines binds to their receptors an increase in cytosolic calcium, which in turn stimulates protein kinases that stimulates acid secretion from a H^+ / K^+ ATPase (proton pump) on the canalicular surface. In close proximity to the parietal cells are gut endocrine cells called Enterochromaffin like cells (ECL cells). ECL cells have receptors for gastrin and acetylcholine are the major source for histamine release. Histamine binds to the H_2 receptor on the parietal cells, resulting in activation of adenyl cyclase, which increases intracellular cyclic adenosine monophophate (CAMP). CAMP activates protein kinase that stimulates acid seceretion by the H^+ /K^+ ATPase. In human, it is believed that the major effect of gastrin upon acid secretion is mediated indirectly through the release of histamine from ECL cells rather than through direct parietal cell stimulation[6].

Mechanism of secretion of gastric hydrochloric acid

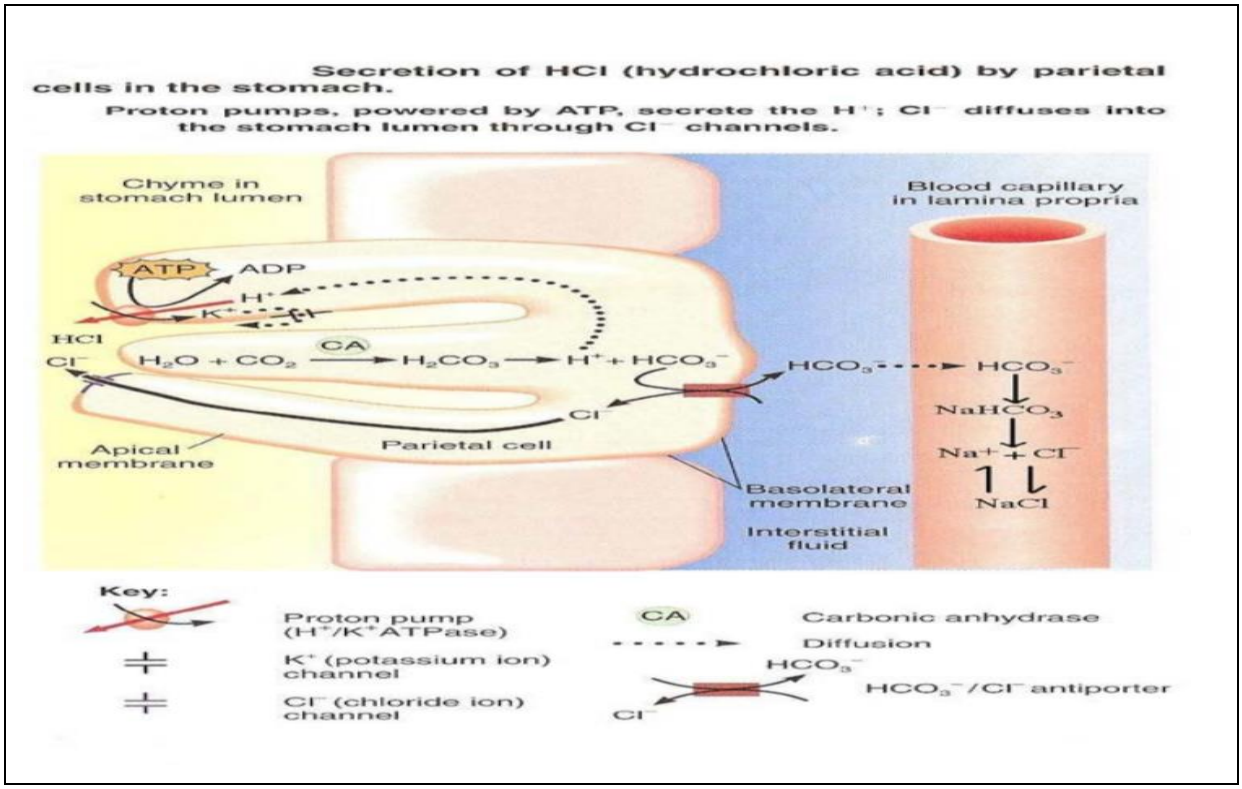


Figure 1 Mechanism of secretion of gastric hydrochloric acid

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

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

2. Proton pump powered by H^+ / K^+ ATPases actively transport H^+ into the lumen while bringing potassium ions into the cell.

3. At the same time Cl^- and K^+ diffuse out through Cl^- and K^+ channels in the apical membrane.

4. The enzyme carbonic anhydrase, which is especially plentiful in parietal cells, catalyzes the formation of carbonic acid (H_2CO_3) from water (H_2O) and carbon dioxide (CO_2).

5. As carbonic acid dissociates, it provides a ready source of H^+ for the proton pumps but also generates bicarbonate ions (HCO_3^-)

6. As a result HCO_3^- builds up in the cytosol; Itexits the parietal cell in exchange for Cl^- via Cl^- / HCO_3^- antiporters in the basolateral membrane. HCO_3^- diffuses into nearby blood capillaries. “This alkaline tide”of bicarbonate ions entering the blood stream.

7.As a result, one molecule of $NaHCO_3$ is formed in the blood against one molecule of HCl formed and excreted into the stomach.

8. The H^+ ions developed as stated in step (5) join with OH^- ions as described in step (1) to form water[7].

1.5Etiology and Pathogenesis

Most peptic ulcers occur in the presence of acid and pepsin when Helicobacter pylori, NSAIDs or other possible factors disrupt normal mucosal defense and healing mechanisms. Hypersecretion of acid is the primary pathogenic mechanism, in hypersecretory states such as Zollinger Ellison syndrome.

Schematic diagram for etiology of ulcer

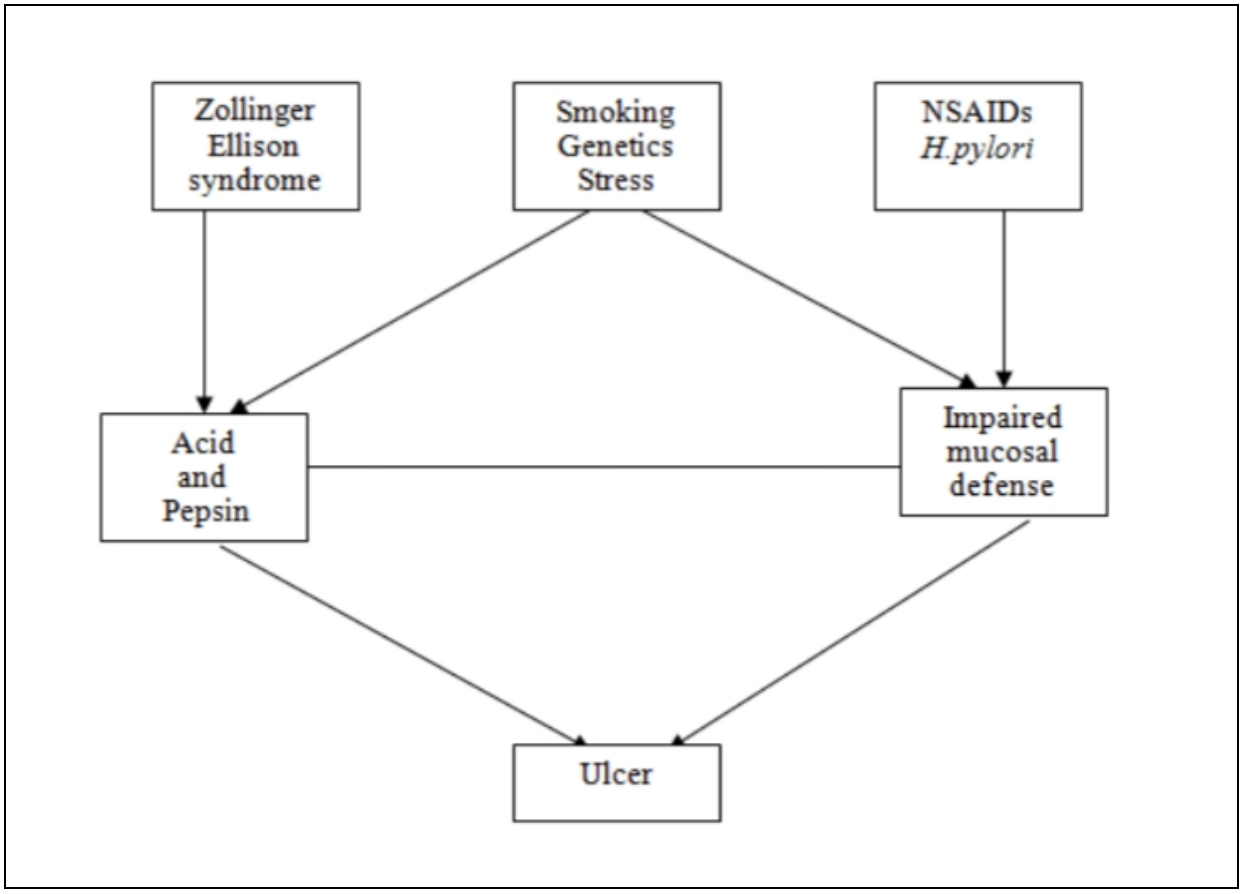


Figure 2 Schematic diagram for etiology of ulcer

The pathogenesis of DU and GU is multifactorial and most likely reflects a combination of pathophysiologic abnormalities, environmental, and genetic factors. Ulcer location appears to be related to a number of etiologic factors, most of the DUs occur in the first part of the duodenum. Benign GUs can occur anywhere in the stomach, although most are located on the lesser curvature, just distal to the junction of the antral and acid secreting mucosa[8].

Clinical features

- Abdominal pain, classically epigastric with severity relating to meal times (duodenal ulcers are classically relieved by food, while gastric ulcers are exacerbated by it).
- Water brash (bitter regurgitation).

- Nausea and sometimes vomiting.
- Loss of appetite and weight loss.
- Hematemesis (vomiting of blood).
- Melena (tarry, foul-smelling feces due to oxidized iron from hemoglobin).

Rarely, an ulcer can lead to a gastric or duodenal perforation. This is extremely painful and requires immediate surgery. A history of heart burn, Gastroesophageal reflux disease (GERD) and use of certain forms of medication can raise the suspicion for peptic ulcer[8].

1.6 A.Factors which responsible for peptic ulcer [9]

1. Helicobacter pylori (HP) 2.NSAID's3.Adrenocorticosteroid 4. Uncommon forms of peptic ulcer

1.6.1 B.Potential risk factors 1. Cigarettesmoking 2. Psychologicalstress 3. Alcohol 4. Genetic factors 5. Dietary factors

A. Factors which responsible for peptic ulcer

1. Helicobacter pylori infection (HP)

Helicobacter pylori is an acid-liable, spiral shaped, gram-negative bacteria that resides between the mucous layer and surface epithelial cells in the stomach or any location where gastric epithelium is found. The shape and motility of the bacterium permit penetration of the mucus layer where the local pH is less acid. Before HP enters the mucous, it produces large amounts of ureases, which breaks down urea in gastric juice and converts it to ammonia and carbon dioxide. This metabolic process continues after HP reaches the "safe haven" of the mucous. The neutralizing effect of ammonia forms a microenvironment that protects the organism from the lethal effect of acid. HP attaches to epithelial surface by adhesions or pedestals specific for gastric type epithelium.

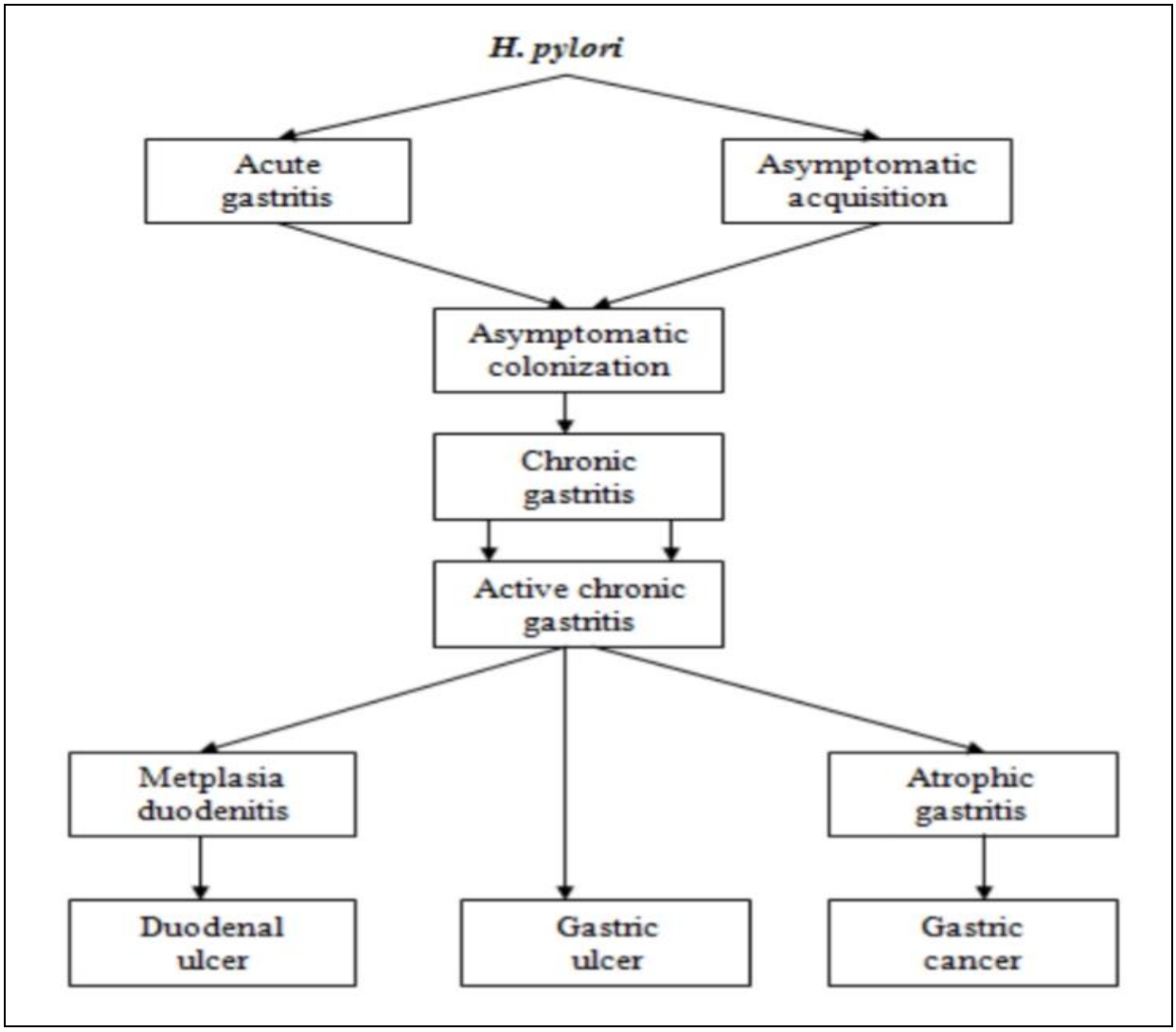


Figure 3 Helicobacter pylori infection

The specific pathophysiologic mechanisms by which HP causes ulcers is contraversial and remain unknown however several theories have been proposed.The defense by the elaboration of toxins, potentially toxic enzymes and inflammation. Candidates include lipopolysaccharide, vacuolizing cytotoxin, urease and ammonia, as well as macrophage and neutrophil activation. The role of the immune system in HP infection requires further study

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

2. NSAID's

There is overwhelming evidence linking chronic NSAID'suse and gastroduodenal ulcers. In patients receiving NSAID's, ulcers occur more frequently in the stomach than in the duodenum. Hospitalizations Complications and mortality are increased in chronic NSAID's users and are in part related to ulcer bleeding and perforation. Several factors, including a history of PUD, NSAID dose duration of exposure, and disability may predispose people to ulcer and complications. Chronic NSAID's therapy produces gastro duodenal injury by two mechanisms, a direct action on the mucosa and a systemic effect where by endogenous PG synthesis is inhibited. NSAID inhibition of cyclooxygenase not only decreases protective PG's, but also generates oxygen derived free radicals and makes available of more Arachidonic acid for metabolism via the lipoxygenase pathway. Leukotrines, products of lipoxygenase metabolism, are inflammatory substances that may contribute to mucosal injury[10].

