EVALUVATION OF ANTI-OXIDANT AND ANTI ULCER ACTIVITIES OF ETHANOLIC EXTRACT OF *DESMOSTACHYA BIPINNATA* BY USING *INVIVO* AND *INVITRO* METHODS

A Dissertation submitted to
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In partial fulfillment of the requirements for the award of the Degree of
MASTER OF PHARMACY
IN
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Submitted by
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MAY – 2017

CERTIFICATES

CERTIFICATE

This is to certify that the dissertation entitled**" Evaluvation of Anti-Oxidant and Anti Ulcer Activity of Ethanolic Extract of *Desmostachya Bipinnata* By Using *Invivo* and *Invitro* Methods"** being submitted to The TamilNadu Dr. M.G.R Medical University, Chennai was carried by **Mr. Shanavas k** to The Tamil Nadu Dr. M.G.R Medical University, Chennai in partial fulfillment for the degree of **Master of Pharmacy** in **Pharmacology** is a bonafied work carried out by candidate under my guidance and supervision in the Department of Pharmacology, Karpagam College of Pharmacy Coimbatore – 32.

I have fully satisfied with his performance and work. I have forwarded this dissertation work for evaluation.

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I have fully satisfied with her performance and work. I have forwarded this dissertation work for evaluation.

Date: **Dr. S. MOHAN, M.Pharm, Ph.D.,**

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DECLARATION

I hereby declare that this dissertation **" Evaluation of Anti-Oxidant and Anti Ulcer Activity of Ethanolic Extract of *Desmostachya Bipinnata* by Using *Invivo* And *Invitro* Methods"** submitted by me , in partial fulfillment of requirements for the degree of **Master of Pharmacy** in **Pharmacology** to The Tamil Nadu Dr.M.G.R Medical University, Chennai is the result of my original and independent research work carried out under the guidance of **Dr. C. Senthil Kumar., M.Pharm.,PhD** Associated Professor , Department of Pharmacology ,Karpagam College of Pharmacy , Coimbatore -32

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EVALUATION CERTIFICATE

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Examination centre:

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SHANAVAS K

(261526154)

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LIST OF ABBREVATIONS

%	:	Percentage
µI	:	micro liter
CPCSEA	:	Committee For the purpose of care and supervision of experimentation on animals
ANOVA	:	Analysis of Variance
Fig	:	Figure
Tab	:	Table
G	:	Gram
M mole	:	Mille moles
Mg/dl	:	Milligram per deciliter
Min	:	Minutes
ml	:	Mille liter
mm	:	mille meter
Mm	:	Mille molar
SD	:	Standard deviation
Kg	:	Kilogram
SR	:	*Sidarhombifolia*
EESR	:	Ethanol extract of *Sidarhombifolia*
N	:	Normality
NaOH	:	Sodium Hydroxide
TCA	:	Trichloroaceticacid
AECD	:	Alcholic Extract of *desmostachya bipinnata*

CHAPTER-I

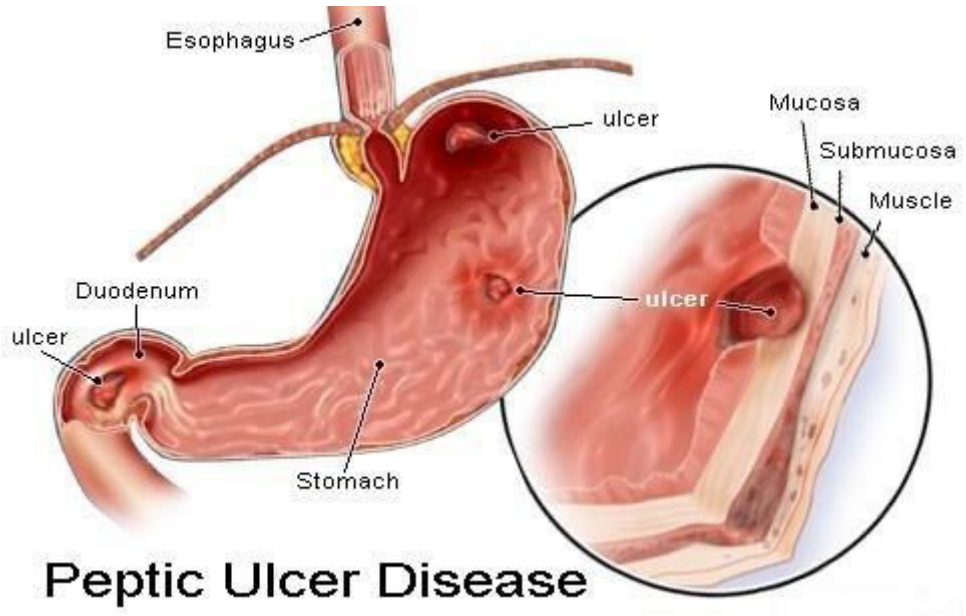
1. INTRODUCTION

1.1. Peptic ulcer

Peptic ulcer and other acidic symptom affect up to ten percentages of the humans with sufficient severity to prompt victims to seek medical attention. The more significant disease condition requiring medical focus is ulcer and gastro esophagealdisease[1]. In the US, approximately 4 million people have peptic ulcer (duodenal and gastric types), and 350 thousand new patient are diagnosed in each year, around 180 thousand peoples are admitted to hospital and treated with drugs yearly, and about five thousand patient from this case die each year as a result of ulcer condition. The lifetime of human being developing a peptic ulcer is about 10 percentages for Americans males and four percentages for female population[2].

Peptic ulcers is wound in the lesions in the stomach and GIT that are most often affected in younger to older adults population, but this may diagnosed in young adult life. They often appear without obvious sign and symptom, after a period of days to months of active phase of disease, it may heal with or without drug treatment. It also affect because of bacterial infections with H. Pylori.

Fig. No: 1: Diagram of Peptic Ulcer [3]



The following statistics relate to the prevalence of peptic ulcer[4]:

Table. No: 1: Prelavance of peptic ulcer

Country/Region	Extrapolated prevalence	Population estimated used
USA	5,398,077	293,655,405
Canada	597,571	32,507,874
India	19,578,503	1,065,070,607
Russia	2,646,581	143,974,059
Australia	366,050	19,913,144

1.2. Risk factor for ulcer[5]

1.2.1. Bleeding

Upper gastrointestinal (UGI) bleeding is the secondary common medical condition that effect high mortality in peptic ulcer. UGI bleeding commonly present along with hematemesis (vomiting with digested food and blood or coffee-ground like substance) and black, tarry stools (melana). Clinical diagnosis of UGI done by nasogastric tube lavage shows blood or coffee-ground like material presence. However this diagnosis may be negative when the bleeding arises beyond a closed pylorus region. Most of the patient's having bleeding ulcers can be treated with fluid and blood resuscitation, drug therapy, and endoscopic surgery.

1.2.2. Perforation: this ulcer may be spread to small intestine, oesophagus and large intestine ulcers account for 60, 20 and 20 percentage of perforations.

1.2.3. Penetration

Ulcer penetration called due to the permeation of the ulcer among the bowel part without free perforation and filtration of whole contents inside the

peritoneal cavity. Surgical treatment regimen recommended that permeation affect in twenty percentage of ulcers, but little proportion of penetrating ulcers become clinically important. The common symptom these complications include acidic irritation, weight reduction and diarrhoea: watery vomiting is an uncommon, but diagnostic symptom. No evident clinical data is available in the treatment regimen and guidance for the curing of penetrating ulcers.

1.2.4. Obstruction

Gastric wall obstruction among the frequent ulcer symptoms. Most of the cases are related with duodenal or pyloric part ulceration are 5 percentage of the patient populations.

1.2.5. Changes in lifestyle and dietary

Aspirin and related drugs (non-steroidal anti-inflammatory drugs), alcohol, coffee (even caffeine) and tea can interfere with the curing of the peptic ulcers. [6,7,8,9&10] Smoking may also low the ulcer healing process[11]. People with ulcer symptom have been evaluated to have more carbohydrate than people with no ulcers[12] from this route may occur with a genetic susceptibility for the ulcer pathogenis[13].

Sugar has also been reported to increase stomach pH[14]. Salt may cause the stomach and intestine irritation. Large uptakes of salt have been linked to higher risk of stomach ulcer[15]

One of the amino acid Known as Glutamine, is the important source in the energy in cells which cover the stomach and intestine[16]. It is also prevent the stress ulcer related by large burns of the preliminary study about the pathogenesis of ulcers[17].

1.3. Types of Peptic Ulcer

1) Gastric ulcer

2) Duodenal ulcer

1.3.1. Gastric ulcer[2]

Gastric ulcers are usually single and less than 20 millimetres in diameters. Ulcers on the small curvature are mainly related for the chronic gastritis condition, whereas those in the larger curvature are often associated to the non-steroidal anti-inflammatory drugs effects.

Gastric ulcers almost invariably arise in the setting of H. pylori gastritis or chemical gastritis that results in injury to epithelium. Most patients with gastric ulcers secrete less acid than do those with duodenal ulcers and even less than normal persons. The factors implicated include:

(1) back-diffusion of acid into the mucosa,

(2) Decreased parietal cell mass,

(3) Abnormalities of the parietal cells themselves.

A minority of patients with gastric ulcers exhibit acid hyper secretion. In these persons, the ulcers are usually near the pylorus and are considered variants of duodenal ulcers. Interestingly, the intense gastric hyper secretion that occurs in the Zollinger-Ellisonsyndrome is associated with severe ulceration of the duodenum and even the jejunum but rarely with gastric ulcers.

1.3.2. Duodenal ulcer

Duodenal ulcers are ordinarily located on the walls of the duodenum, on a short distance of the pylorus region.

The maximal capacity for acid production by the stomach reflects total parietal cell mass. Both parietal cell mass and maximal acid secretion are increased up to twofold in patients with duodenal ulcers. However, there is a large overlap with normal values and only one third of these patients secrete excess acid.

Accelerated gastric emptying, a condition that might lead to excessive acidification of the duodenum, has been noted in patients with duodenal ulcers. However, as with other factors, there is substantial overlap with normal

rates. Normally, acidification of the duodenal bulb inhibits further gastric emptying.

The pH of the duodenal bulb reflects the balance between the delivery of gastric juice and its neutralization by biliary, pancreatic and duodenal secretions. The production of duodenal ulcers requires an acidic pH in the bulb, that is, an excess of acid over neutralizing secretions. In ulcer patients, the duodenal pH after meal decreases to a lower level and remains depressed for a longer time than that in normal persons.

Impaired mucosal defences have been invoked as contributing to peptic ulceration. The mucosal factors, including the function of prostaglandins, may or may not be similar to those protecting the gastric mucosa.

Table NO: 2:Distinguishing features of two major forms of peptic ulcer[23]:

Features	Duodenal ulcer	Gastric ulcer
1. Incidence	a. Four times more common than gastric ulcers and b. Usual age 25-50 years.	Less common than duodenal ulcers and Usually beyond 6th decade.
2. Etiology	Most commonly as a result of H. pylori infection and other factors-hyper secretion of acid-pepsin, association with alcoholic cirrhosis, tobacco, hyperparathyroidism, chronic pancreatitis, blood group O, genetic factors.	Gastric colonisation H. pylori asymptomatic but higher chances of development of duodenal ulcer. Disruption of mucus barrier most important factor. Association with gastritis, bile reflux, drugs, alcohol, tobacco.
3. Pathogenesis	Mucosal digestion from hyperacidity most significant factor. Protective mucus barrier may be damaged.	Usually normal to low acid levels Damage to mucus barrier significant factor.

4.Pathologic changes	(a). Most common in the first part of duodenum. (b).1-2.5 cm in size. Round to oval.	(a) Most common along the lesser curvature and pyloric antrum. (b) Same to duodenal ulcer.
5.Clinical features	Pain-food-relief pattern Night pain common No vomiting No loss of weight No particular choice of diet Marked seasonal variation Occur more in people at greater stress.	Food-pain pattern No night pain Vomiting common loss of weight Patients choose bland diet devoid of curries No seasonal variation More often in labouring groups

1.4.Anatomy of Stomach

1.4.1.Parts of the stomach

The stomach is divided into five parts: a cardiac part, fundus, body, pyloric part and pylorus[25].

1.4.2. Cardiac

This part situated in the left side of the midline behind cardiac orifice region.

1.4.3. Fundus

This is a rounded part of the stomach receive the initial food and it controlled by splinter muscle.