3. Adreno corticosteroids

The association between adreno corticosteroids and PUD remains contraversial. Although it is likely that adreno corticosteroids induce ulcers because of their ability to increase gastric acid secretion and inhibit PG production, sufficient evidence is lacking to support a causal relationship. Discrepant findings among study participants . A recent study suggests that elderly patients on concurrent oral adreno corticosteroids and NSAID's are at a much higher risk for PUD than those receiving either of these agents alone, that ulcer risk is related to adreno corticosteroid dose and duration of therapy. It is possible that adreno corticosteroids either delay or inhibit the healing of ulcers caused by Patients receiving adreno corticosteroids[11] , aspirin and other NSAID's.

4. Uncommon forms of peptic ulcer

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

B. Potential risk factors [12]

1. Cigarette smoking

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

2. Psychological stress

Reports suggest an association between psychological stress and peptic ulcer disease. Stress ulceration of the stomach is associated with clinical conditions like trauma, head injury, burns, shock, sepsis and neurological disorders. It is reported to result from interactions between mucosal vascular and neurohumoral factors and the autonomic nervous system plays an important role. Electric stimulation of different regions of the limbic area modulates gastric acid secretion, motility and mucosal blood flow, all of which are important factors for the stress induced ulcer development. The CNS more importantly, the brain gutaxis are important mediators of stress ulcerogenesis and complex neural mechanisms have been proposed. The disruptive and protective mediators of this neural mechanism now recognized include biogenic amines, amino acids, peptides and neurotransmitters like acetylcholine, Gamma-amino butyric acid (GABA) and several neuropeptides. Stress causes ischemic condition in the gastric mucosa by reducing blood flow following activation of parasympathetic and sympathetic nervous system, resulting in the constriction of the smooth muscles of the blood vessels and gastric tissue. This causes O_2, which is dismuted by super oxide dismutase to form H_2O_2. Stress produces loss of gastro protection, increased acidity, pepsin and histamine release and aggravate the situation.

3. Genetic factors

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

4. Alcohol

Ethanol (50-100 %) rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage, leading to increased intracellular membrane permeability to sodium and water. Ethanol induces solubilization of mucus constituents in stomach with concomitant fall in transmucosal potential difference and increases Na^+ , K^+ flux into the lumen, also depress tissue levels of DNA, RNA and proteins leading to flow stasis in injured areas. Ethanol appears to stimulate gastric secretions by exciting sensory nerves in the buccal and gastric mucosa and promoting the release of gastrin and histamine. Ethanol induced ulcers are inhibited by agents which increases much defensive factors such as PGE_2. The massive intracellular accumulation of Ca_2^+ represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the superficial epithelium. Further, gastric lesions caused by ethanol have been attributed to free radical damage, which results in lipid peroxidation products. Clinically, cirrhosis due to consumption of alcohol is linked to an increased incidence of peptic ulcer.

5. Diet.

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

1.7 Current status of treatment for peptic ulcer[13]

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

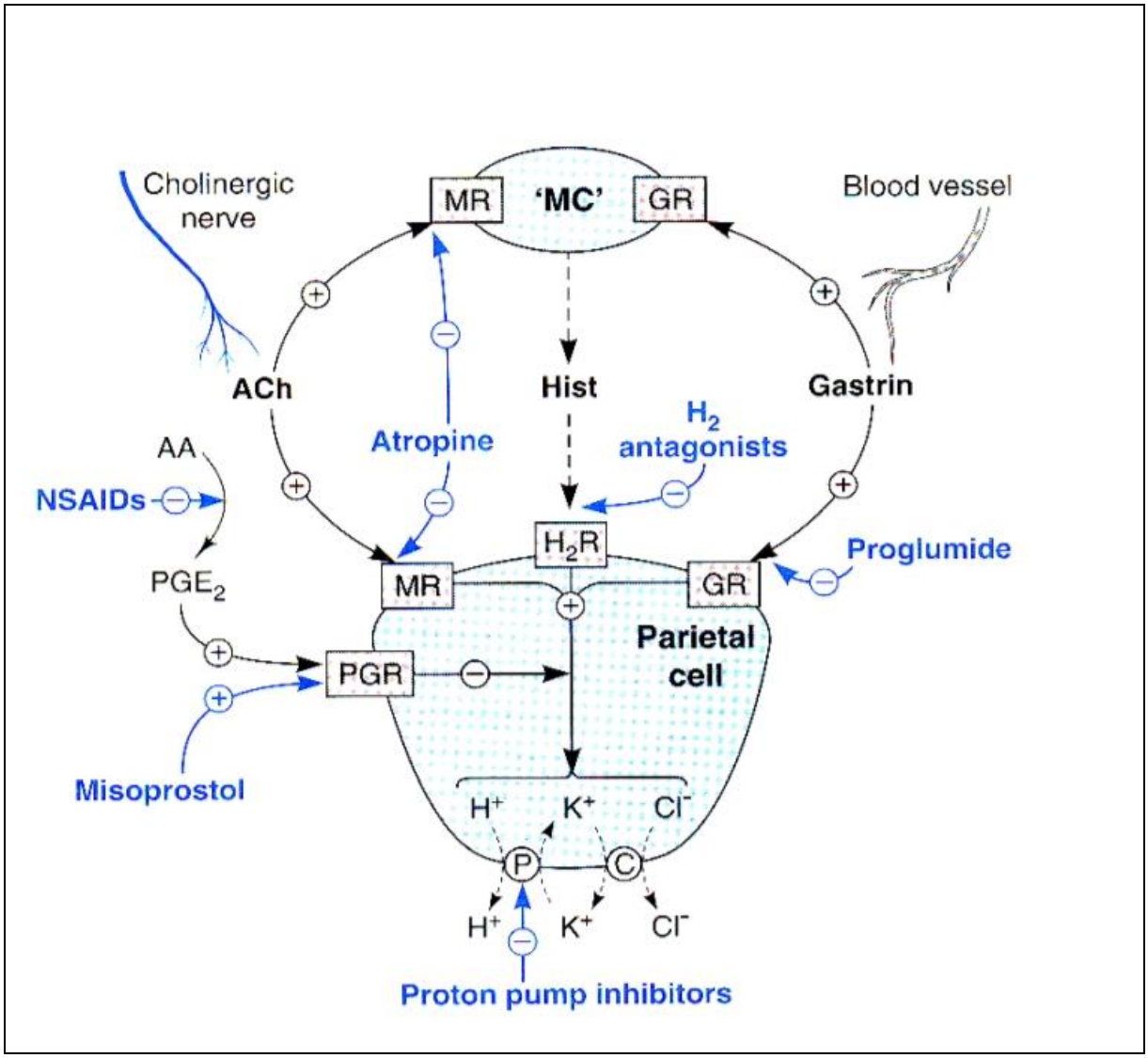


Figure 4 Mechanism of action of antiulcer agents

Approaches for the treatment of peptic ulcer are

1.8 A. Reduction of gastric acid secretion

1. **H2 receptor antagonists**

They are currently the most popular drugs for peptic ulcer. H_2-receptor antagonists reduce acid stimulation by histamine, gastrin, cholinomimetic drugs and vagal stimulation. All phases (basal, psychic, neurogenic and gastric) of secretion are attenuated. The most prominent action is on acid output but volume pepsin content and intrinsic factor secretion are also reduced. They also prevent occurrence of stress induced ulcers. They include drugs like Ranitidine, Cimetidine, Roxatidine, Famotidine and Nizatidine. . H^+ K^+ ATPase (proton pump) inhibitors Blockade of the gastric proton pump constitutes a more direct mechanism for acid secretion inhibition compared to blockade of histamine and cholinergic receptors. They are powerful inhibitors of gastric acid. Examples of this class are Omeprazole, Pentoprazole, Rabeprazole and Lansoprazole. They are found to inhibit the growth of H. pylori. Anticholinergics Anticholinergics or muscarinic cholinergic antagonists can reduce basal secretion of gastric acid by 40 to 50 % without raising the pH. Selective muscarinic M_1 receptor antagonists Pirenzepine and Telenzipine belong to this class. Selective antagonists of M_1 receptors are as effective as Atropine, but are less likely to produce adverse effects that are characteristic of cholinergic blockade (dry mouth and tachycardia). Prostaglandin analogues Prostaglandins E_2 and I_2 the predominant prostaglandins synthesized by the gastric mucosa inhibit the secretion of acid and stimulate the secretion of mucous and bicarbonate. Their most important action appears to be their ability of reinforce the mucous layer covering gastric and duodenal mucosa, which is buffered by HCO_3^- secreted into this layer by underlying epithelial cells. They are known to increase blood flow and are indicated for the prevention of NSAID induced gastric ulceration. Currently available PG's in the market are Misoprostol, Enprostil, Rioprostil, Arbaprostil and Trimoprostil.

B. Neutralization of gastric acid (antacids)

The function of antacids, which are basic substances, is to neutralize the HCl secreted by gastric parietal cells and raise the pH of gastric contents. Peptic activity is indirectly reduced if the pH rises above 4, because pepsin is secreted as a complex with an inhibitory terminal moiety that dissociates below pH 5. i) Systemic antacids They are water soluble, acts instantaneously, but the duration of action is short. E.g: Sodium bicarbonate , Sodium citrate. ii) Non-systemic antacids They are insoluble and poorly absorbed basic compounds. Hydroxides of aluminium and magnesium are the most common constituents of antacids preparation. E.g. Aluminium hydroxide, Magnesium hydroxide and Calcium carbonate etc.

C.Ulcer protective:

i) Sucralfate It is sulfated disaccharide basic aluminum sulfate complex, it forms an adherent coating with pertinacious material at ulcerated mucosal base sites so avidly, that it is difficult to wash the gel from the crater. When pH< 4, there is extensive polymerization and cross linkage of sucralfate forming a sticky, viscid coat and acts as a physical barrier preventing acid, pepsin and bile from coming in contact with the ulcer base. ii) Bismuth compounds They have no substantial capacity to neutralize gastric acids. Their beneficial effects have been ascribed to cytoprotection (enhanced secretion of mucous and HCO_3^- probably through stimulation of mucosal production, inhibition of pepsin activity and accumulation of bismuth sub citrate preferentially at the craters of the gastric ulcer). Bismuth has been shown to promote healing of both gastric and duodenal ulcers. Bismuth serves as an important component in the “Triple therapy” of H. pylori as it detaches H.pylori from the surface of the mucosa and directly kills the organism. Chronic use of other bismuth salts has caused encephalopathy and osteodystrophy.

D. Ulcer healing drugs

Carbenoxolone It is an antiulcer drug obtained from Glycyrrhiza glabra (liquorice root) oleandane derivative of glycyrrhizic acid. It was found to promote healing of gastric ulcer without altering the volume or acidity of gastric juice Anti-H.pylori drugs Examples of drugs under this class include antimicrobials like Amoxicillin, Clarithro-mycin, Metronidazole, Tinidazole, Tetracycline and Cetraxate (mucosal protective agent). F. Miscellaneous groups Proglumide, a cholesystokinin and gastrin receptor antagonist is also found to possess antisecretory and antiulcer activity.

Meciadanol a new synthetic flavonoid, an inhibitor of histidine decarboxylase has been shown to prevent gross and histological induced gastric ulcers as well as those caused by acidified aspirin probably by inhibiting mast cell degranulation. Dopamine receptors may be involved in gastric cytoprotection since duodenal ulcers are in schizophrenics, who have excess hyperactivity of brain dopamine. Protective effects have been seen with parenterally administered Dopamine agonists like Bromocriptine, Lergotrile and Apomorphine as well as the Dopamine precursor- Levodopa and the MAO-B inhibitor – Deprenyl[14].

Diagnosis

The history and physical examination are important to identify patients at risk of ulcer, perforation, bleeding, or malignancy. However, a systematic review of models using risk factors, history, and symptoms found that they did not reliably distinguish between functional dyspepsia and organic disease[15]. Therefore, the test-and-treat strategy for H. pylori is recommended for patients with dyspepsia who have no alarm symptoms[16]. The American College of Gastroenterology (ACG) recommends testing for H. pylori infection in patients with active PUD or history of PUD, dyspepsia symptoms, or gastric MALT lymphoma[17]. The rationale for testing patients with a history of PUD who are currently asymptomatic is that detecting and treating H. pylori infection can reduce the risk of recurrence. The test-and-treat strategy for detecting H. pylori is appropriate in patients with dyspepsia and low risk of gastric cancer (age younger than 55 years and no alarm symptoms such as unexplained weight loss, progressive dysphagia, odynophagia, recurrent vomiting, family history of gastrointestinal cancer, overt gastrointestinal bleeding, abdominal mass, iron deficiency anemia, or jaundice)[18]. Endoscopy is recommended for patients who are 55 years or older, or who have alarm symptoms.

Table 1: Differential Diagnosis of Peptic ulcer Disease

Commonly mistaken for peptic ulcer disease	**Less commonly mistaken**	**Rarely mistaken**
Esophagitis	Celiac disease	Abdominal aortic aneurysm
Functional dyspepsia	Cholangitis	Acute coronary syndrome

Gastritis	Cholecysitis	Barrett esophagus
Gastroenteritis	Cholelithiasis	Gastric cancer
Gastroesophageal reflux disease	Esophageal perforation	Viral hepatitis
	Inflammatory bowel disease	Zollinger- Ellison syndrome
	Irritable bowel syndrome	

Urea breath test

Urea breath tests require the ingestion of urea labeled with the nonradioactive isotope carbon 13 or carbon 14. Specificity and sensitivity approach 100%. Urea breath testing is one option for test of cure and should be performed four to six weeks after completion of eradication therapy. Proton pump inhibitors (PPIs) must be stopped for at least two weeks before the test, and accuracy is lower in patients who have had distal gastrectomy. Cost and inconvenience are disadvantages of this test.

Stool monoclonal antigen test

Stool antigen tests using monoclonal antibodies are as accurate as urea breath tests if a validated laboratory based monoclonal test is used [19].They are cheaper and require less equipment than urea breath tests. Like urea breath tests, stool antigen tests detect only active infection and can be used as a test of cure. PPIs should be stopped for two weeks before testing, but stool antigen tests are not as affected by PPI use as are urea breath tests.

Serologic tests

Serologic antibody testing detects immunoglobulin G specific to H. pylori in serum and cannot distinguish between an active infection and a past infection. Serologic tests may be most useful in mass population surveys and in patients who cannot stop taking PPIs (e.g., those with gastrointestinal bleeding or continuous NSAID use) because the tests are not affected by PPI or antibiotic use[20].

Endoscopy with biopsy

Endoscopy with biopsy is recommended to rule out cancer and other serious causes in patients 55 years or older, or with one or more alarm symptoms. In patients who have not been taking a PPI within one to two weeks of endoscopy, or bismuth or an antibiotic within four weeks, the rapid urease test performed on the biopsy specimen provides an accurate, inexpensive means of diagnosing H. pylori infection. Patients who have been on these medications will require histology, with or without rapid urease testing. Culture and polymerase chain reaction allow for susceptibility testing but are not readily available for clinical use in the United States.

Treatment

Eradication of H. pylori is recommended in all patients with PUD. First-line therapy should have an eradication rate of more than 80%. Because pretreatment susceptibility is rarely known to the primary care physician, therapy must be chosen empirically based on regional bacterial resistance patterns, local recommendations, and drug availability. includes treatment options; standard triple therapy is a reasonable initial therapy where clarithromycin resistance is low. Eradication heals most duodenal ulcers and greatly diminishes the risk of recurrent bleeding. A systematic review found that treatment of H. pylori infection is more effective than antisecretory noneradicating therapy (with or without long-term maintenance antisecretory therapy) in preventing recurrent bleeding from peptic ulcer. Current data suggest that increasing the duration of therapy to 14 days significantly increases the eradication rate[21].

Test of cure

Test of cure for all patients after therapy is neither cost-effective nor practical. Indications for eradication testing with the urea breath test or stool antigen test include H. pylori–associated ulcer, continued dyspeptic symptoms, H. pylori–associated MALT lymphoma, and resection for gastric cancer.When indicated, eradication testing should be performed at least four weeks after completion of therapy.

i.Standard triple therapy

A seven- to 10-day triple drug regimen consisting of a PPI, amoxicillin 1 g, and clarithromycin 500 mg (Biaxin) twice daily has long been the first-line therapy to eradicate H. pylori. However,

increasing resistance to clarithromycin is associated with declining eradication rates, now well below 80%[22].Therefore, this regimen is not recommended where the prevalence of clarithromycinresistant strains of H. pylori exceeds 15% to 20%. An alternative triple drug regimen substitutes metronidazole 500 mg twice daily for amoxicillin. Adding probiotics to triple therapy, specifically Saccharomyces boulardii and Lactobacillus, has been shown to increase eradication rates (absolute increase of 9% and 5%, respectively) and decrease adverse effects of treatment, particularly diarrhea (absolute decrease of 14% and 7%, respectively)[23].

Table 2: Accuracy of Diagnostic Tests for Helicobacter pylori infection

Tests	**Sensitivity (%)**	**Specificity (%)**	**PV + (%)**	**PV- (%)**	**Advantages**	**Disadvantages**
Noninvasive Urea breath test (Carbon13)	97	100	99	1.5	Used for initial diagnosis and test of cure	Expensive inconvenient Patient must fast for six hours
Stool monoclonal antigen tests Enzyme immunoassay	92	94	89	3.9	_	More expensive than immunechromatography
Immuno chromatography	69 to 87	87 to 93	72 to 85	6.6 to 15	May be used in the office for rapid	Varying reliability

					diagnosis	
Antibody tests	76 to 84	79 to 80	64 to 67	9 to 13	Lower cost Easily available	PV+ dependent on prevalence, not useful as test of cure
Invasive Rapid urease test	95	100	98.9	2.4	Rapid Inexpensive	Sensitivity is low in treated patients
Histology	94	99	97	3	_	Expensive
Culture	NR	100	NR	NR	Allows for Susceptibility testing	Not widely available, expensive
Polymerase chain reaction	NR	NR	NR	NR	Allows for Susceptibility testing	Not standardized; not widely available

Table 3: Treatment Regimens for Helicobacter pylori infection

Type	**Regimen**	**Duration**	**Eradication rate**	**Comments**
First line Standard triple therapy	PPI, amoxicillin 1g,and clarithromycin5oomg	7 to 10 days (up to 14 days)	70% to 85%	Preferred

	(Biaxin) twice daily PPI, clarithromycin 500mg, and metronidazole 500 mg (Flagyl) twice daily.			
Sequential therapy	PPI and amoxicillin 1g twice daily, followed by PPI, clarithromycin 500mg, and tinidazole 500mg (Tindamax) or metronidazole 500mg twice daily	10 days (5 days for each regimen)	>84%	Needs validation in the United states
Second line				
Non-bismuth-based quadruple therapy (concomitant therapy)	PPI, amoxicillin 1g, clarithromycin 500 mg, and tinidazole 500 mg or metronidazole 500 mg twice daily	10 days	90%	Less complex than sequential therapy with similar eradication rates
Bismuth-based quadruple therapy	Bismuth subsalicylate 525 mg or subcitrate 300 mg, metronidazole 250 mg, and tetracycline	10 to 14 days	75% to 90%	May also be used if first-line therapy fails

	500mg, four times daily; and PPI twice daily.			
Levofloxacin-based triple therapy	PPI and amoxicillin 1 g twice daily, and levofloxacin 500 mg (Levaquin) once daily	10 days		Needs validation in United states; should be used as salvage therapy only.

ii.Sequential therapy

Sequential therapy consists of a five-day course of a PPI and amoxicillin 1 g taken twice daily, followed by a five-day course of a PPI, clarithromycin 500 mg, and metronidazole 500 mg (Flagyl) or tinidazole 500 mg (Tindamax) taken twice daily. The overall eradication rate is 84%, with an eradication rate of 73% for clarithromycin-resistant strains. A recent meta-analysis of available global data revealed that sequential therapy is superior to seven-day triple therapy, but it is not superior to 14-day triple therapy, bismuth-based quadruple therapy, or non–bismuth-based quadruple therapy. Compliance and tolerance rates of sequential therapy are similar to those of triple therapy but cost is lower, especially when the cost of failure of first-line therapy is considered. However, most studies were performed in Italy, and the ACG guideline states that sequential therapy requires validation in the United States.

iii.Non bismuth- based quadruple therapy concomitant therapy

This approach involves the addition of metronidazole 500 mg or tinidazole 500 mg twice daily to the standard triple regimen. It is less complex than sequential therapy with similar eradication rates. Additionally, non– bismuth-based quadruple therapy may be more effective than sequential therapy in patients with dual antibiotic resistance to clarithromycin and metronidazole. It has the highest eradication rate, about 90%, even in areas with high clarithromycin and metronidazole resistance, but would presumably cost more than sequential therapy because clarithromycin is taken for 10 days.

iv.Bismuth-based quadruple therapy

This is the traditional quadruple regimen and includes a bismuth salt (subsalicylate 525 mg or subcitrate potassium 420 mg), metronidazole 250 mg, and tetracycline 375 to 500 mg, all taken four times daily, in addition to a PPI taken twice per day.Bismuth-based quadruple therapy is often employed as salvage therapy if first-line treatment fails, but it may be used as first-line therapy in areas of high resistance or when cost is an important consideration. A three-in-one combination capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline has been developed to help reduce the pill burden, but patients still have to take three capsules four times per day in addition to a PPI. The regimen is usually given for 10 to 14 days.

V.Levofloxacin-based triple therapy

This is a 10-day regimen of a PPI and amoxicillin 1 g twice daily, and levofloxacin 500 mg (Levaquin) once daily. The ACG states that this regimen requires validation in the United States.It should be reserved for second-line therapy and is better tolerated than bismuthbased quadruple therapy[24].

1.9 NSAIDs and PUD

Prevention

Risk factors for gastrointestinal toxicity from NSAID use include older age; chronic use of high-dose NSAIDs; use of aspirin, anticoagulants, or corticosteroids; and a history of ulcer [25].Therapies aimed at protecting the mucosa include the prostaglandin analogue misoprostol (Cytotec), histamine H2 receptor antagonists, a cyclooxygenase-2 (COX-2) inhibitor instead of a standard NSAID, and PPIs. A Cochrane review on the effectiveness of these therapies compared with placebo suggests that high-risk patients should take a COX-2 inhibitor with a PPI for the greatest gastrointestinal safety. Concerns have been raised about increased cardiovascular risk with the use of COX-2 inhibitors. The ACG and the Canadian Association of Gastroenterology have each developed evidence-based guidelines for the prevention of NSAID-related ulcers in patients at risk of cardiovascular disease, including those with previous cardiovascular events [26].NSAIDs are appropriate for patients with low risk of gastrointestinal complications, whereas cotherapy with a PPI or misoprostol is preferred for patients with gastrointestinal risk factors. Patients at low

cardiovascular risk may take traditional NSAIDs or a COX-2 inhibitor; however, the Canadian Association of Gastroenterology suggests that the use of naproxen may be appropriate for patients at high cardiovascular risk[27].

Complications

Several complications can occur in patients with peptic ulcer disease of any etiology. They are the main reasons for the high morbidity and mortality associated with this disease found until now. The routine application of different gastro protection strategies and eradication therapies for Helicobacter pylori infection, have reduced significantly the incidence relative to that seen in the previous decades. They are more common in habitual smokers and chronic NSAIDs users [28, 29]. There are four major complications of peptic ulcer disease: Bleeding, perforation, penetration and obstruction.

i.Bleeding

Although its incidence has declined a little in the recent years; however remains being the most common complication and appears in around10-20% of patients. It is a frequent cause of admission in emergencies. Ulcers NSAIDs related are more likely to bleed than those caused only by Helicobacter pylori chronic infection. Populations at a greatest risk are the elderly and those with other serious conditions, such as respiratory, cardiac, cerebrovascular renal problems. A total of 80-90% of upper gastrointestinal hemorrhage are from not variceal bleeding origin, and around 40-50% of these are caused by peptic ulcer disease. The mean associated mortality is around, 5% It can take various clinical manifestations: 15% have melenas, 30% hematemesis, 50% have both and about 5% has hematochezia caused by severe bleeding. In other cases, ulcer bleeding may have a chronic course, manifestating as iron deficiency anemia or a positive fecal occult blood. There is a strong correlation between ulcer bleeding and use of NSAIDs or aspirin, because these drugs predispose to ulceration and inhibition of platelet aggregation [30]. Although not seems that the exclusive use of corticosteroids substantially increase the risk of ulcer bleeding, the combined use of these drugs together with NSAIDs, may increase by tenfold the risk of this complication[31,32]

ii.Perforation

It occurs in up to 5% of patients with peptic ulcer. Usually correspond to 60% of duodenal ulcers cases, most of them located at the anterior wall of the duodenal bulb and 40% of gastric ulcers, often affecting lesser curvature. Free perforation of a duodenal or gastric ulcer into the peritoneal cavity may endanger the patient's life. It usually appears as a sudden, severe abdominal pain, located in the epigastrium, which may radiate to back or become diffuse and associated with acute shock suggests a complicated ulcer perforation with peritonitis. Typically the patient usually remains motion less, his thighs flexed on the abdomen giving the impression of gravity. On examination, a hard, rigid abdomen is seen wither bound. Auscultation may initially show increased intestinal noises and as the condition progresses, they diminish and finally almost disappear. Around 70% of cases, present visible pneumoperitoneum at plain radiologic abdomen. The etiology of duodenal ulcer perforated appears to be multi factorial such as alcohol, tobacco, Helicobacter pylori, and especially intake of NSAIDs, since more than a third of the perforations are related to taking these, even to reaching figures of up to 50%, mainly in the elderly and being sometimes the only acetyl salicylic acid NSAID used even in small doses[33,34]. Another cause although much less common, is cocaine chronic consumption. The patho physiology of duodenal ulcer perforated by cocaine remains speculative. Chances are that perforation occurs by a localized vasoconstriction or vascular thrombosis[35]. It has been reported that in these cases the majority (40%) are placed mostly in juxta-pyloric area.

iii.Penetration

This complication occurs when an ulcer cross the wall of the stomach or duodenum, but instead of drilled freely into the peritoneal cavity, burrow into an adjacent organ. It occurs in approximately 25% of duodenal ulcers and 15% of gastric ulcers. Adjacent organs that extend most often are the pancreas, liver or omentum. The clinical presentation may be similar to that of uncomplicated ulcer but the pain is usually more severe and persistent[36].Pain cannot be relieved by eating, or even may worse and more often, wakes the patient at night. Typically, pain radiating to the back, when an ulcer penetrated to the pancreas or to right upper quadrant, appears when penetration is in the gastro hepatic omentum. Rarely, penetrating peptic ulcers may form fistulas between the duodenum and bile duct (choledoco-duodenal fistula) or between the stomach and colon (gastro-colic fistula).

iv.Obstruction

It is an uncommon complication, which represents approximately 5% of ulcer-related complications. Until about 1970, peptic ulcers represented the most common cause of obstruction to gastric emptying[37]. In the last years, however, has decreased the frequency of obstruction secondary to peptic ulcer and currently gastric malignancies are the leading cause of gastric outlet obstruction[38].