1.4.4. Body

This is the main part of the stomach, it situated between the fundus and pyloric region.

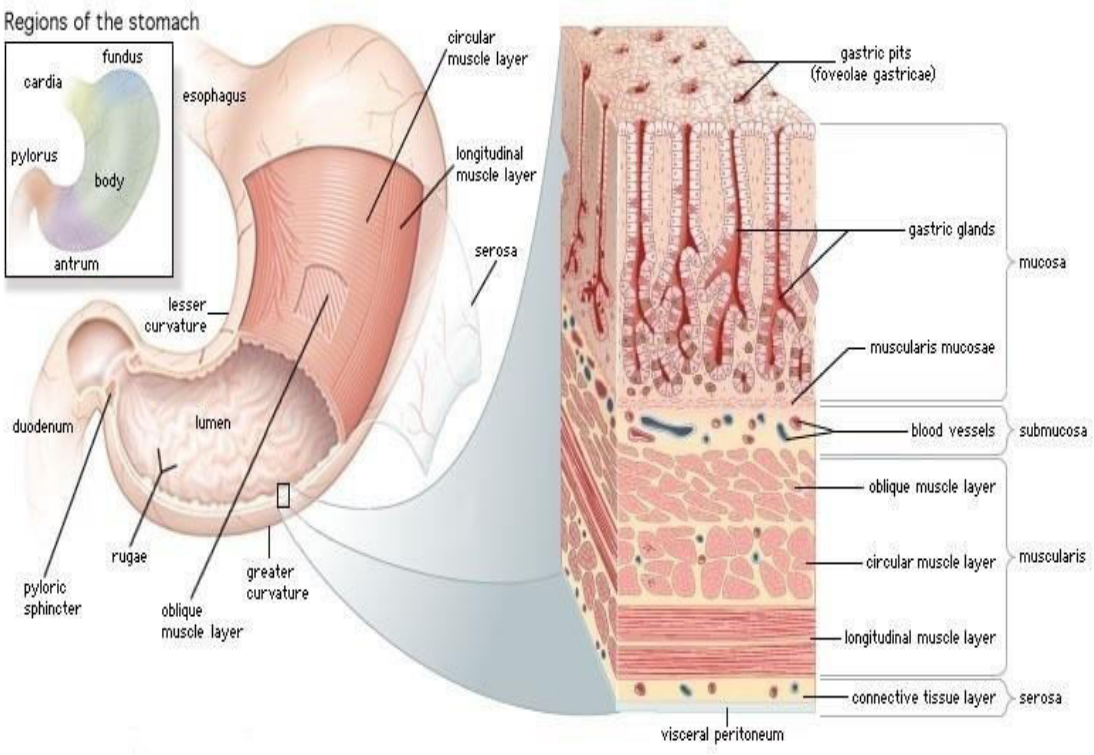
1.4.5. Pyloric Part:

This contain a wide part, the pyloric region and a narrow pyloric tube.

1.4.6. Pylorus

the pylorus wall is thicker part because made up of extra circular smooth muscle.

Fig. No: 2 : Layers of stomach[26]



1. Serosa
2. Connective tissue layer
3. Visceral peritoneum
4. Muscularis

5. Oblique muscle layer
6. Circular muscle layer
7. Longitudinal muscle layer
8. Submucosa
9. Muscularis
10. Gastric surface
11. Epithelium
12. Gastric glands
13. Gastric pits
14. Mucosa

The stomach walls are made up of the 4 basic layers which present in the entire GIT, with little modified anatomy. The surface about mucosa as the layers of simple columnar epithelial cells.

Epithelial cells extends down to the columns of secreting cells are known as gastric pits. Secretions from many gastric glands accumulated inside the gastric pit extended through inside the stomach. The gastric glands contain three types of exocrine secreting cells that release their secreting products inside the stomach it responsible for the absorption of vitamin B12, and hydrochloric acid[27]. The normal adult may produce gastric juice 2000-3000ml per day

1.5. Physiology of Stomach[24]

Stomach is a hollow organ situated just below the diaphragm on the left side in the abdominal cavity. When empty, its volume is 50ml and normally it can expand to accommodate 1 to 1.5 litters of solids and liquids. Gastric juice is the mixture of secretions from different glands of the stomach.

1.6. Properties

Its volume ranges from 1200 to 1500 ml/day. Gastric juice is highly acidic with pH of 0.9 to 1.2. The acidity of gastric juice is due the HCl. The specific gravity ranges from 1.002 to 1.004.

1.7. Composition

It contains 99.5% of water and 0.5% solids. The solids are organic and inorganic substances.

1.8. Organic substances

1.8.1. Gastric enzymes

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

1.8.2. Pepsin

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

1.8.3. Rennin

It is a milk curdling enzyme.

1.8.4. Gastric lipase

It is a weak lipid splitting enzyme.

1.8.5. Other gastric juice

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

1.8.6. Gastric mucus

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

1.8.7. Intrinsic Factor

This is necessary for absorption of the extrinsic factor.

1.8.8. Inorganic substances

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

1.9. Process of Digestion[28]

1.9.1. Mechanical digestion

After the ingestion food it enters stomach through oesophagus it undergoes peristaltic movements called mixing waves happened in every 25 to 30 seconds. These helped the maceration of food particle, mixing it with gastric fluid to form a semisolid consistency is called chyme.

These intense and more vigorous mixing waves promoted to reach the pylorus. Pyloric sphincter normally not fully closed it is partially closed. The chyme reaches the pylorus, these wave forces many millilitres of chyme into the small intestine by the pyloric sphincter. Sometime the chyme is forced return back into the stomach body parts, the mixing process continued. These forward and backward transport of the chyme are the responsible for most mixing process inside the gastro intestinal tract.

Foods may accumulate in the fundus for up to an hour without that mixed with gastric secretion. During this process, digestion process through salivary amylase continues inside the stomach.

1.9.2. Chemical Digestion

The partial cells produce the hydrogen ions ($H^{+)}$ and chloride ions (Cl^-) separately inside the stomach lumen; the total effect is secretion of hydrochloric acid (HCl). Proton pumps powered by H^+ / K^+ ATPase energy used to transport H^+ inside the stomach which transport the potassium ions (K^+) into the cells. The enzyme carbonic anhydrase, which is responsible for the parietal cells, which catalyses the synthesis of carbonic acid from water and carbon dioxide

The strongly acidic nature of the stomach digest many microbes in food and HCl partially digest the proteins inside the food and responsible the secretion of hormones interrelated to promote the secretion of bile in gall bladder and pancreatic juice from pancreases. Enzymatic digestion of proteins started in side stomach lumen. The proteolytic enzyme secreted inside the stomach is known as pepsin, function of these enzyme is the

breakdown of selective peptide bonds between the amino acids making up proteins. Pepsin is most active in acidic environment of the stomach (pH 2): it is inactive in higher pH. Other enzyme inside the stomach is gastric lipase, which break the short chain triglycerides. This enzyme have a little function inside the adult stomach, it active at pH range of 5-6

Only a little concentration of absorption happened through stomach, because epithelial cell of the stomach is impermeable of the chemical substances but it absorbed some amount of water or aqueous vehicle, ions, certain therapeutic agents.

1.10. Phases of gastric secretion[29,30]:

Secretion of gastric juice occurs when the food is taken in the mouth. Neural and hormonal mechanisms are involved in gastric secretion, which occurs in three phases:

Cephalic phases

Gastric phases

Intestinal phases

1.10.1. Cephalic phases

This is solely under nervous control. While taking food, the secretion of gastric juice starts even before food enters the stomach. The impulses are sent from head and so this phase is called cephalic phase. The gastric juice secreted in this phase is called appetite juice. This occur as conditioned and unconditioned reflex. In both, pepsinogen and HCL are secreted.

1.10.2. Unconditioned reflex

This causes gastric secretion when food is placed in the mouth. Afferent impulses arise from taste buds and other receptors in the mouth and reach the appetite center in amygdala and hypothalamus. From here, the efferent

impulses pass through dorsal nucleus of vagus and vagal fibers to the wall of the stomach. The gastric secretion occurs by the release of acetylcholine.

1.10.3. Conditioned reflex

In this, the sight, smell, hearing or thought of food causes gastric secretion. The impulses arising from cerebral cortex reach stomach via vagus.

1.10.4. Gastric phases

This is under both nervous and hormonal control. When food enters the stomach, secretion of gastric juice increases which is rich in pepsinogen and HCL mechanisms involved in this phase of gastric secretion are:

1.10.4.1. Local mesenteric reflex

When food enters the stomach, the food particles stimulate the local nerve plexus in the wall of the stomach. These nerves, in turn activate the glands of the stomach and a large quantity of gastric juice is secreted.

1.10.4.2. Vagovagal reflex

Presence of food in stomach stimulates the sensory nerve endings. Now the sensory impulses pass to the brainstem via sensory fibers of vagus. The efferent impulses pass through the motor fibers of vagus back to stomach and cause secretion.

1.10.4.3. Gastrin mechanism

Gastrin is one of the gastrointestinal hormones. It is secreted by the G cells present in pyloric glands of stomach. Small amount is also secreted in mucosa of upper small intestine. In foetus, it is also secreted by islets of Langerhans in pancreas. Gastrin is a polypeptide containing G14, G17 or G34 amino acids.

The intestinal phase of gastric digestion is due to activation of receptors in the small intestine. Whereas reflexes started from the cephalic and gastric phases initiate the stomach secret ration and gastric motility, these happened during the intestinal phase will be reduce the chime overloading in duodenum. In

addition, response happened during the intestinal phase helped the continuous digestion of foods that passed in the small intestine. The chyme having fatty acids and glucose absorbed the stomach and enter in to the small intestine, it triggers enteroendocrine cells in the small intestinal mucosa to release into the blood two hormones that affect the stomach-secretin and cholecystokinin or CCK. With respect to the stomach, secretin mainly decreases gastric secretion, whereas CCK mainly inhibits stomach emptying. Both hormones have other important effects on the pancreas, liver and gallbladder that contribute to the regulation of digestive processes

1.11. Causes of Peptic Ulcer

1.11.1. Helicobacter pylori (H. pylori)

Helicobacter pylori is a type (spiral shaped) Gram-negative bacterium that grown in the stomach is about fifty percentage of human population. Helicobacter pylori is freely adapted for survival inside environment of stomach. It attaches to the surface epithelial cells of the mucus, have higher urease action produces ammonia which balance the neutral microenvironment for its survival. It is now identified as an important aetiology for the chronic gastritis, and peptic ulcer. The Helicobacter pylori is always responsible for inflammation inside the gastric mucosa identified by inflammatory cells. The Helicobacter pylori infection responsible for higher amount acid secretion from the gastric pits of the stomach. Once identified the H. pylori generally exists for the up to 90 percentage of people having duodenal and gastric ulcer conformed[31].

1.11. 2. Non-steroidal anti-inflammatory drugs (NSAIDs)

Ongoing use of this class of medications is the second most common cause of ulcers. These drugs (which include aspirin, ibuprofen, naproxen, diclofenac, tolmetin, piroxicam, fenoprofen, indomethacin, oxaprozin, ketoprofen, sulindac, nabumetone, etodolac and salicylates) are acidic and they block prostaglandins, substances in the stomach that help maintain blood flow and protect the area from injury. Some of the specific drugs listed are more likely to produce ulcers than others[32].

1.11. 3. Zollinger–ellison syndrome

The Zollinger-Ellison (ZE) syndrome is characterized by autonomous gastrin production by an adenoma or adenocarcinoma of the pancreas or duodenum. The Zollinger-Ellison syndrome is distinguished from peptic ulcer disease by the demonstration of fasting hyper gastrinemia. There are many causes of fasting hyper gastrinemia (gastritis, vagotomy and pyloroplasty, the short bowel syndrome, rheumatoid arthritis, retained antrum, G-cell hyperplasia), but only two conditions-atrophic gastritis and renal failure are associated with gastrin levels increased several times above the upper limit of the normal range. However, in a patient with peptic ulcer disease and hypergastrinemia, it is important to exclude the Zollinger-Ellison syndrome. The Zollinger-Ellison syndrome arises from a gastrinoma, a tumour in the pancreas. This may be a localized or diffuse tumor. The presence of hypergastrinemia leads to hyper secretion; while the maximal acid output may be increased, the major defect is basal hypergastrinemia and a marked increase in the basal acid output. The patient will have aggressive peptic ulcer disease with ulceration in unusual sites or multiple ulcers that fail to heal on medical therapy. Hypertrophic gastric folds and diarrhoea may be prominent features[33].