Epidemiology

The point prevalence of active H pylori ulcer is approximately 1% about 1.5 to 2 million American adults have an active ulcer at any time. As recently as 1990 the burden of peptic ulcer disease in the united states was estimated at 4 million cases, with 500,000 new cases annually[39]. The lifetime prevalence of peptic ulcer ranges from approximately 11% to 20% for men, and 8% to 11% for women[40]. In japan, the male to female ratio for pepticulcer is about 2:1, with the rate of gastric ulcers being about 1.5 times greater than that of duodenal ulcers for either sex[41].In western developed countries, the ratio of duodenal ulcer to gastric ulcer is reversed, with a higher incidence of duodenal ulcers. Peptic ulcer still causes major economic losses and health care expenditures because of lost worker productivity, restricted activity, physician visits, and hospitalizations. Expenditures in the united states for peptic ulcer disease are approximately 20 billion per year. The prevalence of H pylori infection and H pylori peptic ulcers has steadily declined in the united states. In contrast, nonsteroidal anti inflammatory drug (NSAID) use has remained high. In chronic NSAID users the point prevalence of gastric ulcers ranges from 9% to 31% and of duodenal ulcers ranges from 0% to 19%[42,43]. The recent introduction of the selective cyclooxygenase-2 (COX-2) inhibitors may result in a reduction in the incidence of NSAID ulcers but this may be offset in part by the increased use of aspirin for cardiovascular prophylaxis. The risk of major gastrointestinal bleeding from aspirin therapy is about 2.5% per annum, even with low-dose therapy[44].

IN VITRO ANTIOXIDANT ACTIVITY OF *CANTHIUM PARVIFLORUM*

Antioxidant are substances that significantly delay of an oxidizable substrate when present at low concentrations in comparison with those of the substrate. The activities of free radicals have been implicated in aging, destruction of DNA, Obstruction of arteries, cancer, strokes, cardiac and central nervous system disorders which have led to an increase in the investigation of substances that can protect against these reactive oxygen species and thus may play a role in disease prevention.

Endogenous antioxidants are synthesized with in the system of living organisms and repair free radical damage internally by initiating cell regeneration while exogenous antioxidants which are derived from sources outside the living systems such as diets stimulate cell repair externally. The growing need to complement these endogenous antioxidants has to an increased supplementation by exogenous sources. At present, there are interests and widespread researchers on exogenous antioxidants from natural sources perhaps, due to the fact that they are less expensive, readily available and believed to have lesser side effects when compared to their synthetic counterparts

Testing of wound healing activity

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen(connective tissue) production. Later, the epithelial tissue (the outer skin) is regenerated. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling.

The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction. Angiogenesis involves new blood vessel growth from endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts excrete collagen and fibronectin to form a new, provisional extracellular matrix. Subsequently, epithelial cells crawl across the wound bed to cover it and the wound is contracted by myofibroblasts, which grip the wound edges and undergo contraction using a mechanism similar to that in smooth muscle cells.

CHAPTER II

PLANT PROFILE

Figure 5 Plant Profile

2.1. SCIENTIFIC CLASSIFICATION[45]

The scientific classification of *Canthium Parviflorum* as following,

Division : Phenerogams
Class : Gamopetalae
Order : Gentianales
Family : Rubiaceae
Genus : *Canthium*
Species : *Parviflorum*

2.2. VERNACULAR NAMES OF *CANTHIUM PARVIFLORUM*[45]

Tamil : Karaichedi

Hindi : Kirma

Malayalam : Cherukara

Telugu : Karai

English : Carraycheddie

2.3. Morphology[46]

The Rubiaceae are trees, shrubs or infrequently herbs comprising about 450 genera and 6500 species, including some lianous forms. The leaves are simple and usually entire, and are opposite or sometimes whorled; stipules are present and interpetiolate. The flowers are nearly always bisexual and actinomorphic, often heterostylous, and usually are in cymose inflorescences. The calyx is somewhat reduced and 4-5 lobes or sometimes the lobes are absolute or rarely one of them greatly expanded and brightly colour. The sympetalous corolla is mostly 4-5 lobed, occasionally with 3 or upto 10 lobes. The androecium consists of as many stamens as corolla lobes and is adnate to the corolla tube or epigynous zone, alternate with the lobes. The gynoecium consists of a single compound pistil of 2 or seldom more carpels, a single style, and a nearly always inferior ovary with the number of locules.

2.4. MEDICINAL IMPORTANCE[47]

- *Canthium parviflorum* used as laxative and also to cure gout.
- *Canthium parviflorum* plant is having germination problems and is frequently attacked by meliola fungi.
- This plant material is used for its pharmacological importance as an anthelmintic, antidysenteric, antispasmodic and as a diuretic.
- The leaves are used to cure vitiated conditions of kapha in fever and constipation.

CHAPTER-III

REVIEW OF LITERATURE

1. **Adebiyi et al., (2017)[48]** Free radicals are reactive molecules involved in many physiological processes and have been associated with many diseases, such as cancer, arthritis and liver injury. As a results, there is need to explore substance with free radical scavenging and or antioxidant activity. The present study was designed to evaluate the free radical scavenging activity of ethanol extract of leaf and stem of Grewia carpinifolia using various in vitro models. Ascorbicacid was used as the reference in the study. 1,1-Diphenyl-2-picrylhydroxyl(DPPH)quenchingassay, 2,2'-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS) cation decolorization test, ferric reducing antioxidant power(FRAP) Assay systems were selected for the present experiment. The ability of the extracts to inhibit lipid oxidation was measured using Thiobarbituric Acid Reactive substances (TBARS) assay. The extracts were used at 2.0, 0.4, 0.6, 0.8 and 1 mg/ml concentrations and radical scavenging activity was determined in terms of inhibition percentage. The IC_{50}-(concentration required for 50% inhibition) was also calculated for each radical. The study revealed that Grewia carpinifolia has a high radical scavenging activity in the various radical systems. The total phenolic content was 19.08 ± 1.21 mg gallic acid equivalent (GAE)/g extract and 14.85 ± 1.09 mg GAE/g extract for the leaf and stem respectively while the flavonoid content was 9.00 ± 0.13 and 13.22 ± 1.53 mg quercetin/g extract. The antioxidant activity of Grewia carpinifolia extract may be due to the high level of flavonoids and phenols in the plant.
2. **Sabiu et al., (2015)[49]** This study investigated polyphenolic constituents and gastroprotective effects of aqueous leaf extracts of spondias mombin and ficus exasperate against indomethacin-induced gastric ulcer in rats. Ulceration was induced by a single oral administration of indomethacin (30mg/kg body weight). Wistar rats were pretreated with esomeprazole (reference drug) at a dose of 20 mg/kg body weight,s.mombin or F.exasperata at 100 and 200 mg/kg body weight once daily for 21 days prior to ulcer induction. At the end of the experiment, gastric secretions and antioxidant parameters were evaluated. We observed that the significantly increased ($p<0.05$) ulcer index, gastric volume, malondialdehyde

level and pepsin activity were effectively reduced following treatment with S.mombin and F.exasperata. The extracts also markedly attenuated the reduced activity of superoxide dismutase as well as pH and mucin content in the ulcerated rats. These findings are indicative of gastroprotective and antioxidative potentials of the extracts which is also evident in the degree of % inhibition against ulceration. The available data in this study suggest that the extracts of S.mombinand F.exasperata proved to be capable of ameliorating indomethacin-induced gastric ulceration and the probable mechanisms are via antioxidative and proton pump inhibition.

3. **Partap et al., (2014)[50]** The free radical scavenging potential of methanolic extract of Leptadenia pyrotechnica was studied on in vitro antioxidant models. The antioxidant potential was evaluated by determining the activity of hydrogen peroxide (H2O2) radicals scavenging and 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay. In all these studies, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. The extract was also shown to have high phenolic content i.e. 99.09±0.10 µg/mg. These results clearly indicated that MELP could be a potential source of natural antioxidant and effective against free radical mediated diseases.
4. **Lian et al., (2014)[51]** In this study, ultrasound-assisted extraction (UAE) and other methods of extracting flavonoid compounds and ferulic acid (FA) from S. sinensis were investigated. Five different extraction methods, including water extraction (W), water extraction using UAE (W+U), 75% ethanol extraction (E), 75% ethanol extraction using UAE (E+U), and supercritical CO2 extraction (SFE) were applied in the extraction of bioactive compounds (flavonoids and ferulic acid) in order to compare their efficiency. The highest yield of flavonoids (4.28 mg/g) and ferulic acid (4.13 mg/g) content was detected in the E+U extract. Furthermore, S. sinensis extracts obtained by E+U show high antioxidant activity, and IC50 values of 0.47 mg/mL for DPPH radicals and 0.205 mg/mL for metal chelating activity. The total antioxidant assay shows superoxide radical scavenging capacity and in vitro mushroom tyrosinase inhibition in a dose-dependent manner, suggesting that E+U can be used for extraction of bioactive compounds from S. sinensis.
5. **White et al., (2014)[52]** Chronic diseases such as cancer, diabetes, neurodegenerative and cardiovascular diseases are characterized by an enhanced

state of oxidative stress, which may result from the overproduction of reactive species and/or a decrease in antioxidant defenses. The search for new chemical entities with antioxidant profile is still thus an emerging field on ongoing interest. Due to the lack of reviews concerning the antioxidant activity of lichen-derived natural compounds, we performed a review of the antioxidant potential and mechanisms of action of natural compounds isolated from lichens. The search terms "lichens", "antioxidants" and "antioxidant response elements" were used to retrieve articles in LILACS, Pubmed and web of science published until February 2014. From a total of 319 articles surveyed, 32 met the established inclusion and exclusion criteria. It was observed that the most common isolated compound studied was usnicacid, cited in 14 out of the 32 articles.The most often described antioxidant assays for the study of in vitro antioxidant activity were mainly DPPH, LPO and SOD. The most suggested mechanisms of action were scavenging of reactive species, enzymatic activation and inhibition of iNos. The compounds isolated from lichens are possible candidates for themanagement of oxidative stress, and may be useful in the treatment of chronic diseases.

6. **Reddy Palvai et al., (2014)[53]** *Canthium parviflorum*leaves were analyzed for their proximate and phytochemical composition. The leaves were extracted with methanol (ME) and analyzed for antioxidant activity by radical scavenging method, reducing power, ferric reducing capacity, and in vitro inhibition of Fenton's reagent induced oxidation in oil emulsion and microsomes. In addition, the effect of high temperature (100^{o} c , 15 and 30min) and pH (4.5,7,and 9) on the antioxidant activity of ME was investigated. The leaves were rich in polyphenols, flavonoids β-carotene, glutathione, α-tocopherol, and ascorbic acid. The ME exhibited varying degree of antioxidant activity in a dose dependent manner. The RSA was 68%-500µg. Reducing potencywas 0.34 and FRAP was 1.377. *Canthium* exhibited greater inhibition of oxidation in microsomes (73%) than in the oil emulsion (21%) . Heat treatment resulted in reduction of radical scavenging activity of extract from 68% to 40%. At pH 4.5 and 7 methanol extract exhibited some percent of antioxidant activity which ranged between 18 and 32% . Data indicates *canthium* as a good source of antioxidants and methanol extract exhibited good antioxidant activity.
7. **Patro et al., (2014)[54]** medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day to day practice. *Canthium*

coromandelium (Rubiaceae) is one of traditional medicinal plant in India which is used for treatment of various ailments. Different parts of plants (ie., leaves, bark,stem,fruits,root and even whole plant) have shown to have various pharmacological activities like antimicrobial activity, antioxidant activity, hepatoprotective activity, antimalarial activity,anti-diabetic activity,anti asthmatic and antibacterial activity.Phytochemicals reported in the plants have been listed based on their pharmacological activity.Although Phytopharmacological reports are very less, still it is c.onsidered as a valuable source of treatment against various diseases. The present review highlights a literatue on botanical, chemical and pharmacological discussionof *canthium coromandelicum*.

8. **Roy et al., (2013)[55]** Peptic ulcer, also known as PUD or peptic ulcer disease, is an ulcer (defined as mucosal erosions equal to or greater than 0.5 cm) of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. Symptoms include abdominal pain with severity relating to mealtimes, after around 3 hours of taking a meal; bloating and abdominal fullness; nausea, and copious vomiting; loss of appetite and weight loss etc. There are many herbs, nutrients, and plant products that have been found to play a role in protecting or helping to heal stomach and peptic ulcers. Few human trials are available, but many have show good potential in animal or in vitro studies. And the present study was aimed to collect information on various herbs which are used in treating Peptic Ulcer in various parts of the world, depending upon the data's provided by various researchers.
9. **Owoyele et al., (2013)[56]** NSAIDS (drugs use in pain management) have been linked with ulcer and employed in several animal experiments, but the ulcer dose has been conflicting. In this regard, an animal model experiment was carried- out to determine the ulcer- dose of indomethacin on female wistar rats. Based on this objective, three varying doses (30,40 and 50mg/kg/bw) of indomethacin were respectively given orally to three groups (B,C and D) of 48hr- fasting rats weighing 200±25g. Eight hours later, the animals were sacrified and the stomach harvested and compared with 48hr- fasting/untreated control (group A) for ulcer index (UI) and macroscopic examination (ME) using standard procedures.Results showed different degrees of gastric ulcers in a dose dependent fashion in all the treated groups and were supported by macroscopic features. Specifically, the 30mg/kg/bw treated group presented a mean UI of 3.34±0.30mm while the 40mg/kg/bw and

50mg/kg/bw groups presented a UI of 11.02±1.31mm and 19.53±2.87mm respectively. The 50mg/kg/bw treated rats however, presented a high degree of weakness, behavioural changes and reduced physical activity;suggesting therefore, that for experimental purposes, the physical and behavioural influence of indomethacin should be considered in the determination of ulcer-doses, since it may likely affect the outcome.