1.11.4. Hereditary factors

Hereditary factors are important in the pathogenesis of peptic ulcer disease, as suggested by a higher prevalence of peptic ulcer disease in certain genetic syndromes. A number of familial aggregations have been noted in patients with peptic ulcer disease. These include hyperpepsinogenemia 1, normal pepsinogenemia 1, antral G-cell hyper function, rapid gastric emptying, childhood duodenal ulcer and immunologic forms of peptic ulcer disease. Heredity also plays a role in the development of ulceration and is associated with the syndrome of multiple endocrine adenomatosis 1 (adenomas of the pancreas, pituitary and parathyroid). Parents, siblings and children of ulcer patients are more likely to have peptic ulcer disease than control individuals. There is greater concordance for ulcer disease in identical than in fraternal twins. Hyperpepsinogenemia 1 appears to be an autosomal dominant trait.

There is increasing evidence to suggest that familial peptic ulcer disease is related to Helicobacter pyloric infection amongst family members[34].

1.11. 5. Male sex

Among the male and female ratio for gastric ulcers up to 1.5:2.1 percentage and duodenal ulcers about three: one[35].

1.11. 6.Cigarette smoking

Smoking is associated with a higher prevalence of peptic ulcer disease and may be associated with impaired healing of duodenal and gastric ulcer disease. Also, death rates from peptic ulcer disease are higher in individuals who smoke. Smoking increases a person's risk of getting an ulcer because the nicotine in cigarettes causes the stomach to produce more acid.

1.11. 7. Pepsinogen-1

It is secreted by the chief and mucous neck cells of the gastric mucosa and appears in the gastric juice and urine. Serum levels of this proenzyme correlates the gastric capacity for acid secretion and are considered a measure of parietal cell mass. A person with a high level of pepsinogen-1 is at five times the normal of developing a duodenal ulcer. Hyperpepsinogenemia is present in half of children of ulcer patients with hyperpepsinogenemia[37].

1.11. 8. Alcoholic cirrhosis

Ulcers are more common in people who have cirrhosis of the liver. Since cirrhosis of the liver is associated with alcohol consumption, alcohol reduction may work as a preventive measure to reduce the risk of peptic ulcer. Also alcohol is thought to slow wound healing increasing the risk of bleeding ulcers. Consumption of alcohol significantly increases the risk of gastric ulcers[38]. The cyclo-oxygenase-2 (COX-2) inhibitors exert their therapeutic effect by blockage of the synthesis of prostaglandins which mediate the inflammation. At therapeutic dose regimen doses they are very little or no secretary effect on cyclo-oxygenase-1, which found in various tissues which is responsible for the production of the prostaglandins which prevent the upper gastrointestinal tract ulceration. The COX-2 inhibitors will be decrease gastro

duodenal toxicity comparing to the conventional NSAIDs treatment regimen. The COX-2 inhibitors, like NSAIDs, may also aggravate the inflammatory bowel symptoms and leading to intestinal strictures[39].

1.11.9. COPD

Gastrointestinal symptoms could be related to the high incidence of peptic ulcer disease in patients with Chronic obstructive pulmonary diseases (COPD), to abdominal discomfort caused by bronchodilator drugs or corticosteroids, or to early satiety caused by flattened diaphragm or air swallowing[40].Glucocorticoids increase the secretion of hydrochloric acid, pepsinogen and pancreatic trypsinogen. They increase both basal and nocturnal gastric secretion. They decrease the resistance of gastric mucosa to the irritant action of the gastric secretions, and produce or aggravate gastric ulceration. They are ulcer genic, the risk is doubled, bleeding and silent perforation of ulcers May occur[41].

1.12. Aggressive and Defensive Factors of The Gastrointestinal Tract[42]

Peptic ulcer is precipitated by an imbalance of the aggressive and defensive factors of the GI tract

Table No: 3 :Aggressive and defensive factors in GI tract for pepticulcer.

AGGRESSIVE FACTORS	DEFENSIVE FACTORS
Gastric acid	Mucus
Pepsin	Bicarbonate
Bile	Prostaglandins
Acetylcholine	Phospholipid membrane
Histamine	Cellular repair and migration
Gastrin	Mucosal blood flow
Medications	
Helicobacter pylori	

1.13. Approaches for the Treatment of Peptic Ulcer:

1.13.1. Antacids

Antacids have been used for conventional in the treatment of patients with dyspepsia and peptic-ulcer disorders. These are the important agent used to treatment for acid-peptic disorders up to discovery of H_2-receptor antagonists and proton pump inhibitors. Now also continuously used in patients as a non-prescription drugs for cure of heartburn and dyspepsia. These agents having weak basic character and react with gastric hydrochloric acid to convert a salt and water. The mechanism of action of these agents are reduction of gastric acidity, promote the mucosal defence mechanisms by the stimulation of mucosal prostaglandin production. After ingestion of food, about 45 m Eq/h quantity hydrochloric acid is released. A single dose volume of 156 m Eq of antacid administration after the one hour from the food consumption fruitfully neutralizes gastric acid concentration for up to 2 hours. However, these capacity in different type of the branded formulations of antacids is significantly variable, it depends up on their dissolution rate, water solubility, kinetic of neutralization process, and the time of gastric emptying.

Sodium bicarbonate (eg, baking soda, Alka Seltzer) combine faster with HCl to give carbon dioxide and NaCl. Neutralization of carbon dioxide affect the gastric distention and belching effect. The unaffected alkali is easily absorbed, may cause metabolic alkalosis at high doses or to patients with renal insufficiency syndrome. Sodium chloride absorption may elevate the fluid retention in patient's having congestive failure, hypertension, and renal insufficiency.

Calcium carbonate is sparingly soluble and reacts lower rate than sodium bicarbonate with HCl to give a molecule of carbon dioxide and $CaCl_2$, it may produce belching or metabolic alkalosis. Higher doses of sodium bicarbonate or calcium carbonate alone calcium-containing dairy products can precipitate to hyperkalcemia, renal damage.

Formulations having magnesium hydroxide or aluminium hydroxide slow reaction with HCl to produce magnesium chloride or aluminium chloride

and a molecule of water. Because no gas is formed so belching will not appear. Because unabsorbed magnesium salts may lead to osmotic diarrhoea and aluminium salts may lead to constipation, hence these substances commonly administered combined form in the branded proprietary products (eg, Gelusil, Maalox, Mylanta) to reduce patience compliance of bowel function the patients. These agents (magnesium and aluminium) are absorbed and bio transformed by the kidney so the patients having renal insufficiency should not use these agents for long term usage.

All the antacids interfere the absorption among other medications by linking of the drug (retard its absorption) or by elevate the pH. It may affect the dissolution or solubility profile of drugs (commonly weakly basic or acidic drugs) is altered. Hence the antacids should not be administered with in two-hour between tetracycline's, fluoroquinolones, azoles, and iron ingestion.

1.13.2. H_2-Receptor Antagonists[53]

The H_2 antagonist's exhibit competitive binding in the parietal cell H_2 receptor and retard the basal and food-induced acid secretion in a normal, posology relate manner. They are specifically selective and should not infers H_1 or H_3 type receptors. The amount of gastric secretion and volume of the pepsin are also decresed.H_2 antagonists retard the acid secretion induced by histamine, gastrin and cholinomimetic agonists by two mechanisms. One is the histamine secreted from ECL cells in gastrin or vagal stimulation have blocked through binding inside the parietal cell H_2 receptor. Second, direct effect of the parietal cell by gastrin or acetylcholine may affect in suppress the acid secretions. It appears that reduced parietal cell cAMP levels attenuate the intracellular activation of protein kinases by gastrin or acetylcholine.

Cimetidine, ranitidine, famotidine, nizatidine:

The potencies of the four H_2-receptor antagonists vary over a 50-fold range. When given in usual prescription doses however, all of the H_2 antagonists inhibit 60-70% of total 24-hour acid secretion. H_2 antagonists are especially effective at inhibiting nocturnal acid secretion (which depends

largely on histamine) but have a lowest impact in meal mediated the acid release (this induced by gastrin, acetylcholine or histamine). Thus, they block more than 90% of nocturnal acid but only 60-80% of daytime acid secretion. Therefore, nocturnal and fasting intra gastric pH is raised to 4-5 but the impact upon the daytime, meal-stimulated pH profile is less. Recommended prescription doses maintain greater than 50% acid inhibition for 10 hours; hence, these drugs are commonly given twice daily. At doses available in over-the-counter formulations, the duration of acid inhibition is less than 6 hours.

1.13.3. Proton Pump Inhibitors (PPI_s) [54]

Proton pump inhibitors are pro drugs it is activated inside the acidic environment. After administration it enter in the GIT it enter by systemic circulation through absorption, again it transported to parietal cells and activated by acidic secretory pits. This place these drug activated via proton-catalysed production of a tetracyclic sulphonamide. The activated form then drugs directly binds covalently via sulfhydryl groups of cysteines in the H^+, K^+-ATPase pump, may cause deactivation of pump irreversible manner. Acid secretion start only, when the new pump molecules are synthesized and inserted into the luminal membrane, these agents have a prolonged duration of action (up to 24- to 48-hour) and retardation of acid secretions.

Omeprazole[55] can cause maximal inhibition of HCl secretion. Given orally in gastric juice-resistant capsules, it reaches parietal cells via the blood. In the acidic milieu of the mucosa, an active metabolite is formed and binds covalently to the ATP-driven proton pump (H+/K+ ATPase) that transports H+ in exchange for K+ into the gastric juice.

Lansoprazole and pantoprazole produce analogous effects. The proton pump inhibitors are first-line drugs for the treatment of gastroesophageal reflux disease (GERD).

1.13.4. Anticholinergics[56, 57]

Anticholinergic drugs reduce in inter and post prandial secretion of gastric juice, since these phases are partially under cholinergic control. They

reduce basal acid secretion by about 50% and histamine and gastrin - induced secretion by 40%. They do not reduce food stimulated secretion. They inhibit gastric motility and prolong the gastric emptying time.

Pirenzepine: This drug has selective antimuscarinic action on M_1muscarnic receptors in the stomach.

Propantheline and oxyphenonium are the other examples of this class.

1.13.5. Mucosal Protective Agents[58]

Sucralfate contains numerous aluminium hydroxide residues.it is not an antacid after oral intake, sucralfate molecules reacted to form cross-linking in gastric secretion, forming a semisolid consistency this attached to the mucosal layers hence it Protected from acid digestion, and it also helped ulceration due to the pepsin, trypsin and bile acids related damage, improve ulcer wound more rapidly. Sucralfateis taken on an administered in empty stomach (one hour before meals and at bedtime). It is higher tolerated but it may release Al_3^+ ions can lead constipation.

1.13.6. Prostaglandin Analoges

Misoprostol is a semi synthetic prostaglandin derivative with having higher stability compared to natural prostaglandin analogues, oral administration is compatible absorbed in local membrane and act as a prostaglandins synthesis promotes mucus production and inhibits acid secretion. Additional systemic effects (frequent diarrhoea; risk of precipitating contractions of the gravid uterus) significantly restrict its therapeutic utility.

1.13.7. Colloidal Bismuth Compounds[59]

The bismuth may create coats ulcers and erosions, can produce the protective layer about acid and pepsin enzymes. It stimulate prostaglandin, mucus and bicarbonate secretion. This product reduces stool formation. It has direct antimicrobial effects and binds enterotoxins, direct antimicrobial activity against H pylori.

1.13.8. Ulcer Healing Drugs

Carbenoxolone is a semisynthetic product of glycyrrhetinic acid, which derived from sap of liquorice root (belonging to the family succusliquiritiae) it stimulate mucus production and it has a mineralocorticoid like effect (inhibition of 11-β-hydroxysteroid dehydrogenase) that promote renal reabsorption of NaCl and water. It may, therefore, exacerbate hypertension, congestive heart failure or edemas.

1.13.9. Eradication Of Helicobacter Pylori

This bacteria plays a vital role for the pathogenesis of long term gastritis and peptic ulcer condition. The combine formulation of antimicrobial agents and proton pumb inhibitor (omeprazole) is proven effective. Some patients are tolerance to amoxicillin or clarithromycin, hence regimen is substituted with metronidazole.

1.14. herbs helpful in antiulcer therapy[18, 19]

Now a day's world population have economical interest for the natural substance for the diagnosis and treatment of the traditional systems of medicine. Diet constriction and lifestyle change are the basis of Ayurvedic treatment regimen, with herbal medical formulas which will cure out therapeutic effect. Ayurvedic formulation contain many herbs (polyherbal formulation) having a high margin of safety and efficacy. Peptic ulcer is a serious health hazard occurs due to the imbalance between offensive factor (acid secretion, H.Pylori infection, bile, and increased free radical concentration) and impaired mucosal resistance factor (mucus, bicarbonate secretion, prostaglandins etc). Literature found that the conventional therapy have the serious adverse effects low patient complaince.

Various herbal drugs like Shilajit, Ginger, Bael have been tried for their ulcer protective effectsand have promising results. Therefore, the search for an ideal antiulcer drug continues and has also been extended to herbal drugs for their better protection, easy availability, low cost and toxicity.

Acacia Arabica, Acacia catechu, Amaranth, Barberry (Berberis vulgaris), Black berry, Calendula, Dong Quai (Angelica sinensis), Ghee, Grapefruit (Citrus paradisi), Liquorice (Glycyrrhizaglabra), Olive oil, Piper betle, Plumbagozeylanica, Solon, Tea root extract.

A peptic ulcer cause severe erosion of the gut lining inside the stomach, intestine or oesophagus. An ulcer occurs when the lining of these organs is corroded by an acid and digestive juices which are released inside stomach cell lines[20].

Ulcer may be lead to disintegration, loss and death of tissue as they erode the layers in the wall of the stomach or duodenum. There is an old saying in medicine, “No acid, and no ulcer’’ If the protective layer of mucus is insufficient or if there is inadequate dilution and buffering of acid gastric juices by swallowed food and the alkaline juices of the small intestine, it can result in ulcer. Hyperacidity is influenced by nervous system factors by anxiety, other emotional states and stress[21].

Bari ilayachi (*Elettariacardamomum* and *Amomumsubulatum*)Both drugs has gastro protective effect may be due to a decrease in gastric motility[43]. They cause relaxation of circular muscles which may protect gastric mucosa through flattening of the folds. This will increase the mucosal area exposed to narcotizing agents and release the volume of gastric agents on rugal crest. Such action has been postulated to play a role in cytoprotective effect of prostaglandins[44].

Black berry one of the most interesting substances that has been obtained from chili peppers and present in spicy plants such as ginger or black pepper is capsaicin. This substance acts on sensory neurons to stimulate their membrane receptors, predominantly vaniloid (VR)-1 receptors, and release various kinins such as substance P. When applied in large dose capsaicin destroys selectively C-fibre neuronal endings leading to inactivation of sensory nerves and the loss of all reflexes in which these nerves are involved. In smaller dose, capsaicin is the potent gastro protective agent and stimulant of gastric microcirculation[45].

Chamomile is an herb that has been used traditionally as a mild sedative to relieve anxiety and in treating digestive disorders including peptic ulcers. Chamomile also may be effective in relieving inflamed or irritated mucous membranes of the digestive tract and in promoting digestion. Chamomile has a soothing action on the digestive system. Its gentle soothing action is beneficial in digestive disorders like indigestion, acidity and peptic ulcers. It is also high in the flavonoidapigenin-another flavonoid that has inhibited growth of H. pylori in test tubes[46].

Dong quai (Angelica sinensis) Animal studies suggest that dong quai may soothe ulcers, but studies in people are needed before a definitive conclusion can be drawn.

*Licorice (Glycyrrhizaglabra)*Liquorice root has a high background in effect of soothing effect in the inflamed and damaged mucous membranes of the digestive tract. Licorice also protect the stomach and intestinal parts by increased production of mucin, which protects the lining of the HCl and other substances. According to Preclinical research, Flavonoids ofliquorice may also supressed the growth of H. Pylori[47, 48].

*Marshmallow root (Althaea officinalis)*For decades, marshmallow has been used in folk medicine to help cure gastric ulcers. The roots of the marshmallow contain mucilage, a gelatinous substance found in plants. When it comes into contact with water, this mucilage swells, forming a soft, protective gel. This is believed to provide a protective barrier against irritating substances that may aggravate ulcers.

Tea root extract (Camellia sinensis) Tea root extract might primarily decrease the leakage of plasma proteins into the gastric juice with strengthening of the mucosal barrier and increase in its resistance to the damaging effect of ethanol induced ulcer[49].

Turmeric (Curcuma longa) Constituents of Curcuma longa exert several protective effects on the gastrointestinal tract. A salt of curcumin, sodium curcuminate, was found to inhibit intestinal spasm, and p-tolymethylcarbinol, a turmeric component, was found capable of increasing gastrin, secretin,

bicarbonate, and pancreatic enzyme secretion. Turmeric has also been shown in rats to inhibit ulcer formation caused by stress, alcohol, indomethacin, pyloric ligation, and reserpine. This study demonstrated turmeric extract significantly increased the gastric wall mucus in rats subjected to these gastrointestinal insults[50, 51].

1.15. Screening Methods for Antiulcer Activity[60, 61]

1.15.1. Pylorus ligation in rat (SHAY rat)

This model is a simple and convention method for induction of gastric ulceration in the rat through ligature in the pylorus region, the ulceration is affected by accumulation of acidic juice inside the stomach. Ulcer index & pH of gastric content of treated animals are compared with control groups. Different cumulative group administration followed by dose - response curves establishment for ulcer formation can be measured in these method.

Female Wister rats weighing 150-170 g are starved for 48 hours having access to drinking water ad libitum. During this time they are housed single in cages in prevent coprophagy. Six animals are used per dose and as control groups. Under mild ether anaesthesia an incision is made at the abdominal midline. The pylorus is closed by using small nylon the higher supervision is required to avoid he damage of blood vessels inside the pylorus region. Grasping the stomach with instruments is to be meticulously avoided; else ulceration will invariably develop at such points. The abdominal wall are sutured through surgical procedure. The test sample are administrated through oral ingestion or injected subcutaneous route. The animals are placed for 19 hours in a suitable plastic container. Afterwards, these animals are sacrificed in CO_2 anaesthesia. The abdomen is re ligated and a ligature is placed above the oesophagus region and closer to the diaphragm area. The stomach is replaced to a watch glass and the materials are collected in to a centrifugal tube. Above the longer curvature the stomach fully opened and pinned between a cork plates. The mucosal layer is observed with the help of a stereomicroscope.

1.15.2. Stress ulcer through immobilization stress

Psychogenic factors, such as stress, produce a major role in etiology of gastric ulcers in human beings and animals. Hence not only antacids ingestion, anticholinergic, H_2–antagonists, proton pump inhibitors treatment, and also psychotropic agents such as neuroleptics have also effective for the treatment.

Groups of 6 female Wistar rats per dose of test drug and for controls weighing 150-170 g are used. Food and water are removed 24 hours before the experimental procedure. After oral or subcutaneous ingestion of the test substance or the placebo drug in animal's extremities are fixed combine and the animals are tied in wire gauze. These animals are horizontally suspended in a dark room at 20^{o}C for one day and last animals are sacrificed in CO_2 anaesthesia method. The stomach has been cut, fixed in a cork plate and the count and score the severity of ulcers by the help of video recorded stereo-microscope.

1.15.3. Stress ulcers by cold water immersion

Cold water treatment of rats in during the restraint duration boost the appearance of gastric ulcers and reduce the time necessary immobilization process.

Groups of 8-10 Wister rats weighing 150-200 g are used. After oral administration of the test compound, the rats are placed vertically individual restraint cages in water at 22^{o}C for 1 hour. These are removed, allowed to dry and inject Evans blue (30mg/Kg) intravenously through tail vein. After ten minutes, these animals are sacrificed by CO_2 anaesthesia, stomachs was collected in Formal - saline (2%v/v) overnight storage about 24 hours. After that the stomachs are opened the greater curvature, washed via warm water and examined through 3-fold magnifier.

1.15.4. Indomethacin induced ulcers in rats

Non-steroidal anti- inflammatory agents, like indomethacin and acetyl-salicylic acid, produce a gastric lesions in human beings and in rodents by the inhibition of gastric cyclooxygenase leading for the formation of prostacyclin.

Groups of 5-6 Wister rats weighing 150 - 200 g are used. The test drugs are administered orally in 0.1 % tween 80 solution 10 minutes before the oral indomethacin at a dose about 20 mg/kg (4 mg/ml dissolved in 0.1 % tween 80 solution). Six hours later, the rats are euthanatized through CO_2 anaesthesia, these animals stomach was removed and inject with Formol-saline (2%v/v) for storage about one days. After that the stomachs are opened the greater curvature, washed via warm water and examined through 3-fold magnifier.

1.15.5. Ethanol induced mucosal damage in rats (Cytopotective activity)

Intra gastric application of absolute ethanol is a reproducible method to produce gastric lesions in experimental animals. The lesions may be blocked by some drugs (ex:-prostaglandins). These protective activity opposite to the irritants are known as cytoprotective activity.

Male Wistar rats weighing 250 - 300 g are deprived of food 18 hours prior to the experiment but are allowed free access to water. During this time they are kept in restraining cages to prevent coprophagy. The rats are administered either are appropriate vehicle or the cytoprotective drugs, for example a prostanoid, intra gastrically 30 minutes prior to administration of 1 ml absolute ethanol. Untreated animals are included as controls. One hour after administration of ethanol, the animals are sacrificed in CO_2 anaesthesia and their stomachs exercised, cut along the greater curvature and gently rinsed under tap water. The stomachs are stretched on a piece of foam core mat, mucosal site up. The subjective scores of the treated tissues are recorded.

1.15.6. Sub acute gastric ulcer in rats

This is a method for producing standard sub-acute gastric ulcers in rats and for the quantitative evaluation of the healing process.

Female Wistar rats weighing 120-150 g are fasted for 24 hours having access to water ad libitum in cages with wire sieves at the bottom. The rats are anesthetized with ether and a polyethylene catheter including a fine steel wire with a needle tip (1.2 mm diameter) at the lower end is orally inserted into the stomach. After the cannula reaches the gastric wall, the upper end of the steel wire is pressed in a definitive manner, so as to puncture the gastric wall. Each rat is kept in the same position during the intervention in order to localize the puncture at nearly the same region of the glandular part of the stomach. The test substances are administered orally, 30 minutes prior or 24 hours after puncture. Free access to food and water is provided from 2 hours up to the end of the experiment. Each group consists of 8 - 15 rats. The animals are sacrificed by overdose of ether at definitive time intervals after puncture. The stomach is dissected and opened along the lesser curvature, extensively rinsed in tap water and fixed to the end of a polyethylene tube of 10 mm diameter (plastic tip of an automatic pipette) in a position with the punched ulcer in the centre. The end of the tube with the gastric wall is suspended in a beaker containing physiological saline, and the pressure in the tube is gradually increased with a valve rubber ball connected to the other end of the tube. The value of tension at which bubbles appear at the ulcerous gastric wall is noted. This value is termed as tensile strength and can be expressed in mm Hg.

CHAPTER II

2. LITERATURE REVIEW

AshishPandey, et al, (**2013**)[62] was reviewed the pharmacognastical ,taxonomical and pharmacological use of desmostachyabipinnata. He reported it have Desmostachyabippinata is reported to have several medicinal properties for jaundice, menorrhagia, haemorrhoides, asthma, galactogogue, antihelicobactor, antidiarrhoeal and diuretic property as per literature.

Audil Rashid et al, (2001)[63] was studied the in-vitro antibacterial effect of the of soil pollutants on soil incubating root-associated fungi. Roots of partheniumhysterophorous(L) subjected to fungal isolation of desmostachyabipinnata (L).Aspergillustype of was isolated from roots of both the plants. From this study 7 different fungal species were isolated and characterised. .The root extract of both plants were found to be have bacterial agent.