10. **Suleyman et al.,(2012)[57]** studied the antiulcer activity of lacidipine was investigated on indomethacin-induced gastric ulcer model and it was examined whether the antiulcer effect of lacidipine was related to oxidant/antioxidant parameters in rats.Anti-ulcerative effects of lacidipine were investigated on an indomethacin-induced gastric ulcer model in rats. The antiulcer capability of lacidipine was compared to 20 mg kg^{-1} famotidine. Results showed that lacidipine prevented the formation of indomethacin-induced ulcers at 2 and 4 mg kg^{-1} doses significantly. Enzymatic and non-enzymatic antioxidant parameters, such as total glutathione,superoxide dismutase and glutathione peroxidase, were found low and enzymatic and non-enzymatic oxidant parameters, such as myeloperoxidase and malondialdehyde, were found high in the gastric tissues of indomethacin-given rats. Indomethacin acts through not only the inhibition of the synthesis of cytoprotective prostaglandins but also by affecting enzymatic and non-enzymatic, oxidant and anti-oxidant mechanisms.
11. **Polat et al., (2011)[58]** A gastroprotective effect occurs when α(2) receptors are innervated. The dextro isomer of medetomidine, dexmedetomidine, is a highly selective α(2)- adrenoreceptor agonist. The aim of this study was to investigate whether dexmedetomidine has an antiulcerative effect and to show whether the antiulcer mechanism of dexmedetomidine is linked with oxidant/ antioxidant parameters. The antiulcerative effect of dexmedetomidine was studied in an indomethacin-induced ulcer model, and some oxidant/antioxidant parameters were measured in these gastric tissues. Whereas the average ulcerous areas for the groups that received 10,25,50, and 100 µg/kg dexmedetomidine doses were 29 ± 4.2, 8 ±2.1, 0 ± 0 and 0 ± 0 mm(2), respectively, the ulcerous area was 52.1 ± 4.5 mm(2) in the indomethacin control group and 0.5 ± 0.2 mm(2) in the famotidine group.In conclusion, the α(2)-adrenoreceptor agonist dexmedetomidine showed a significant antiulcerative effect in rat gastric tissue at all doses.This antiulcerative effect is

stronger with increasing dosage; at the 50 and 100 µg/kg doses, no ulcerous areas were observed. In light of these results, we conclude that there is a correlation between antiulcer mechanisms and α(2)-receptor activation. In rats given dexmedetomidine, all of the investigated antioxidant parameters increased, except for catalase (CAT). Conversely, aside from myeloperoxidase (MPO), all oxidant parameters decreased. Therefore, antioxidant/ antioxidant parameters play a role in the antiulcer mechanisms of dexmedetomidine.

12. **Pasumarthi et al., (2011)[59]** investigated the presence of potential phytochemicals like tannins, alkaloids, flavonoids, saponins, steroids, anthraquinones and reducing sugars in medicinal plants like Hemidesmus indicus, *Canthium parviflorum* and Canavalia gladiata for testing their effect on Caco-2 cell viability. The plant extracts were tested for their cytotoxic effect on the colon adenocarcinoma cell line (Caco-2). MTT assay was used to evaluate the viability of cells in the presence of the extracts. Methanolic extract of *Canthium parviflorum* extract showed to be a potent cytotoxic with an IC50 at 52 µg/ml. H. indicus showed an IC50 at 60 µg/ml and C. gladiata was nontoxic.
13. **Gadekar et al., (2010)[60]**Peptic ulcer are a broad tem that includes ulcers of digestive tract in the stomach or the duodenum. The formation of peptic ulcers depends on the presence of acid and peptic activity in gastric juice plus a breakdown in mucosal defenses. There are two major factors that can disrupt the mucosal resistance to injury: non-steroidal anti-inflammatory drugs (NSAIDS) example, aspirin and Helicobacter pylori infection.Numerous natural products have been evaluated as therapeutics for the treatment of a variety of diseases, including peptic ulcer. There has been considerable pharmacological investigation in to the antiulcer activity of some compounds. In this work, we shall review the literature on different medicinal plant and alkaloids with antiulcer activity. This article reviews the antacids/ anti- peptic, gastroprotective of the most commonly employed herbal medicines and their identified active constituents. The experimental parameters used for antiulcer activity were cold restraint stress-induced ulcer model, diclofenac –induced ulcer model in rats, (HCl-ethanol)- induced ulcer in mice and water immersion stress-induced ulcer in rats. The ideal aims of treatment of peptic ulcer disease are to relieve pain, heal the ulcer and delay ulcer recurrence. About 70% of patients with peptic ulcer disease are infected by Helicobacter pylori and eradication of this microorganism seems to be curative for this disease. This article reviews drugs derived from medicinal plant more commonly used

in the world for peptic ulcer and if reported the antiulcer activity. This article will be concerned only with the antiulcer and gastro-protective effects.

14. **Suleyman et al., (2010)[61]** Indomethacin is an indol derivative, non-steroidal, anti-inflammatory drug with anti-inflammatory, analgesic, and antipyretic effects. Indomethacin became the first-choice drug to produce an experimental ulcermodel as a results of having ulcerogenic potential than other non-steroidal anti-inflammatory drugs (NSAIDS). There have been several conflicting reports about the ulcerogenic mechanism of indomethacin ; the mechanism is still unclear. It has been suggested that indomethacin induces gastric damage via inhibiting the release of protective factors like cyclooxygenase-1 (cox-1), prostaglandin E2 (PGE2), bicarbonate, and mucus; increasing aggressive factors like acid; and increasing oxidant parameters while decreasing antioxidant parameters. Classic antiulcer drugs are known to produce antiulcer effects by activating against indomethacin (increasing PGE2, mucus , and bicarbonate production; inhibiting acid secretion; decreasing oxidant parameters; and increasing antioxidants). However, some antiulcer drugs have been shown to inhibit indomethacin-induced ulcers without affecting acid and mucus secretion or oxidant parameters, as well as to inhibit the production of protective factors like COX-1, PGE2, and bicarbonate, and to reduce antioxidant parameters. In order to resolve the contradictions in the abovementioned data, this review hypothesized a relationship between indomethacin- induced ulcers and α 2 adrenergic receptors. It is suggested that blockage of α 2 adrenergic receptors may be responsible for the increase in the aggressive factors induced by indomethacin, and stimulation of α 2 adrenergic receptors may be responsible for the increase of protective factors induced by antiulcerdrugs.
15. **Sachin et al., (2009)[62]** Gastric ulcer is one of the most prevalent gastrointestinal disorders, which affects approximately 5-10% of people during their life. In recent years, abundant work has been carried out on herbal medicine to clarify their potential efficacy in gastric ulcer prevention . Here present study was carried out to investigate antiulcer activity of methanol extract of Erythrina indica (Family: Fabaceae) leaves in pylorus ligated and indomethacin induced ulceration in the albino rats. Preliminary methanol extract of E.indica was subjected to the acute oral toxicity study according to the OECD guideline no.423. Based on which, three dose

levels i.e.125, 250 and 500mg/kg were selected for the further study. In pylorus ligation induced ulcer model, various parameters were studied viz. gastric volume , pH, total acidity, and ulcer index. Ulcer index and percentage inhibition of ulceration was determined for indomethacin induced ulcer model. Ranitidine at 100mg/kg was used as the standard drug.Pretreatment of methanol extract of E.indica leaves showed significant ($P<0.01$) decrease in the gastric volume, total acidity and ($P<0.01$) decrease in number of ulcers and ulcer score index in pylorus and indomethacin induced ulceration models. The methanol extract of E.indica leaves possess significant antiulcer properties in a dose dependent manner.In conclusion the antiulcer properties extract may be attributed to the polyphenolic compounds that are present in it.

16. **Sahu et al., (2009)[63]** Antioxidant activity of methanolic and extract of Amorphophallus campanulatus tuber was studied for its free radical scavenging property on different in vitro models e.g – 1,1- diphenyl-2- picryl hydrazyl (DPPH) method,nitric oxide method and reducing power method.The extracts showed good dose dependent free radical scavenging property in all the models. IC_{50} values for water and methanolic extract were found to be 59.91 and 99.40 µg/mL in DPPH method and 77.02 and 70.20 µg/mL in nitricoxide method.In reducing power method, aqueous shows more reducing poweras compared to methanolic extract. Ascorbic acid was used as standard.It is concluded that the aqueous extract shows more antioxidant activity as compared to methanolic extract.
17. **De Lira Mota et al., (2009)[64]** Peptic ulcers are a common disorder of the entire gastrointestinal tract that occurs mainly in the stomach and the proximal duodenum. This disease is multifactorial and its treatment faces great difficulties due to the limited effectiveness and severe side effects of the currently available drugs. The use of natural products for the prevention and treatment of different pathologies is continuously expanding throughout the world. This is particularly true with regards to flavonoids, which represent a highly diverse class of secondary metabolites with potentially beneficial human health effects that is widely distributed in the plant kingdom and currently consumed in large amounts in the diet. They display several pharmacological properties in the gastroprotective area, acting as anti-secretory, cytoprotective and antioxidant agents. Besides their action as gastroprotectives, flavonoids also act in healing of gastric ulcers and additionally these polyphenolic compounds can be new

alternatives for suppression or modulation of peptic ulcers associated with H. pylori. In this review, we have summarized the literature on ninety-five flavonoids with varying degrees of antiulcerogenic activity, confirming that flavonoids have a therapeutic potential for the more effective treatment of peptic ulcers.

18. **Zahin et al., (2009)[65]** Methanolic extracts of Plumbago zeylanica (Root), Acorus calamus (Rhizome), Hemidesmus indicus (Stem) and Holarrhena antidysenterica (Bark), used in Ayurvedic medicines for number of ailments were evaluated for their antioxidant activity by ferric thiocyanate (FTC) assay and compared with thiobarbituric acid (TBA) method. The order of antioxidant potential according to FTC assay was found to be highest in Plumbago zeylanica followed by Holarrhena antidysenterica, Acorus calamus and Hemidesmus indicus.Whereas there is slightly difference in activities as measured by TBA method. The antioxidant activity of medicinal plants was at par with the commercial antioxidant butylated hydroxy toluene (BHT), L-Ascorbic acid and α- tocopherol. Further, the radical-scavenging activity of the extracts was measured as decolourizing activity followed by the trapping of the unpaired electron of DPPH. The percentage decrease of 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) standard solution was recorded maximum for Hemidesmus indicus (77.0%) followed by Plumbago zeylanica (73.41%), Acorus calamus (20.88%) and Holarrhena antidysenterica (20.06%) extracts at a concentration of 100 µg/ml. Phytochemical analysis revealed the presence of major phytocompounds like alkaloids, glycosides, phenolics and saponins. Moreover, total phenolics concentration equivalents to gallic acid was found in the range of 59.50 to 109.0 mg/g of plant extracts, which correlated with antioxidant activity. The findings indicated promising antioxidant activity of crude extracts of the above plants and needs further exploration for their effective use in both modern and traditional system of medicines.

19.Mohideen et al., (2003)[66] studied wound healing and diuretic activities of *Canthium parviflorum*. In ayurvedic system of medicine, the shrub *Canthium parviflorum Lam*. is used as laxative and to cure gout. Tribes of Orissa use its fruits to treat headache. Basing upon the reports on astringent activity of leaves of this thorny shrub, pharmacologist evaluated the wound healing and diuretic activity of the aqueous and alcoholic leaf extracts. The extract of leaves in the form of ointment was applied on excision wound in male wistar albino rats with body weight between 175-225 g. A significant healing process as evidenced by increased rate of wound contraction as

compared to control was observed. The aqueous extract of 10% w/w ointment exhibited equivalent wound healing activity as nitrofurazone ointment. Significant diuretic activity and dose dependent response was exhibited by both the extracts.

20. Inauen et al., (1988)[67] We investigated whether the trophic actions of prostaglandins, omeprazole, and indomethacin on gastric mucosa lead to accelerated healing of gastric ulcers in the rat. Cryoulcers were produced in the corpusarea and treated with 16,16-dimethyl prostaglandin E_2 (5 or 100 µg/kg b.i.d., intragastrically), omeprazole (40 µmol/kg once daily, subcutaneously), indomethacin (2 mg/kg b.i.d., subcutaneously). A the end of the treatment, plasma gastrin, cell labeling index (autoradiography with (^{3}H)thymidine), and the size and depth of mucosaldefects were measured. Compared with placebo, omeprazole accelerated ulcer healing as indicated by a smaller ulcer area (1.1± 0.2vs. 4.8 ± 1.2 mm^2 (mean ± SEM) and smaller ulcer depth (383 ± 31 vs. 488 ± 41 µm) after 10 days of treatment. Prostaglandins did not affect ulcer healing despite thickening of gastric corpus mucosa. Indomethacin delayed ulcer healing and reduced the labeling index. Omeprazole induced a marked hypergastrinemia (208- F 12 vs. 66 ± 12 pmol/L on day 5, and 469 ± 23 vs. 58 ± 16 pmol/L on day 10). The results indicate that abolishment of acid secretion by omeprazole accelerates healing. Trophic actions and "cytoprotective" effects by prostaglandins are not relevant for ulcer healing in this model.