K. Ashok Kumar et al, (2010)[64] studied the phytochemical analysis and antibacterial use of Desmostachyabipinnata whole plant extracts. The extracted essential oil obtained from the aerial parts of this species through hydro distillation and characterised via GC–MS analysis. From this study the extracts having 16 compounds representing 99.97% of the oils are appear as the principle secondary metabolite from this species. The in-vitro antibacterial screening was conducted through agar diffusion and broth dilution methods indicate the significant inhibitory effect against all four bacteria strains**.**

Mohammed et al, (2009)[65] investigate the potential in vitro antihelicobacter activity of selected Egyptian plants, focusing on the determination of the main component responsible for such activity. The main objective is to obtain a natural product have antihelicobacter activity. Antimicrobial screening for wild Egyptian medicinal plant extracts, revealed that five methanolic extracts have good antihelicobacter activity. Determination of MICs, revealed that the wild plant, Desmostachyabipinnata (DEM) extract proved to be the most active one, where its MIC was 40 µg/ml.

After fractionation of the DEM extract, ethyl acetate fraction exhibited excellent antihelicobacter activity. By further fractionation and purification, using TLC and column chromatography, a flavonoid compound was isolated, with MIC value of 62 µg/ml. The isolated compound was spectroscopically identified as 4`- methoxy quercetin-7-O-glucoside. DEM plant (available as a wild plant in Egypt) containing a flavonoid compound which possesses a good in vitro antihelicobacter activity. The isolated compound (Quercetin) might be useful as a chemo-preventive agent for peptic ulcer in H. pylori-infected individuals, after its clinical valuation.

Hafiz et al, (2012)[66] studied the crude aqueous-methanolic extract of *D. bipinnata* was evaluated through *in vivo* and *in vitro* experiments. The antidiarrheal study was conducted through mice against castor oil–induced diarrhoea, which will be conducted similar comparing toloperamide. These extract will be treated in gut preparations produced an effect similar like atropine-sensitive spasmogenic effect in rabbit jejunum up to 5 mg/mL, followed by a partial relaxation at 10 mg/mL. The maximum stimulant effect was comparable with the acetylcholine-induced maximum contraction and was similarly reproducible in guinea pig ileum preparation.

Rajasekaranet al, (2015)[67] evaluated the effect of the polyphenolic fraction of Desmostachyabipinnataroot extracts on tamoxifen induced liver necrosis in female Sprague-Dawley rat models.The roots of Desmostachyabipinnata were extracted through 70% methanol, and the polyphenolic fraction was isolated. Protection of BRL3A cells againstethanol-induced damage was determined by 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. This study results indicate that it have the protective action against the tamoxifen induced hepatocytes.

Gouriet al, (**2014**)[68] studied hepatoprotective potential of dried powdered root extract of Desmostachyabipinnata against paracetamol-induced liver damage in wistar rat model. From this perspective aqueous extract of the roots of D.bipinnata at a dose of 100mg/kg and 200mg/kg wasadministered orally for 7 days. Silymarin at a dose 25mg/kg was used as

standard drug. On the 7th day of the experiment paracetamol was administered orally to induce toxicity. After the 24hrs of final drug administration, blood was collected and serum marker enzymes and bilirubin levels were determined. Animals pre-treated with aqueous extract of D. bippinata showed have the significant reduction in the elevated level of serum marker enzymes compared to paracetamolinduced liver damage in rat model.

MedhaM.Hegde et al, (2010)[69] studied the alcoholic and aqueous root extract of Desmostachyabipinnatafor antidiarrheal property in rats by castor oil induced diarrhoea and charcoal meal test atthe doses of 200 and 400 mg/kg body weight. The alcoholic extract and to a less effective compared to the aqueous extract significantly. The phytochemical study of these extracts showed the presence of alkaloids, glycosides, flavonoids, tannins, phytosterol, terpenoids, polyphenolics, protein and carbohydrates.

Amani S. Awaadet al[70] (2011) Five main flavonoid glycosides were isolated, for the first time, from the ethanol extract of Desmostachiabipinnata (L.)Stapf (Gramineae). They were identified as kaempferol(1), quercetin(2), quercetin-3-glucoside(3), trycin(4) and trycin-7-glucoside(5). The structure elucidation was based on UV, Electrospray ionization mass spectrometry (ESIMS), 1 H and 13 C NMR, proton- proton correlation spectroscopy (1 H-1 H Cosy), distortionless enhancement by polarization transfer (DEPT), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlations spectrum (HMBC). The total extract (200 and 300 mg/kg) and two of the isolated compounds (trycin and trycin-7-glucoside.100 mg/kg each) showed a very promising antiulcerogenic activity with curative ratios; 68.31, 70.54, 77.39 and 78.93%, respectively.

Ashoka M. Shenoy*et al,* (2012)[71] The antiulcer activity of methanolic extract of *Aeglemarmelos* leaves was investigated by aspirin plus pylorus ligation induced as in gastric ulcers in rats, Indomethacin induced ulcer in rats, water immersion stress test induced ulcer in rats. In aspirin plus pylorus ligation model, Aeglemarmelos at doses of 200 and 400mg/kg produced significant reduction in gastric volume, free acidity, and ulcer index compared to control. In Indomethacin and water immersion stress test induced ulcer models both doses (200mg/kg & 400mg/kg) of Aeglemarmelos extract significantly reduced severity of ulceration.

CHAPTER III

3. AIM AND OBJECTIVE

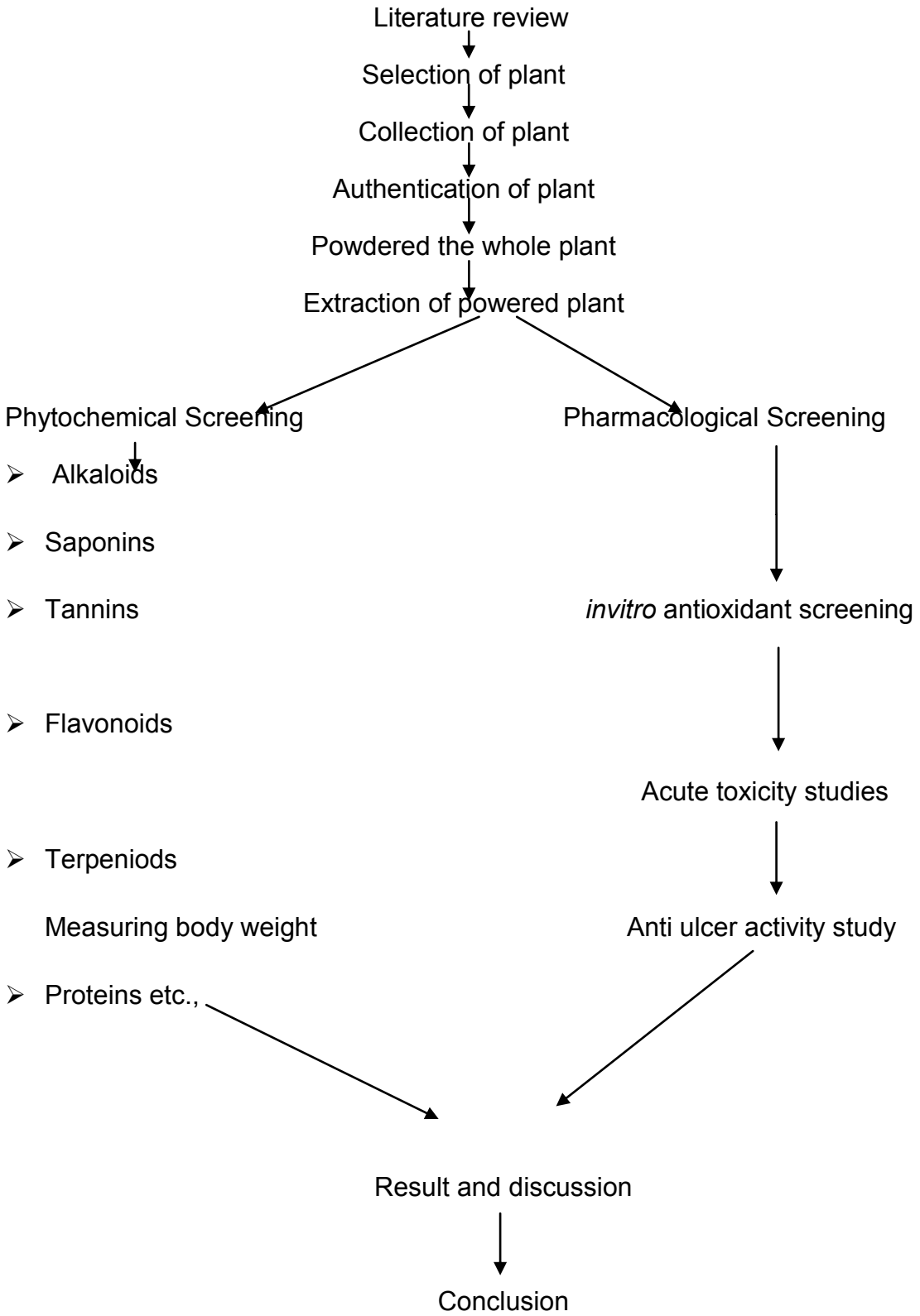
GIT disease against few effective drugs available for modern therapy, it produce side effect during the treatment is worse than the condition of rebound acidity. as per the literature review selected plant that cure ulcer diseases so considerable interest has developed in the examination of these numerous plants remedies which are useful for antiulcer activity. So it is necessary to find new drugs of importance in antiulcer effect with fewer side effects. Moreover it is necessary to required scientific validation traditionally using medicinal plants on various systems such as Ayurvedic Siddha Unani systems.

In our study *Desmostachyabipinnata* plant extracts its phytochemical investigation will be a useful tool for the identification and authentication of the plant for industrial and further research purpose, which will be related to the antioxidant activity. Antioxidants, which can scavenge free radicals, have an important role in pharmacological systems. Antioxidants are emerging as prophylactic and therapeutic agents. Hence, antioxidant was also evaluated for the potent extract. And now I have under taken the study of evaluation anti-oxidant and antiulcer activity of *Desmostachyabipinnata* plant extracts by using *invivo* and *invitro* methods

- To select plant based on their ethnomedical uses and preparation of their extracts.
- To screen phytochemical profile.
- To screen the selected extract for antioxidant using various *invitro* methods
- To screen the potent plant extract for their *invitro* antioxidant,
- To evaluate *invivo* antiulcer activities by NSAIDs induced animal model.

CHAPTER IV

4. PLAN OF WORK



CHAPTER V

5. PLANT PROFILE

Figu. No : 3 : Photograph of Desmostachyabipinnata[73]

5.1. Taxonomical Classification[74]

Table No : 4 : Taxonomical classification of *Desmostachyabipinnata*

Kigdom	*Plantae*
Division	*Magnoliphyta*
Class	*Monocotyledonae*
Order	*Poales*
Family	*Poaceae*
Genus	*Desmostachya*
Species	*D. bipinnata*

5.2. Vernacular Names[75]

Table No : 5 : Vernacular names of *Desmostachyabipinnata*

Tamil	*Kusa, Taruppaipullu*
Kannada	*Darpha*
Malayalam	*Darphapullu, Kusa*
English	*Sacrificial grass*

5.2. Distribution

Global Distribution **:** South Asia and Europe

5.3. Plant Description[76]

A rhizomatous perennial of dry areas with an extensive system of rhizomes 2–3 mm thick at 0.1-02mdeep.The leafsare coarse, narrow, tough, up to 0.5 m length.Culms with glossy yellow leaf sheaths at the rhizomatous perennial of dry areas with an extensive system of rhizomes. Leaves are coarse, narrow, tough, up to 50 cm long. The whole plant materials are useful for the symptoms of different diseases. Desmostachyabippinata is indicated by several medicinal treatments for jaundice and other gastro intestinal disorder, Plant is a common weed plant mainly cultivated Northern parts of India. From these plants lot of chemical constituents have isolated including tannins, flavanoids, terpenoids, carbohydrates and essential oils.. The alcoholic and aqueous extract are useful of this plant for several diseases.

5.4. Chemical constituents

The sample of essential oil is collected from the aerial parts of the plant via hydro distillation process. The literatures indicates it contains 16

compounds representing 99.97% of the oils: camphene (16.79%), isobornyl acetate (9.92%), tricyclene (4.30%), (+,-) trans-2,6-gamma-Irone (2.21%), Caryophyllenediepoxide (12.29%) , β-eudesmol (11.16%) Eseroline (25.15%) and Calarene(3.48%) appear as the main components.[3] Plant contain xanthenes also.[2]The plant contain flavonoids and carbohydrates also,

CHAPTER VI

6. MATERIALS & METHODS

6.1. Collection and Authentication of Plant

Desmostachyabipinnata plan twas procured from the Botany Central council for Research in Ayurvedia and Siddha Govt of India and authenticated by Chelladurai.V research officer Botany Central council for Research in Ayurved and Siddha Govt of India. CERTIFICATE NO:UWAN/344/16

6.1.1. Extraction Procedure[78]

Preparation of *Desmostachyabipinnata* whole plant extract.