CHAPTER-IV

AIM AND OBJECTIVES

4.1. AIM

The aim of our study is **"Pharmacological evaluation of ethanolic extract of leaves of *Canthium parviflorum* (*Invivo* and *In vitro*)".**

4.2. OBJECTIVE

To emphasize the above aim, we has followed certain objectives

- Extraction of leaves of *Canthium parviflorum* by using ethanol as solvent
- The extracts are Concentrated and subjected for Preliminary phytochemical investigation
- *In vitro* anti-oxidant activity of *Canthium parviflorum*
- *In vivo* antiulcer activity of *Canthium parviflorum*
- In vitro wound healing model of *Canthium parviflorum*

4.3. PLAN OF WORK

The detailed plan of work laid in to the following levels,

- Collection and Extraction of *Canthium parviflorum* leaves by using ethanol as solvent
- The extracts are concentrated and subjected for preliminary phytochemical investigation and Thin Layer chromatography
- The *Canthium parviflorum* and ethanolic extract are subjected for *In vitro* Antioxidant activity such as,
    - DPPH photometric assay
    - Superoxide scavenging activity
    - Nitric Oxide Radical scavenging activity

- The *Canthium parviflorum and* ethanolic leaf extract is screened for *In vivo* antiulcer activity by Indomethacin of ulcer in rat model

- The *Canthium parviflorum* and ethanolic extract is screened for *in vitro* wound healing activity

CHAPTER-V

MATERIALS AND METHODS

5.1. MATERIALS

Methanolic solution was obtained from Sigma Aldrich, India,DPPH was obtained from sigma Aldrich, India , Nitro blue tetrazolium was obtained from sigma Aldrich, India, EDTA was obtained from shanghai youPeng Chemical Co.Ltd., Riboflavin was obtained from sigma Aldrich, Dimethyl sulfoxide was obtained from Thermo Fisher scientific, Sodium nitroprusside was obtained from loba chemie, Phosphate buffer saline was obtained from Thermo Fischer scientific , Nitrite was obtained from Thomas scientific, sulphanilicacid was obtained from sigma Aldrich, Naphthyl ethylene diamine dihydrochloride was obtained from Sigma Chemical Co, India, Diclofenac sodium was obtained from Titan Pharmaceuticals Ltd. (Mumbai). Indomethacin was obtained from sigma-Aldrich (st. Louis, MO, USA). Ranitidine was obtained from Cipla Pharmaceutical company, Pune. Formalin was obtained from Thermo Fischer Scientific India Pvt. Ltd. *Canthium parviflorum* leaves were collected from surulacode. All the chemicals and reagents used in this experiment are of analytical grade.

METHODOLOGY

5.2.1. COLLECTION OF PLANT MATERIALS

Canthium parviflorum leaves were collected locally from the Surulacodu (Kanyakumari Dist,Tamilnadu). The leaves were separated from the plant and washed with water and chloroform to remove soil particles, spread them and dried in the shade for 10 days.

5.2.2. PREPARATION OF EXTRACTION[68]

The leaves were shade dried on the laboratory bench for 10 days. The dry sample was milled and ground in to powder. Extraction involves the separation of a bioactive portion of the plant tissues from the inactive components by using selective solvents in standard extraction procedure. The dried coarse powder of leaves of *Canthium parviflorum* was extracted with 500ml of ethanol by Cold maceration method. After 72 hours, the extract was collected by filtration, evaporated to

dryness. The collected .extract was stored in desiccators at room temperature and used for further pharmacological study.

5.2.3. PRELIMINARY PHYTOCHEMICAL STUDIES

The extract of *Canthium parviflorum* obtained was subjected to qualitative analysis to test the presence of various phytochemical like alkaloids, saponins, Tannins, phlobatannins, flavonoids, glycosides, reducingsugar, terpenoids etc[69].

A) PROCEDURE

I. Tests for alkaloids

a. Hager's Test: To the 2-3ml of extract, few drops of dil.HCL and Hager's reagent was added and shake well. Yellow precipitate was formed showing the presence of alkaloids.

b. Mayer's Test: To the 2-3ml of extract, few drops of dil.HCL and Mayer's reagent was added and shake well. Formation of yellow precipitate showed the presence of alkaloids.

c. Dragendroff's Test: To the 2-3ml of extract, few drops of dil.HCL and Dragendroff's reagent were added and shake well. Formation of orange-brown precipitate showed the presence of alkaloids.

II. Test for saponin

Foam Test: To 1ml extract 20ml distilled water was added and shakes well in measuring cylinder for 15 min. Then 1cm layer of foam was form.

III. Tests for Tannins

a. $Fecl_3$ Solution Test: On addition of 5% $Fecl_3$ solution to the extract, deep blue black colour appeared.

b. Lead Acetate Test: On addition of lead acetate solution to the extract white precipitate appeared.

IV. Test for Phlobatannins

Take 2ml of plant extract, boiled with 1% aqueous hydrochloride acid, red colour precipitate was deposited.

V. Test for steroids

Salkowski Test: To 2ml of extract, 2ml of chloroform and 2ml of conc, H_2So4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

VI. Test for Flavonoids

Shinoda Test: The extract of the plant taken in a test tube, add 5ml of 95% ethanol with conc.Hcl and magnesium turnings. The appearance of intense cherry red colour indicates the presence of flavonoids or orange- red colour indicates the presence of flavonoids.

VII. Test for Anthraquinones

a.Legal's Test: To the 3ml extract, few ml of pyridine, 2 drops of sodium nitroprusside and a drop of 20% NaOH solution were added which gives pink colour indicates the presence of glycosides.

b.Borntrager's Test: To the 3ml extract was mixed with dilute.H_2So_4, and filtered. The filtrate was shaken with chloroform and the chloroform layer was separated. To this dilute ammonia was added which ammonical layer turns pink colour,indicates the presence of glycosides.

VIII. Test for Cardiac glycosides

Keller-Killiani Test: To the 5ml of extract, 1ml of conc. H_2So4, 2ml of Glacial acetic acid and 1 drop of $Fecl_3$ solution was added. Appearance of Brown ring shows the presence of cardiac glycosides.

IX.Test for Reducing sugar

a.Fehling's Test: To the 1ml of Fehling's A solution and 1ml of fehling's B solution were mixed and boiled for one minute. Now the equal volume of test solution was added to the above mixture. The solution was heated in boiling water bath for 5-10 minutes. First a yellow, then brick red precipitate was observed.

b. Benedict's test: To the Benedict's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water for 5 minutes. Solution appeared green showing the presence of reducing sugar.

c.Molisch's test: To the molisch's reagent and test solution were mixed in a test tube. The mixture was heated in boling water for 5 minutes. Appearance of violet purple colour ring showing the presence of reducing sugar.

X. Test for Terpenoids

Add a small amount of plant extract in separate test tube was taken with 2ml of chloroform; 5ml of concentrated sulphuric acid was carefully added to form a layer and observed for presence of reddish brown colour interface to show positive results for the presence of terpenoid.

B.THIN LAYERCHROMATOGRAPHY [70]

Thin layer chromatography (TLC) technique for separation of active compounds was carried out by the method of Hao et al. (2004). Slurry was prepared by mixing 30gm of silica gel G with 100ml of water. The slurry was poured into glass plate and the slurry was spreaded uniformly on the surface of the glass plate. After setting the glass plates were dried in hot air oven at 110^0c for 1hr. Baseline was drawn on the TLC plate.Small spot of solution containing the sample is applied to a plate and dried. Small amount of an appropriate solvent (eluent) Ethylacetate : Chloroform (9:7) which shows better separation of compounds, poured in to a TLC chamber to a depth of less than 1 cm. The container is closed with a cover glass or lid and is left for 10 minutes for saturation. The TLC plate is then placed in the chamber and allowed to run the chromatogram. The solvent moves up the plate by capillary action meets the sample mixture and carries it up the plate (elutes the sample). The dried plate is placed in a chamber containing a few crystals of iodine. The iodine vapor in the chamber oxidizes the substances in the various spots making them visible to the eye. Once the spots are visible they may be outlined with a pencil before the iodine coloration fades.

$$R_f \text{ value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

5.2.4. IN VITRO ANTIOXIDANT ACTIVITY OF *CANTHIUM PARVIFLORUM*

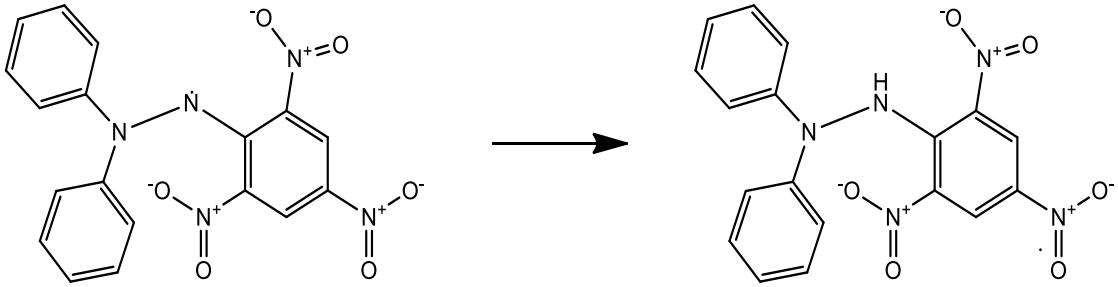
The following In vitro models were carried out to evaluate antioxidant activity for *Canthium parviflorum.*

- DPPH photometric assay
- Superoxide scavenging activity
- Nitric oxide scavenging activity

a)DPPH photometric assay

Principle

The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1-Diphenyl -2- picryl hydrazine. A coloured complex is formed which can be measured colorimetrically at 518 nm



DPPH **1,1-Diphenyl-2-picryl hydrazine**

Figure 6 DPPH photometric assay

Procedure

The effect of extract on DPPH radical was assayed using the method of Mensor *et al*., (2001). A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavenging activity(\%) = \frac{A_{518}\,\text{Control} - A_{518}\,\text{Sample}}{A_{518}\,\text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

b) Superoxide scavenging activity

Principle

NBT dye reduction method

In this method, the superoxide is produced by riboflavin. The superoxide anions are subsequently made to reduce nitroblue tetrazolium which yield a chromogenic product, which is measured at 560nm.

Procedure

Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne *et al.*, (1975). The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control a and test samples.

c) Nitric oxide scavenging activity

Principle

Nitric oxide is a very unstable species under aerobic conditions. It reacts with O_2 to produce the stable product nitrates and nitrite through intermediates through NO_2 and N_3O_4. It is estimated by using Garrat method (Garrat, 1964). In the presence of the test compound, which is a scavenger, the amount of nitrous acid will decrease. The extent of decrease will reflect the extent of scavenging, which is measured at 540nm.

Procedure

Preparation of Garrat' reagent

Three ml of reaction solution containing 2ml of sodium nitroprusside (10 mM) and 0.5ml phosphate buffer saline (1M) were incubated at 25ºC for 2.5h. After incubation, 0.5mL of the reaction mixture containing nitrite was pipetted and mixed with 1 mL of sulphanilic acid reagent (0.33%) and allowed to stand for 5min for completing diazotization. After that 1ml of naphthalene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 0.5h. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generate nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of Griess Illosvery reaction at 540nm.

5.2.5. *IN VIVO* OF *CANTHIUM PARVIFLORUM* EXTRACT BY INDOMETHACIN OF ULCER IN RAT MODEL

a. **Material**

Sterilized cotton buds, Gloves, Face mask, Micrometer,Dissection box.

b. **Animals**

Healthy Swiss albino rats of either sex weighing obtained from the AKCP animalhouse**(CPCSEA approval no: 509/02/C/CPCSEA/2018)**,housed under specificpathogen-free conditions were used for the study. The animals were placed at random and housed in polypropylene cages and were left 7 days for acclimatization to animal room maintained under controlled condition (a 12 h light–dark cycle at 22 ± 2^0C and relative humidity of 30-70%). All animals were allowed to free access to water and fed with standard commercial pellet diet. All animals were taken care of under ethical consideration as per the guidelines of CPCSEA. The Institutional Animal Ethics Committee (IAEC) approved the protocol. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee Animals were used for anti-ulcer study.

a. Grouping of animals

The animals were divided in to 5groups of 8 animals in each group. Group I was kept as normal without any treatment. All other groups were fasted for 36 hours and administered

with indomethacin (30 mg/kg b.wt). Group II (control) animals receive indomethacin alone. Group III, IV and V were pretreated with ranitidine (50mg/kg),Canthium Parviflorum extract 250mg/kg b.wt., and 500mg/kg b.wt. respectively, 1hour prior to the administration of indomethacin. After 6 hours, the animals were sacrified and stomach were removed and opened along the greater curvature to determine the ulcer index[74,75,76].

Determination of ulcer index (UI):

The ulcerative index was calculated by severity of gastric mucosal lesions and graded as follows:

Table 4 Determination of ulcer index(UI):

Erosions	Score
1mm or less	1
1-2 mm	2
More than 2mm	3

Then the UI was calculated by using the formula:

UI= 1 × (no.of lesions of grade1) + 2 × (no.of lesions of grade2) + 3 × (no. of lesions of grade 3)

Then the overall score was divided by a factor 10, which was designated as ulcer index (main and white, 1975).

5.2.6. IN VITRO WOUND HEALING ACTIVITY

CHORIOALLANTOIC MEMBRANE MODEL[77,78,79]

Embryonated chicken eggs (9 days old) were selected then divided into four groups and a small window, (1cm2) was made in the shell follows

GROUP I : Negative Control saline.

GROUP II : Positive Control Diclofenac sodium (50 µg/ml)

GROUP III : Ethanolicextract of *Canthium parviflorum*(100 µg/ml)

GROUP IV : Ethanolicextract of *Canthium parviflorum*(200 µg/ml)

GROUP V : Ethanolicextract of *Canthium parviflorum*(300 µg/ml)

GROUP VI : Ethanolicextract of *Canthium parviflorum*(400 µg/ml)

Whatman No. 1 filter paper was Small disks were generated using a standard 5 mm hole puncher, sterilized by autoclaving and stored for further use. The pre-sterilized filter disks were saturated with different concentrations of the crude extract, from 500 µg/ml, and the control solutions. Diclofenac sodium 50 µg/ml and sterile saline were used as positive and negative controls respectively. Eggshell window was closed and incubated at 370 C for 72 hrs. The window was then opened and the growth of new capillary blood vessels were observed and finally compared with the positive and negative control.