The plants were initially collected from the soil body and rinsed with distilled water and shade dried and then homogenized into fine powder and stored in air tight bottles. A total of 10 g of air dried powder was weighed and was placed in 100 mL of organic solvents (methanol and ethanol) in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 h. And then it was filtered with the help of muslin cloth and the solvent was evaporated by solvent distillation apparatus to make the final volume of one-fourth of the original volume, giving a concentration of 40 mg/mL. It was stored at 40 °C in air tight bottles for further studies.

6.2. Phyto Chemical Screening[79-80]

The plant may be containing the following compound such as carbohydrate, protein, and lipids. That is utilized as food by man. It also contains the compound like. Tannins, Glycosides, alkaloids. Volatiles oils. The compound that is responsible for lots of medicinal properties

6.2.1. Test for carbohydrates

6.2.1.1. Molisch test

The sample powdered was added with 1 ml of alpha napthol solution along with conc Sulphuric acid solution in the test tube reddish colour was produced at the junction between 2 liquid

6.2.1.2. Fehling test

To the sample powder was added with both Fehling A and Fehling B solution and placed in the water bath for a sufficient time. This shows the brick red colour.

6.2.1.3. Benedicts test

To the sample powder add 8 drops of benedict's reagents and Boil the sample vigorously for 5 min it shows the red ppt.

6.2.2. Test for alkaloids

To the small of stored powder (sample) was taken and add few drops of hydrochloric acid and filtered. The filtered was tested with various alkaloid agents.

6.2.2.1. Mayer's reagents

To a small of above filter add small quantity of Mayer's reagent to form cream precipitate.

6.2.2.2. Dragendorffs reagents

From the above filter add small amount of Dragendorffs reagents it forms a orange brown precipitate.

6.2.3. Test for flavonoids

To the filter of the plant extract add 5 ml of dilute ammonia solution and followed by the addition of concentrated sulphuric acid. It forms a yellow colour.

6.2.4. Test for steroids.

6.2.4.1. Salkowski test

Few amount of plant extract was mixed with chloroform and the same volume of sulphuric acid is added on it. Cherry red colour was obtained in the chloroform layer.

6.2.4.2. Libermannburchard test

The extract is dissolved in 2 ml of chloroform 10 drops of acetic acid and conc. Sulphuric acid were added. Now the solution becomes reddish colour then it turns to bluish green colour.

6.2.5. Test for tannins

From few amount of plant extract is treated with vanillin hydrochloric acid reagent. It forms, pink or red colour due to the formation of phloroglucinol.

6.2.6. Test for protein.

6.2.6.1. Millon's reagents

Mellon's reagents (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitate on addition to a protein solution which turns red on heating.

6.2.6.2. Ninhydrin Test

From the sample solution add 2 drops a freshly prepared 0.2% ninhydrine reagent was added to the extract and heating. Development of blue colour may indicate the presence of peptide, amino acid (protein).

6.2.7. Test for glycosides

6.2.7.1. Keller- killani test

From the small quantity of small powder acetic acid was dissolved and adds few drops of ferric chloride and transferred to the surface of concSulphuric acid. At the junction, reddish brown colour was formed, which gradually becomes blue indicates the presents of cardiac glycosides.

6.2.8. Test for saponins

6.8.1. Foam test

1 ml of extract solution is diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicates the presence of Saponins.

6.3. In Vitro Antioxidant Activities

6.3.1 Superoxide Radical Scavenging Activity[81]

6.3.1.1. Principle

The superoxide anion radical scavenging activity was determined by nitro blue tetrazolium (NBT) reduction method of Mc Cord and Fridovich (1969). The assay is based on the ability of drug to inhibit the reduction of nitro blue tetrazolium (NBT) by Superoxide, which is generated by the reaction of photo reduction of Vitamin C within the system. The superoxide radical thus generated reduce the NBT to a blue coloured complex.

6.3.1.2. Reagents

- Nitro blue tetrazolium (NBT) - 1.5nm (12.3mg/10ml)
- Vitamin C - 0.12µm (4.5mg/100ml)
- NaCN/EDTA - 0.0015% NaCN in 0.1M EDTA
- Phosphate buffer - 0.06M (pH 7.8)

6.3.1.3 Procedure

The reaction mixture contained EDTA (0.1 M), 0.3mM NaCN, Vitamin C (0.12mM), NBT (1.5 n moles), Phosphate buffer (67mM, pH 7.8) and various concentrations of the alcoholic extract in a final volume of 3ml. The tubes were illuminated under incandescent lamp for 15min. The optical density at 560 nm was measured before and after illumination. The inhibition of superoxide radical generation was determined by comparing the absorbance values of the control with that of alcoholic extract and fraction-IV. Vitamin C was used as positive control. The concentration of fraction-IV required to scavenge 50% superoxide anion (IC_{50} value) was then calculated.

CALCULATION

$$\% \text{ inhibition} = \frac{ODofcontrol - ODofsample}{ODofcontrol} \times 100$$

6.3.2. DPPH Radical Reducing Activity[82]

6.3.2.1. Principle

It is a rapid and simple method to measure antioxidant capacity. It involves the use of free radical, DPPH (2, 2- Diphenyl - 1- picrylhydrazyl) (Aquino et al, 2001). The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and is purple in color. The color turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolourization is stoichiometric with respect to the number of electrons captured.

6.3.2.2. Reagents

- DPPH - 3mg in 25ml methanol (stored in dark bottle)
- Methanol

6.3.2.3. Procedure

Freshly prepared DPPH (187 µl) was taken in different test tubes protected from sunlight. To this solution added different concentrations (0, 25, 50, 75,100,150,200µg/ml) of alcoholic extract and fraction-IV. The volume was

made up to 1ml with methanol. Keep the tubes in dark and after 20 min absorbance was measured at 515nm. Methanol was used as blank and vitamin C was used as positive control. The concentration of test materials to scavenge 50% DPPH radical (IC_{50} value) was calculated from the graph plotted with % inhibition against Concentration.

Calculation

$$\% \text{ inhibition} = \frac{OD of control - OD of sample}{OD of control} \times 100$$

6.4. Pharmacological Screening

6.4.1. Animals

The albino rat (average body weight 200-300g), used from in house laboratory. The animals were maintained under standardized environmental conditions (22-28^{o}C , 60-70% relative humidity, 12 hr dark/light cycle) in animal house, Department of uwin life science malappuram. The animals were provided with standard mouse chow (SaiDurga Feeds and Foods, Bangalore, India) and water *ad libitum*. All animal experiments were conducted during the present study got prior permission from Institutional Animal Ethics Committee (IAEC approved) and following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division, Government of India (No:-KU/IAEC/M.pharm/167).

ACUTE TOXICITY STUDIES (Dose Fixation[83])

ACUTE TOXICITY STUDY

Experimental Protocol

Guideline	:	OECD – 420-fixed dose method
CPCSEA Reg. No	:	KU/IAEC/M.Pharm/167
Test	:	Limit test
Species	:	*Rattusnorvegicus*
Strain	:	Albino Wistar rats
Number of animals	:	24
Sex	:	Male/female
Dose	:	5, 50, 300, 2000 mg/kg
Route of administration	:	Oral
Duration	:	3hr close observation, followed by 14 days observation

Table No : 6 : Experimental Design for Acute Toxicity Studies

Group	Dose(mg/kg)
Group I	5
Group II	50
Group III	300
Group IV	2000

6.4.2. Study Design

Test animal – 6-8weeks old Adult Wistar rats of male and female, nulliparous and non-pregnant animals were obtained from centralized animal house from uwin life science malappuram and acclimatized to holding for 1 week prior dosing.

Housing conditions

Temperature – The experimental animal room temperature maintained at 22°C±3°C OECD guideline-423, 2001. These ranges are designed to allow homeotherms to maintain metabolic rate or to be within their thermo neutral zones. Because, temperature below the recommended range leads to increased food intake, increased energy expenditure but decrease in efficiency. In contrast, temperature above the recommended range leads to decreased food intake, decreased weight and decreased energy expenditure. Toxicity can vary with temperature might increase with linearity with temperature.

Humidity – The relative humidity maintained at 40%-60% preferably not exceeds 70% (OECD-423, 2001).The relative humidity below the recommended range can develop lesions such as ring tail and food consumption may be increased.

Light – 12-12 hours, Light/dark cycle. Appropriate lighting and light cycle play a key role in maintaining the physiology and the behaviour rat. Light provided for adequate vision and for neuroendocrine regulation of diurnal and circadian cycles (CPCSEA guidelines for laboratory animal facility 2003).

Light intensity – The light intensity maintained at 325 lux approximately 1m above the floor. Consideration of variations in light intensity, for the arrangement of animals on cage rack for toxicology study is necessary.

Caging – Polypropylene cages with solid bottom and walls. Lids made up of stainless steel grill capable of holding of both feed and water.**Feeding condition** – Sterile laboratory feed (*ad libitum*) and RO water bottles daily.

Feed – Brown colored chow diet

Drug administration – Animals were fasted for 12hour prior to dosing on day 0. Treatment rats were dosed by oral gavages, using a curved and ball tipped stainless steel feeding needle, with 20% gum acacia solution.

Clinical observations – All rats were monitored continuously for 4 hour after dosing for signs of toxicity. For the remainder of the 14 days study period,

animals were monitored and any additional behavioural or clinical signs of toxicity. Animal's body weight was measured prior to dosing and on days 7 and 14.On all animals were killed and at the end of the study LD_{50} value was established. Clinical observations and gross pathological examination was carried out.

6.4.3. Aspirin Induced Ulcer[84]

Table No : 7 : Dose dependent studies: (Animal: rats)

SI NO	DRUG	DOSE	ROUTE OF	NO. OF ANIMALS	PARAMETERS FOR STUDY
1.	Control (water)	----------	Oral administration	6	1.Ulcer index& Ulcer score 2.Total acidity 3.Acid volume 4.pH 5.Glutathione 6.Total protein
2.	Standard (famotidine)	3mg/kg	Oral	6	
3.	AEDB	100mg/ kg	Oral	6	
4.	AEDB	200mg/ kg	Oral	6	
5	AEDB	400 mg/kg	Oral	6	

6.4.3.1. Ulcer index& ulcer score

Procedure

1. Animals in the group of aspirin induced ulcer were starved for 36 hours having access to drinking water ad libitum.

2. 1ml of 80% ethanol would be administered orally. Famotidine is given to one group and leaf extractsto the other groups 1 hour before the administration of ethanol.
3. After 2 hours of ethanol administration, animals will be sacrificed by overdose of ether.
4. The stomach was removed and fixed on a cork plate and the number of and severity of ulcers was registered with a stereo-microscope using the following scores.

Severity score

0 = Normal coloured stomach

0.5 = Red colouration

1 = Spot ulcer

1.5 = Haemorrhagic streaks

2 = Ulcers ≥ 3 but ≤5

3 = ulcers > 5

Calculation

Ulcer index is determined by using following formula;

$UI = UN+US+ UP \times 10^{-1}$

Where,

UI = ulcer index

UN = average of number of ulcers per animal

US = average of severity score

UP = percentage of animal suffered ulcer

6.4.3.2. Determination of total acidity[86]

Principle

A known amount of gastric residue was titrated with 0.1 N sodium hydroxide to a pH of 3.5. If pH meter is not available, add two drops of Topfer's reagent which changes to a salmon colour when all the free hydrochloric acid is neutralized. The total acidity however was determined by titration using phenolphthalein as indicator.

Reagents:

(a) **Sodium hydroxide solution (0.1N NaOH):** Stock Sodium hydroxide solution (0.1N NaOH) was diluted ten-folds. Alternatively, 4g of NaOH was dissolved in fresh distilled water and made up to 1000 ml.

(b) **Phenolphthalein solution (1% alcoholic):** 1 g of phenolphthalein was dissolved in 100 ml of 95% alcohol.

(c) **Topfer's reagent (Dimethylaminoazobenzene), 0.5% alcoholic solution:** 0.5 g of Topfer's reagent was dissolved in 100 ml of 95% alcohol.