CHAPTER-VI

RESULTS AND DISCUSSIONS

6.1. a. PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary screening showed the presence of various phytoconstituents in ethanolic extract of *canthium parviflorum* and are Presented in **Table no:5**

Table no: 5 Result Preliminary phytochemical screening of the ethanolic extract of *Canthium parviflorum*

S.No	Constituents	Observation	Inference
1.	**Alkaloids**	**a.Hager's test-** Yellow precipitate was formed showing the presence of alkaloids. **b. Mayer's Test-** Formation of yellow precipitate showed the presence of alkaloids. **c. Dragendroff's Test-** Formation of orange-brown precipitate showed the presence of alkaloids.	**Presence of alkaloids**
2.	**Saponins**	**Foam test-** foam was form.	**Presence of Saponins**
3.	**Tannins**	**a.Fecl$_3$ test-Formation *of*** deep blue black colour appeared. **b.Lead acetate test-** Formation of white precipitate appeared.	**Presence of Tannins**
4.	**Phlobatannins**	Formation of red colour precipitate was deposited.	**Presence of Phlobatannins**

5.	**Steroids**	**Salkowski-** As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.	**Absence of Steroids**
6.	**Flavonoids**	**Shinoda test-** The appearance of intense cherry red colour indicates the presence of flavonoids or orange- red colour indicates the presence of flavonoids.	**Presence of flavonoids**
7.	**Anthraquinones**	**a.Legal's test-** Formation of pink colour indicates the presence of glycosides. b.Borntrager'stest- Formation of pink colour, indicates the presence of glycosides.	**Absence of Anthraquinones**
8.	**Cardiac glycosides**	**Keller-killiani test-** Appearance of Brown ring shows the presence of cardiac glycosides.	**Presence of Cardiac glycosides.**
9.	**Reducing sugar**	**a.fehling's test-**. First a yellow, then brick red precipitate was observed. **b.Benedict's test-** Solution appeared green showing the presence of reducing sugar. **c.Molich's test-** Appearance of violet purple colour ring showing the presence of reducing sugar.	**Presence of Reducing sugar**
10.	**Terpenoids**	presence of reddish brown colour interface to show positive results for the presence of terpenoid.	**Presence of Terpenoids**

Where + indicates Presence, - indicates Absence

6.1. b.THIN LAYERCHROMATOGRAPHY

$$R_f \text{ value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

$$= 0.31$$

Figure 7 Thin layer chromatography of *Canthium parviflorum* extract

6.2. *IN VITRO* ANTIOXIDANT ACTIVITY OF *CANTHIUM PARVIFLORUM*

6.2.1 DPPH photometric assay

The percentage of DPPH radical scavenging activity of *Canthium parviflorum a*re presented in Table 6. The ethanolic extract was found to be more effective when compared with standard rutin. The IC_{50} of the *Canthium parviflorum* and Rutin were found to be 280µg/ml and 470µg/ml respectively.

Table : 6 Results of *Canthium parviflorum extract* on DPPH assay

Concentration (µg/ml)	% of activity (±SEM)*	
	Sample	Standard (Rutin)
125	35.09 ± 0.05	17.85 ± 0.076
250	47.57 ± 0.03	21.08 ± 0.054
500	62.64 ± 0.11	51.21 ± 0.022
1000	68.04 ± 0.02	67.83 ± 0.014
	IC_{50}=280 µg/ml	**IC_{50}=470 µg/ml**

*All values are expressed as mean ± SEM for three determinations.

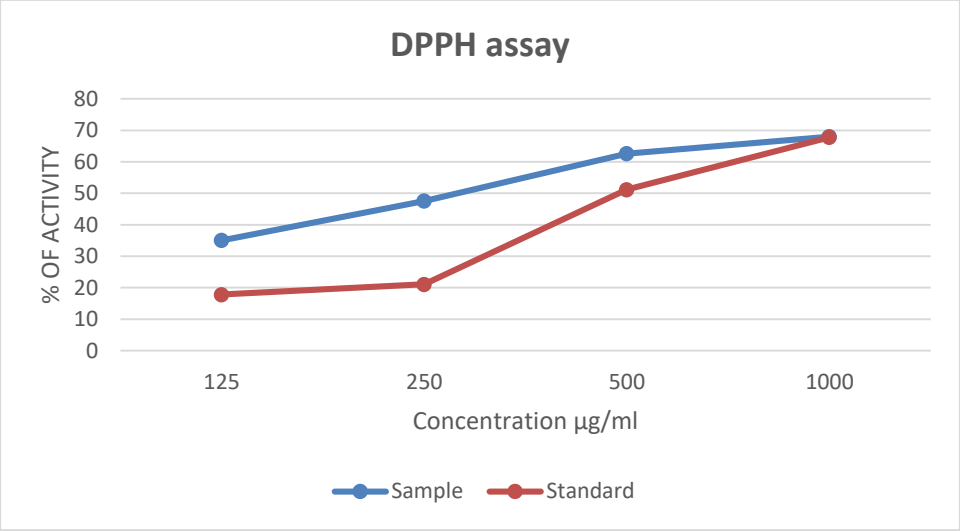


Figure 8 DPPH photometric assay

6.2.2. Superoxide scavenging activity

NBT dye reduction method

The percentage scavenging of superoxide anion examined at different concentrations of various extracts of *Canthium parviflorum* (125, 250, 500, 1000 µg/ml) were presented in Table7. The IC_{50} values of *Canthium parviflorum* were found to have strong superoxide radical scavenging activity;

when compared to that of standard Ascorbate. The IC_{50} of the *Canthium parviflorum* and Ascorbate were found to be 400µg/ml and 50µg/ml respectively.

Table: 7 Effect of *Canthium parviflorum* Superoxide anion scavenging activity

Concentration (µg/ml)	% of activity (±SEM)*	
	Sample	Standard (Ascorbate)
125	28.51 ± 0.53	72.81 ± 0.01
250	34.30 ± 0.59	90.31 ± 0.01
500	57.63 ± 0.67	91.99 ± 0.02
1000	74.92 ± 0.93	97.01 ± 0.01
	IC50= 400 µg/ml	**IC50 = 50 µg/ml**

*All values are expressed as mean ± SEM for three determinations

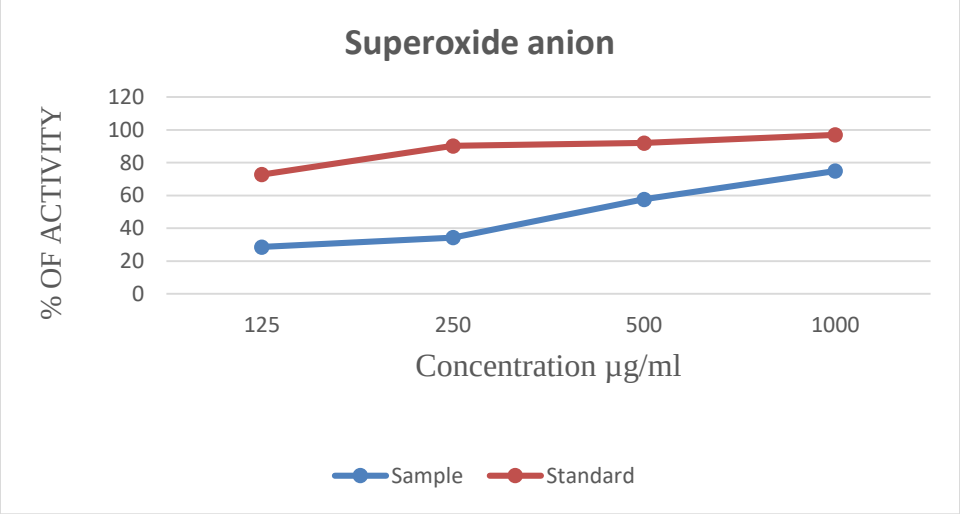


Figure 9 Superoxide anion scavenging activity

6.2.3. Nitric oxide scavenging activity

The reduction of nitric oxide radical by the various extracts of *Canthium parviflorum* and ascorbate was noted to be concentration dependent and was illustrated in Table 8. The sample most effective in scavenging nitric oxide radical activity than that of lower concentration. But when

compared with Ascorbate (standard), the sample showed significant result. The IC_{50} of the *Canthium parviflorum* of Plant 1 and Ascorbate were found to be 260µg/ml and 420µg/ml respectively.

Table: 8 Effect of *Canthium parviflorum* on Nitric oxide scavenging activity

Concentration (µg/ml)	**% of activity (±SEM)***	
	Sample	**Standard (Ascorbate)**
100	42.75 ± 0.36	27.15 ± 0.09
200	51.21 ± 0.13	31.35 ± 0.04
400	59.50 ± 0.20	61.62 ± 0.03
800	66.68 ± 0.64	56.45 ± 0.03
	IC50 = 260 µg/ml	**IC50 = 420µg/ml**

*All values are expressed as mean ± SEM for three determinations

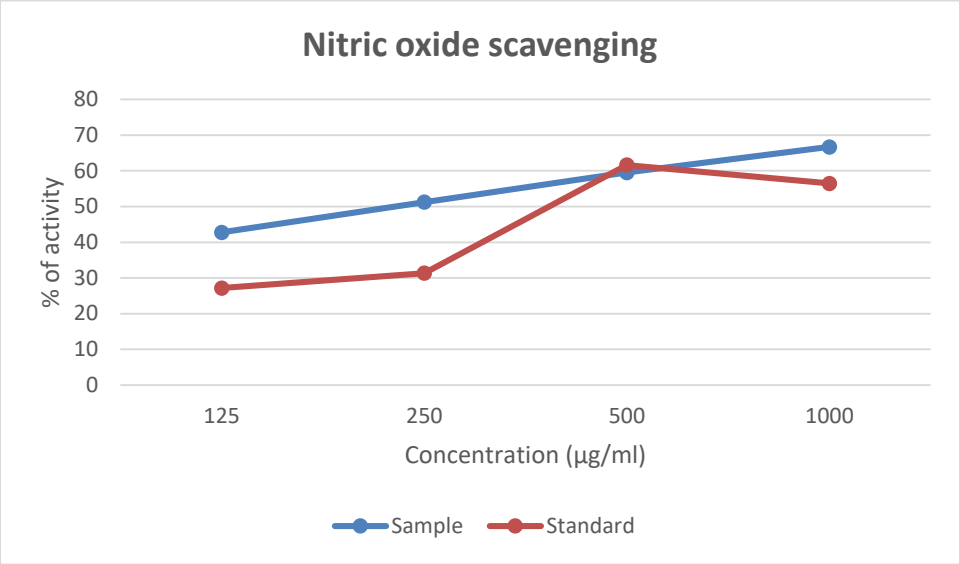


Figure 10 Nitricoxide scavenging activity

6.3. ANTI-ULCERACTIVITY

6.3.1. Effect of Indomethacin -InducedUlcer

The results obtained in the experimental model of indomethacin -induced gastric ulceration in rats are presented in **Table 9**. The ethanolic extract *Canthium parviflorum* was found to possess remarkable ulcer-protective properties 250mg/kg b.wt.and 500mg/kg b.wt. The effect of ulcer protection of ethanolic extract of *Canthium parviflorum*(46.67%) was observed at 250mg/kg and (51.16%) was observed at 500mg/kg, whereas the standard drug ranitidine gave (69.65%) of ulcerprotection.

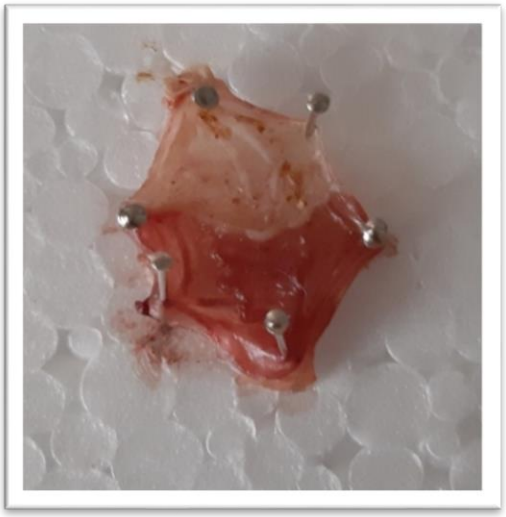


Fig 11 Normal control(No treatment)