Procedure:

1. 10ml of gastric juice specimen was transferred in a porcelain evaporating dish.
2. 1-2 drops of Topfer's reagent is added.
3. A colour change was observed; a bright red colour appears if free hydrochloric acid is present. 1-2 drops of phenolphthalein was added to the gastric juice with Topfer's reagent.
4. Titrated with 0.1 NaOH from a burette, mixing was done after each addition until the last trace of red colour disappeared and was replaced by a canary yellow colour.
5. The numbers of millilitres of NaOH used was read from the burette. This represents the amount of free hydrochloric acid.

6. The titration was continued until the red colour of phenolphthalein appeared (deep pink), titrated to the point at which the further addition of alkali did not deepen the colour.

7. Reading was taken (ml NaOH) for total acidity.

calculation

Y = ml of 0.1 N NaOH x 10

Where,

Y= Total acidity (mEq/L)

6.4.3.3. Acid volume

Procedure

1. Animals in the group of aspirin induced ulcer were starved for 36 hours having access to drinking water ad libitum.

2. 1ml of 80% ethanol would be administered orally. Famotidine is given to one group and plant extracts to the other groups 1 hour before the administration of ethanol.

3. After 2 hours of ethanol treatment, animals will be killed by overdose of ether.

4. The stomach was removed and the contents were drained into a graduated centrifuge tube through a small nick along the greater curvature.

5. The volume of the juice was measured.

6.4.3.4. pH

Procedure

1. Animals in the group of ethanol induced ulcer were starved for 36 hours having access to drinking water ad libitum.

2. 1ml of 80% ethanol would be administered orally. Famotidine is given to one group and AEDB to the other groups 1 hour before the administration of ethanol.
3. After 2 hours of ethanol administration, animals will be sacrificed by overdose of ether.
4. The stomach was removed and the contents were drained into a graduated centrifuge tube through a small nick along the greater curvature.
5. The tubes were centrifuged at 3000 rpm for 10 minutes and the centrifuged samples were decanted and analysed for pH (using digital pH meter, Type DPH – 100- Data instruments).

6.4.3.5. Estimation of glutathione[87]

Reagents

DTNB reagent (5-5 dithiobis-2 nitrobenzoic acid): 39.6 mg of DNTB dissolved in 100 ml of 1 % of sodium citrate solution.

Trichloroacetic acid (TCA).

Procedure

The mucosa of glandular stomach was removed by scraping with a blunt knife and 10% homogenate was prepared. The homogenate was precipitated with 25% trichloro acetic acid (TCA) and centrifuged. The supernatant was taken for GSH estimation using freshly prepared DTNB solution. The supernatant was taken for GSH estimation using freshly prepared DTNB solution. The intensity of the yellow colour formed was read at 412 nm parallel blank for each sample without reagent was run.

Calculation

The amount of Glutathione was determined using molar extinction coefficient.

Calculate the enzyme activity by the following formula:

$$A = Є. b. c$$

Where, A = Absorbance of the solution.

Є = molar extinction coefficient.

b = path length of the light.

c = Concentration of absorbing solute.

6.4.3.6. Estimation of total protein[88]

Biuret method: This method is easy to follow and provide accurate results.

Principle

Proteins and peptides react with alkaline copper tartrate solution to give a violet coloured complex. The intensity of the final colour complex is measured calorimetrically at 540 nm and is proportional to the concentration of the total protein in the specimen under test. Under carefully controlled conditions this method can prove to be very useful but the reagents must be prepared carefully.

Reagents

a) Working biuret solution.

(b) Saline (NaCl, 0.85%w/v in water): 8.5 g of sodium chloride was dissolved in about 800 ml of water and placed in a one litre volumetric flask. The solution was brought up to the 1000 ml mark with water and mixed by inversion. The solution was kept in a stoppered glass bottle.

(c) Standard protein solution: The value of the protein concentration was in the range of 6 to 8 g protein per 100 ml.

Procedure

1. Three test tubes were set up marked as T, S and B for test, standard and blank respectively.
2. 5 ml of working Biuret reagent was pipetted out into the above test tubes.
3. 100µL of undiluted specimen and standard was added in T and S tubes respectively, and 100µL of water in tube B.
4. Contents were mixed thoroughly and incubated for 15 minutes at 37^0C in a water bath or alternatively for 30 minutes at room temperature.
5. Absorbance of the test and standard was measured against the blank at 540nm. The readings were completed within one hour.

Calculation

Total protein concentration in test specimen (g/dL) = $(A_t/A_s) \times 6$

Where,

A_t = Absorbance of testA_s = Absorbance of standard

CHAPTER VII

7. RESULTS AND DISCUSSION

7.1. Soxhlet Extraction Of *Desmostachya Bipinnata*

The percentage yield of the Desmostachyabipinnata was found to be 20.88 %w/v.

Table No : 8 : Extraction of *Desmostachyabipinnata*

Plant	Part used	Method of Extraction	Solvents	Percentage Yield (%W/V)
Desmostachyabipinnata	Whole plant	Maceration	Ethanol (95%)	20.88

Preliminary phyto chemical screening.

Alcoholic Extract of Desmostchyabipinnata was subjected various chemical tested as per the standard methods for the identification of the various constituents. The result if this phyto chemical analysis is listed below.

7.2. phytochemical evaluation

The preliminary phytochemical screening of whole plant extracts indicate in presence of flavonoid, alkaloid, proteins,amino acids and terpenoids, fixed oil and glycosides may accounts antioxidant and anti-ulcer potential.

7.2.1. Test for Proteins

I. Biuret Test:

A violet colour indicates the presence of proteins.

II. Xanthophoretic Test:

Orange colour indicates the presence of aromatic acids.

III. Lead acetate Test:

A white precipitate indicates the presence of proteins.

7.2.2. Test for Amino acids

I. Ninhydrin Test:

A blue colour indicates the presence of proteins, peptides or amino acids.

7.2.3. Test for Fats and Oils

I. Red globules in the section when viewed under the microscope show the presence of fats or oils.

7.2.4. Test for Cardiac glycosides

I. Keller-killiani Test:

At the junction, reddish brown colour was formed, which gradually becomes blue indicates the presence of cardiac glycosides.

7.2.5. Test for Flavonoids

I. On addition of sulphuric acid solution gives deep yellow solution.

II. Heat the test solution with Zinc and HCl, pink to red colour observation shows the presence of flavonoids.

7.2.6. Test for Alkaloids

I. **Dragendroff's Test:**

An orange red coloured precipitate indicates the presence of alkaloids.

II. **Wagner's Test:**

Reddish brown coloured precipitate indicates the presence of alkaloids.

III. **Mayer's Test:**

A dull white coloured precipitate indicates the presence of alkaloids.

7.2.7. Test for Phenolic compounds and Tannins

I. The respective observations may be deep blue black colour, white precipitate, white precipitate, decolouration of bromine water showing the presence of tannins and phenolic compounds.

Table No : 9 : Qualitative phyto chemical screening of AEDB

PLANT CONSTITUENT	INFERENCE
	Ethanol Extract
Carbohydrate	-
Alkaloids	+
Flavonoids	+
Proteins and amino acids	+
Glycosides	+
fixed oil	+
Terpenoids	+
Volatile oil	-
Tannins	-

"+" Presence,"-" Absence.

7.3. In Vitro Antioxidant Activities

7.3.1. Effect of superoxide radical scavenging activity:

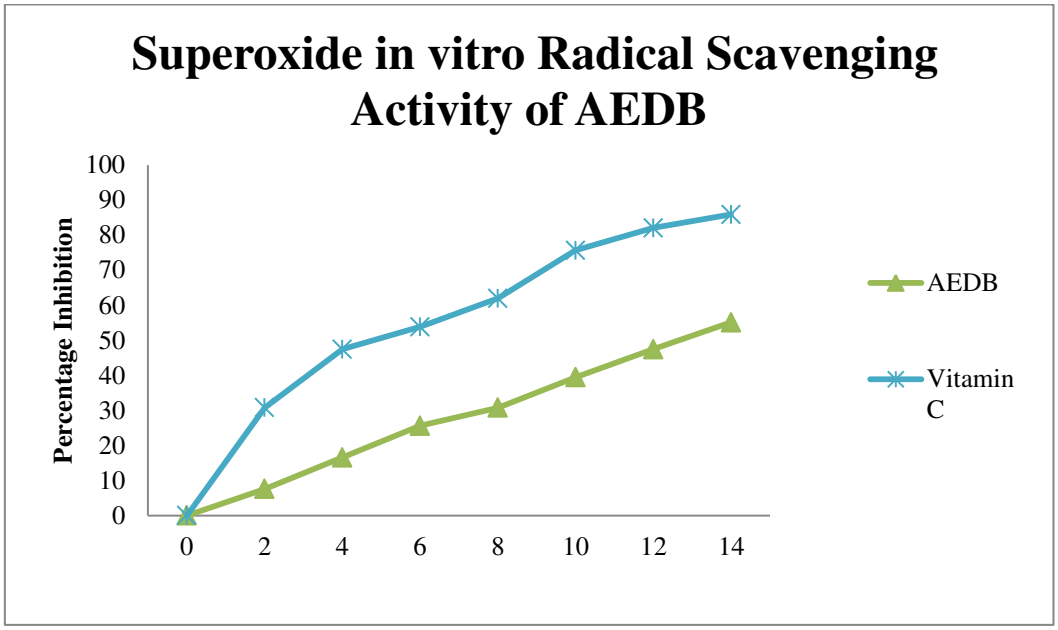
Superoxide generated in the photo reduction of vitamin C was effectively inhibited by the addition of varying concentrations (0-14μL/ml). The concentration of the AEDB needed to scavenge 50% superoxide anion (IC_{50}) was found to be 8 μg/ml (figure 4) Vitamin C which was used as a positive control had an IC_{50} value of 4.5 μg/ml.

Table No : 10 : Effect of AEDB on Superoxide *in vitro* Radical Scavenging Activity

Concentration (µg/ml)	Absorbance		Percentage inhibition	
	Ethanol extract	Vitamin C	Ethanol extract	Vitamin C
0	0.78±1.22	0.78±1.5	0±0.00	0±0.00
2	0.72±2.31	0.54±3.5	7.6±1.35	30.76±0.71
4	0.65±3.1	0.41±0.78	16.6±4.71	47.43±1.79
6	0.58±1.27	0.36±1.55	25.64±3.6	53.84±2.53
8	0.54±1.72	0.28±2.3	30.76±1.55	61.94±4.22
10	0.47±5.5	0.19±3.6	39.47±2.44	75.64±1.67
12	0.41±3.7	0.14±2.3	47.43±3.39	82.05±2.36
14	0.35±1.78	0.11±3.2	55.12±0.67	85.89±0.37

Results are mean ± SD of three individual experiments

Fig. No : 4 : Effect of AEDB on Superoxide *in vitro*Radical Scavenging Activity



7.3.2. Effect of AEDB on DPPH radical reducing activity :

The DPPH radical was effectively scavenged by AEDB. A dose dependent reduction of was observed within the range of concentrations (0-100μg/ml) of reaction system The IC_{50} value of various type of extracts was form the figure no -5. Vitamin C which was used as the positive control exhibited an IC_{50} value of 21.6 μg/ml.