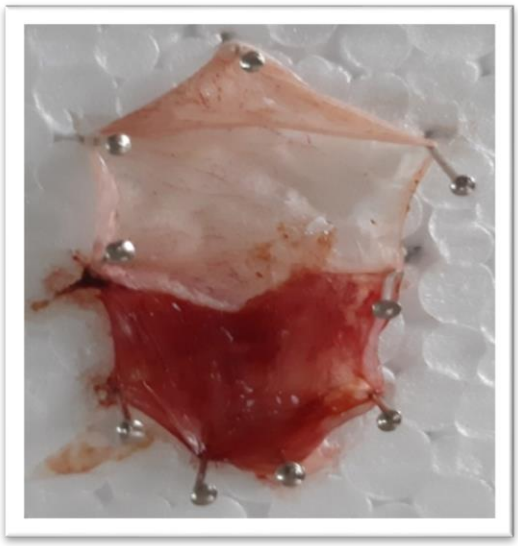


Fig no 12 Negative control (Treated with Indomethacin

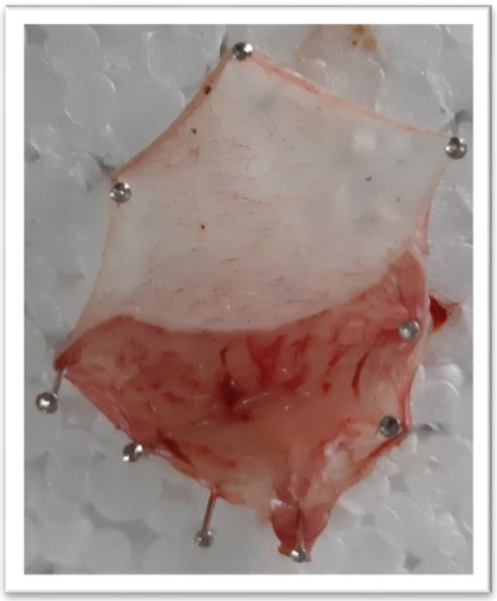


Fig 13 Standard treated with Ranitidine 50mg/kg b.wt-oral

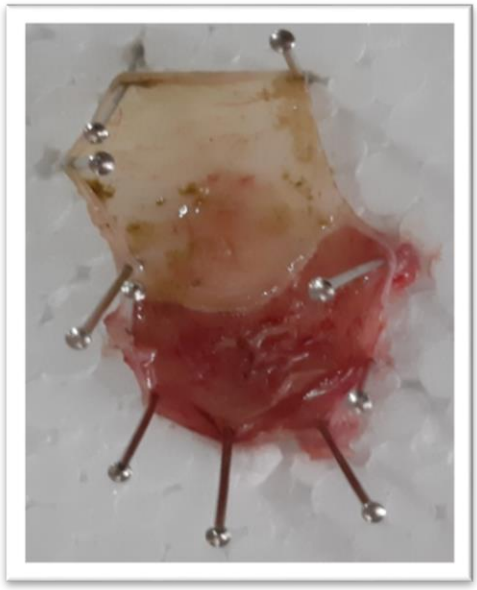


Fig 14 Test-Canthium parviflorum extract(250mg/kg b.wt-oral

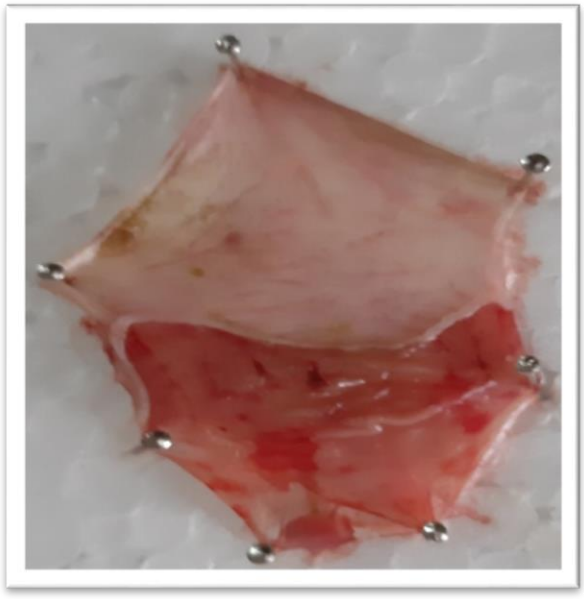


Fig1 5 Test- canthium parviflorum extract

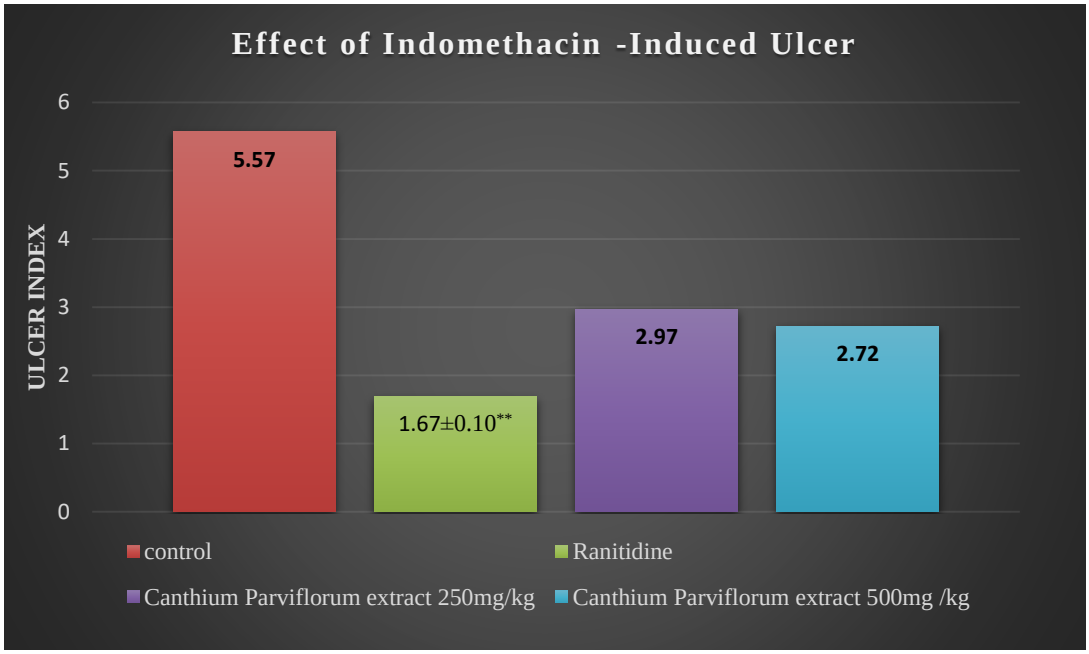
(500mg/kg b.wt)-oral

Table 9: Effect of *Canthium parviflorum*extracts on Indomethacin induced ulcer model.

S.No	Group	Treatment	Dose (mg / kg)	Ulcer index	Protection %
1.	Group I	Without any treatment	-	-	---
2.	Group II	Control(indomethacin)	30 mg / kg	5.57 ± 0.23	---
3.	Group III	Ranitidine	50 mg / kg	1.69 ± 0.10**	69.65%
4.	Group IV	*CanthiumParviflorum* extract	250 mg / kg	2.97 ± 0.20	46.67%
5.	Group V	*CanthiumParviflorum*e xtract	500 mg / kg	2.72 ± 0.09**	51.16%

Results are expressed as mean ±SEM from four observations as compared to standard group the one way ANOVAis Graph Pad‟s software method, (**P< 0.0001) by conventional criteria; this difference is considered to be extremely statistically significant.

Fig 16:Effect of *Canthium parviflorum* extracts on Indomethacin induced ulcer model.



6.4. *IN VITRO* WOUND HEALING ACTIVITY

6.4.1. Chorioallantoic membrane model (CAM)

The result was tabulated by counting the number of blood vessels in various treatments. Ethanolic extract of *Canthium parviflorum* various concentration and positive control promoted an increase in number of blood vessels compared to negative control saline. Treated 100µg/ml, 200µg/ml, 300µg/ml and 400µg/ml of *Canthium parviflorum* Ethanolic extract and 50 µg/ml Diclofenac sodium positive control treated group was observed formation of new blood vessels.(Fig no: 18,19,20,21,22)

The negative control does not show the formation of blood vessels. (Fig no: 17)

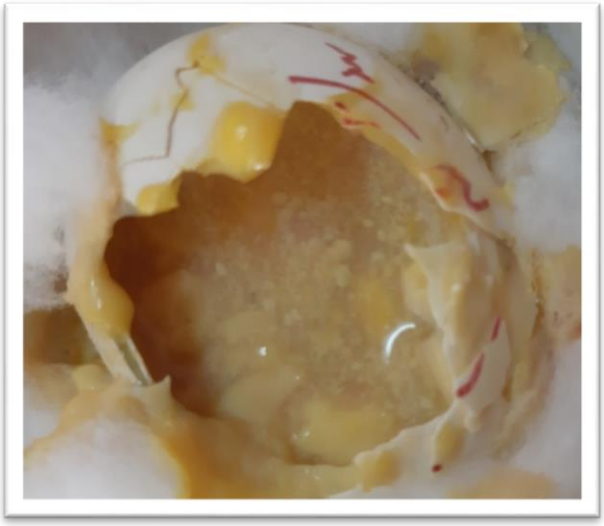


Fig 17-Negative control saline

Fig 18- Positive control Diclofenacsodium50µg/ml

Fig19-Ethanolic extract of Canthiumparviflorum(100µg/ml)

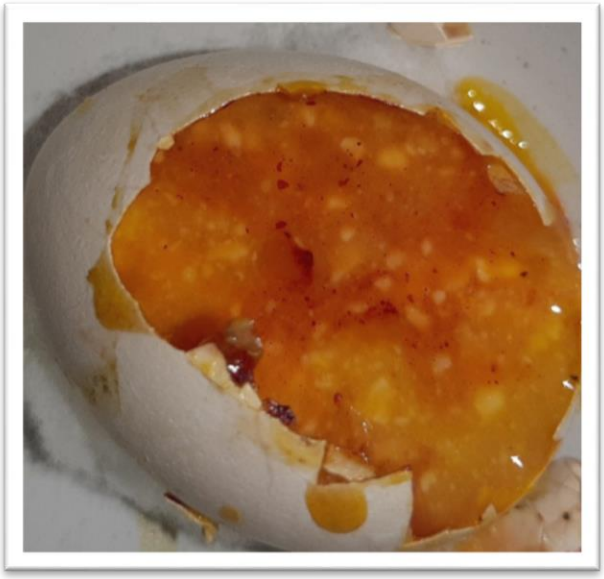


Fig20- Ethanolic extract of Canthium parviflorum(200µg/ml)

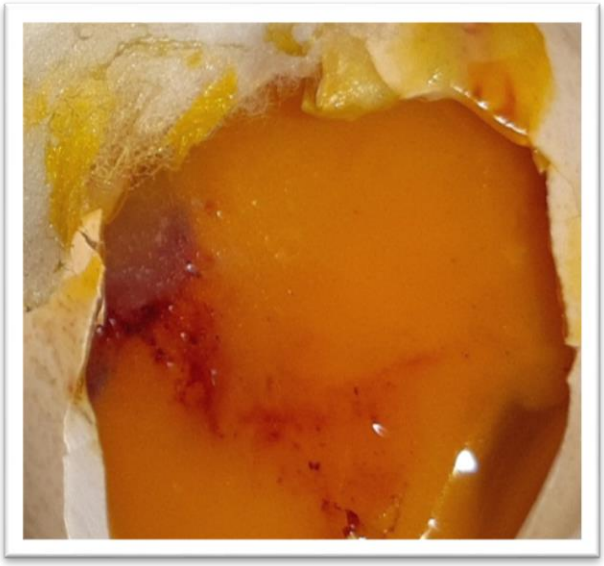


Fig21-Ethanolic extract of Canthium parviflorum(300µg/ml)

Fig 22- Ethanolic extract of Canthium parviflorum(400µg/ml)

Preliminary Phytochemical screening

The Phytochemical studies revealed the presence of alkaloids, Saponins, Tannins, Phlobatannins, Flavonoids, Glycosides, reducing sugars and terpenoids.

In vitro antioxidant activity

DPPH Method

The free radical scavenging activity of *Canthium parviflorum* and rutin against DPPH was presented in Table 6. The IC_{50} was calculated using the regression analysis was found to be 280µg/mL and 470µg/mL for rutin and *Canthium parviflorum* respectively. The DPPH has single odd electron which becomes paired off in presence of free radical scavenger which reduces the characteristic absorption of DPPH at 517nm so decolourisation of the purple colour occurs. The free radical scavenging power of the extract increases as the concentrations of the extract increases and showed more decolourisation and the solution turned yellow. Hence the free radical scavenging capacity of the extract is dose dependent.

Superoxide anion scavenging activity

The Superoxide anion scavenging activity of *Canthium* parviflorum and ascorbate against superoxide anion was presented in Table 7. The IC_{50} was calculated using the regression analysis was found to be 400µg/mL and 50µg/mL for ascorbate and *Canthium parviflorum* respectively. The superoxide anion has single odd electron which becomes paired off in presence of superoxide anion which reduces the characteristic absorption of superoxide anion at 517nm so decolourisation of the purple colour occurs. The superoxide scavenging power of the extract increases as the concentrations of the extract increases and showed more decolourisation and the solution turned yellow. Hence the superoxide anion scavenging capacity of the extract is dose dependent.

Nitric oxide scavenging activity

The nitric oxide scavenging activity of *Canthium parviflorum* and ascorbate against nitric oxide was presented in Table 8.The IC_{50} was calculated using the regression analysis was found to be 260µg/mL and 420µg/mL for ascorbate and *Canthium parviflorum* respectively. The nitric oxide has single odd electron which becomes paired off in presence of nitric oxide scavenging activity

which reduces the characteristic absorption of nitricoxide at 517nm so decolourisation of the purple colour occurs. The nitricoxide scavenging activity power of the extract increases as the concentrations of the extract increases and showed more decolourisation and the solution turned yellow. Hence the nitricoxide scavenging capacity of the extract is dose dependent.

Antiulcer activity of Canthium parviflorum

In Indomethacin -induced gastric ulcer model, the ulcers were induced in rats by the administration of indomethacin (30mg/ kg p.o). There will be eradication of the prostaglandins from the stomach mucosal region, which may cause ulceration, such as the prostaglandins need the major role in the gastric mucosa production. Hence, the purging of prostaglandins it reduces the protective mucosal secretion and increases acid secretion, thereby leading to ulceration in the stomach. In the present study, in gastric ulcer model induced by indomethacin (30mg/kg p.o) in rats the values of ulcer index were reduced in the treated group by ethanolic extract *Canthium parviflorum* 250mg/kg (2.97 ± 0.20) and extract *Canthium parviflorum* 500mg/kg (2.72 ± 0.09) there was a significant reduction in the ulcer index (**P<0.0001) as standard compared to control group.

***In vitro* wound healing activity of Canthium parviflorum extract**

In Chorioallantoic membrane Assay model, Angiogenesis is vital in normal processes of the development of blood vessel of embryo, formation of corpus luteum and wound healing.

Angiogenesis during wound repair helps the two fold function of providing the nutrients required by the healing tissue and contributing to structural repair through the formation of granulation tissue.

Canthium parviflorum Ethanolic extract in various concentration stimulated angiogenesis as proved by CAM model showed a faster wound contraction. By comparing various concentration extract show better activity than the Ethanolic extract of *Canthium parviflorum* 400 µg/ml.

CHAPTER_VII

CONCLUSION

- The leaves Plant under investigation (*Canthium parviflorum*) was successfully extracted using ethanol as the solvent.
- The Phytochemical studies revealed the presence of alkaloids, Saponins, Tannins, Phlobatannins, Flavonoids, Glycosides, reducing sugars and terpenoids.
- Thin layer chromatography of the extracts were performed using ethylacetate and chloroform as the solvent system and the R_f value was found to be 0.31.
- Antioxidant activity of *Canthium parviflorum* by *in vitro* was assessed using DPPH photometric assay, Superoxide anion scavenging method and nitricoxide scavenging activity. The Free radical scavenging power was increased exponentially with increase in the concentration and the free radical scavenging activity was dose dependent. Thus the extract was found to possess significant antioxidant activity.
- Antiulcer activity was performed *in vivo* using indomethacin induced gastric ulceration rat model and the extract was found to possess good ulcer protective properties. The extract at concentration of 500mg/kg, produced significant reduction in the ulcer index as that of standard.
- Wound healing activity *in vitro* was performed using chorioallantoic model. The extract at various concentration stimulated the angiogenesis and the extract with concentration of 400µg/ml showed better activity when comparing other concentrations.

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