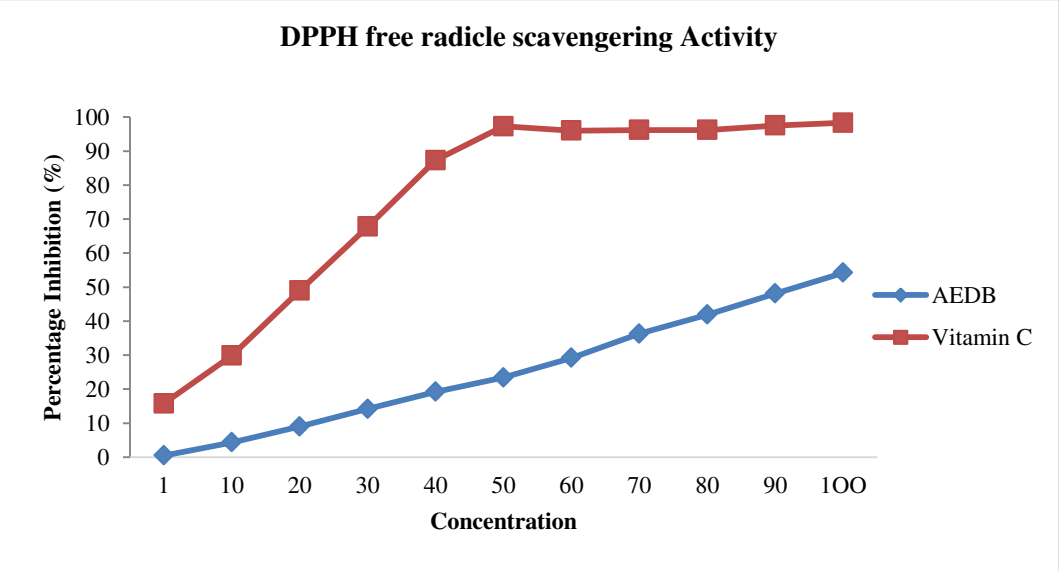
The antioxidant screening shows that that it showed reducing power to DPPH radicals. But the efficiency showed that far below from Vitamin C. The concentration of the AEDB needed to scavenge 50% superoxide anion (IC_{50}) equal to that of standard hence the plant extract have the significant antioxidant activity

Table No : 11 : study of *in vitro* DPPH Radical Scavenging Activity

Concentration (µL/ml)	Absorbance		Percentage inhibition	
	Ethanol extract	VitaminC	Ethanol extract	VitaminC
1	0.694±0.21	0.58±1.23	0.5±0.71	15.8±2.3
10	0.676±1.31	0.487±3.5	4.35±.56	29.9±2.9
20	0.640±3.22	0.361±1.23	9.03±0.78	49±5.6
30	0.60±1.52	0.121±1.5	14.2±1.3	67.8±4.9
40	0.566±4.35	0.101±3.2	19.2±1.27	87.3±4.3
50	0.530±2.33	0.046±4.2	23.4±1.32	97.3±4.2
60	0.461±3.5	0.06±4.9	29.2±.79	96.1±3.2
70	0.459±3.6	0.05±4.1	36.3±0.96	96.2±4.56
80	0.40±4.6	0.05±0.22	41.9±0.95	96.2±4.32
90	0.36±2.5	0.04±0.3	48.13±1.32	97.5±3.78
1OO	0.327±3.72	0.03±.52	54.23±1.56	98.3±3.96

Results are mean ± SD of three individual experiments.

Fig. No : 5 :DPPH radical reducing activity of AEDB and vitamin C.



7.4. Pharmacological Study

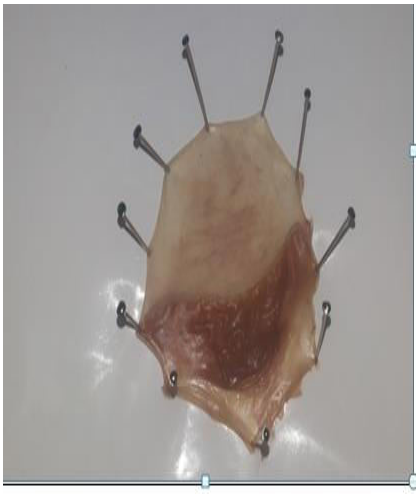
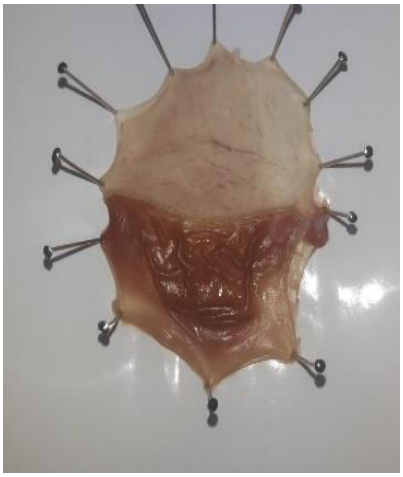
7.4.1. Acute Toxicity Studies

Acute toxicity studies on the albino rats show no morality at a dose of 2000mg/kg of AEDB during a time period of 14 days. In this study, NOAEL were seen in the rats. This help to predict that it does not contain any type of toxicity and it is fully safe for therapeutics uses. So 200 mg/kg between ($1/10^{th}$ dose) of ethanol extract were selected of that dose for the further study.

7.4.1.1. Anti-Ulcer Study

Antiulcer activity of *Alcoholic Extract of Desmostachyabipinnata* (AEDB) on Aspirin induced rat model and it was found to be it has shown significantly increased anti-ulcer activity by increasing doses. The maximum effect was observed at administration of 400mg/kg of AEDB Hence this study was dose depended. The result was shown Table 12 .

Fig. No : 6 : Photographs Showing Aspirin Induced Gastric Ulcers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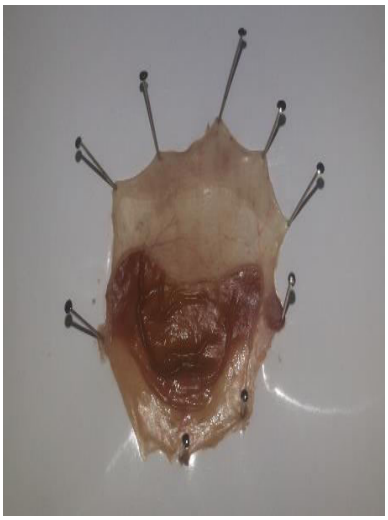
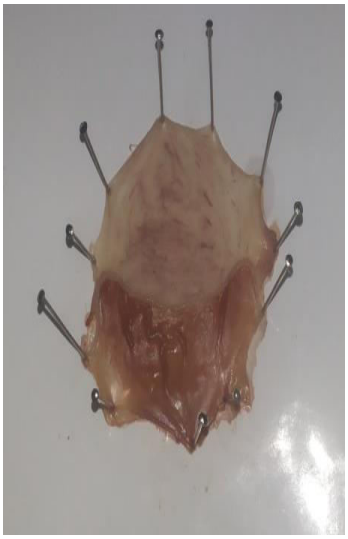
	
Low dose	**High Dose**

Effect of AEDB on Ulcer Index on NSAID induced Ulcer model

AEDB showed reduction in ulcer index at all doses tested when compared with control as reflected by the ulcer index values. The ulcer index value of group I which served as control (water) was 4.11±0.25. The total acidity of group III (100mg/kg), group IV (200mg/kg) and group V (400mg/kg) was 3.32±0.28, 2.29±0.21, 1.89±0.11 respectively, as shown in Table 12. Famotidine, the reference standard (group II) had total acidity 1.68±042, as shown in Table 12. Dose has been increased the ulcer index significantly reduced which showing the table no:12

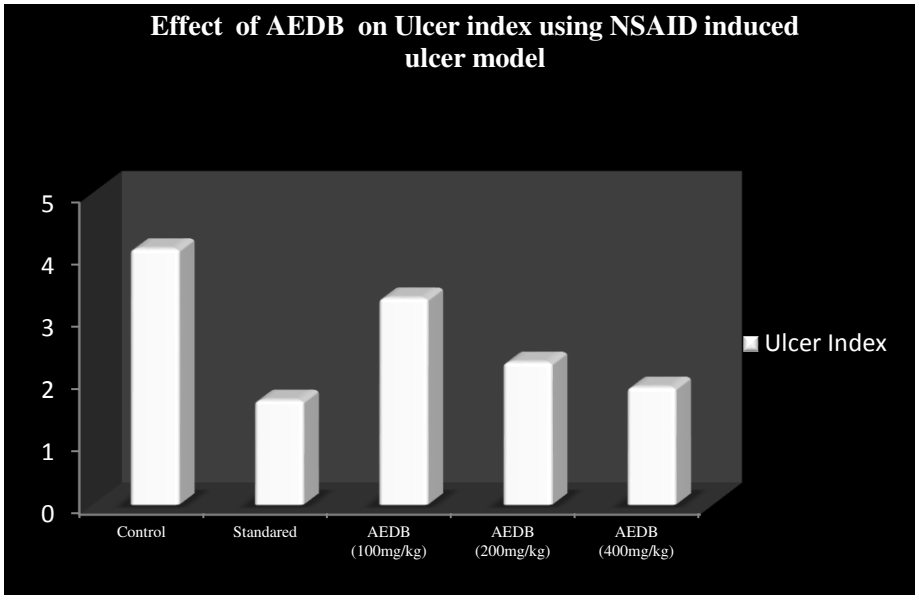
Table No : 12 : Effect of AEDB on Ulcer Index on NSAID induced Ulcer model

S.No	Treatment	Dose	Ulcer Index
1	Control (water)	-	4.11±0.25
2	Famotidine	3mg/kg	1.68±0.42**
3	AEDB	100mg/Kg	3.32±0.28
4	AEDB	200mg/Kg	2.29±0.21*
5	AEDB	400mg/Kg	1.89±0.11**

AEDB - *Alcoholic Extract ofDesmostachyabipinnata*

**P<0.001, *P<0.05, compared with control

Fig. No : 7 : Effect of AEDB on Ulcer Index on NSAID induced Ulcer model



Effect of AEDB on Total Acidity on NSAID induced Ulcer model

AEDB showed reduction in total acidity at all doses tested when compared with control as reflected by the total acidity values. The total acidity of group I which served as control (water) was 73.14±1.13. The total acidity of group III (100mg/kg), group IV (200mg/kg) and group V (400mg/kg) was 75.6±0.22, 67.06±0.516, 40.20±0.21respectively, as shown in Table 7. Famotidine, the reference standard (group II) had total acidity 28.1±0.84, as shown in Table 13. Dose has been increased the totel acidity significantly reduced which showing the table no:13

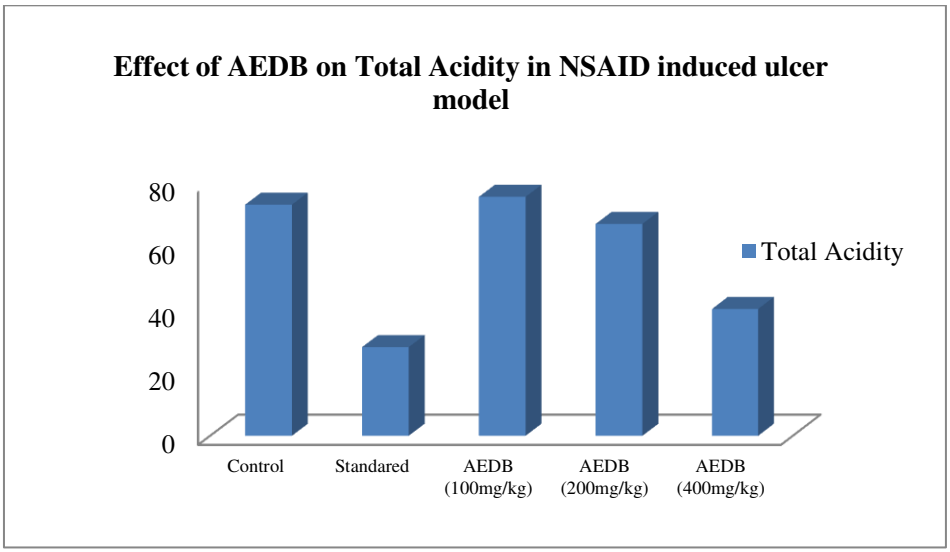
Table No :13 : Effect of AEDB on Total Acidity on NSAID induced Ulcer model

S.No	Treatment	Dose	Total Acidity (m Eq/L)
1	Control (water)	-	73.14±1.13
2	Famotidine	3mg/kg	28.1±0.84**
3	AEDB	100mg/Kg	75.6±0.22*
4	AEDB	200mg/Kg	67.06±0.516*
5	AEDB	400mg/Kg	40.20±0.21**

AEDB - *Alcoholic Extract ofDesmostachyabipinnata*

**P<0.001, *P<0.05, compared with control

Fig. No : 8 : Effect of AEDB on Total Acidity on NSAID induced Ulcer model



Effect of AEDB on Acid Volume on NSAID induced Ulcer model

AEDB showed reduction in acid volume at all doses tested when compared with control as reflected by the acid volume values. The acid volume value of group I which served as control (water) was 5.30±0.21. The total acidity of group III (100mg/kg), group IV (200mg/kg) and group V (400mg/kg) was 5.10±0.11, 4.32±0.09, 4.04±0.04 respectively, as shown in Table 7. Famotidine, the reference standard (group II) had total acidity 3.93±0.33, as shown in Table 14.

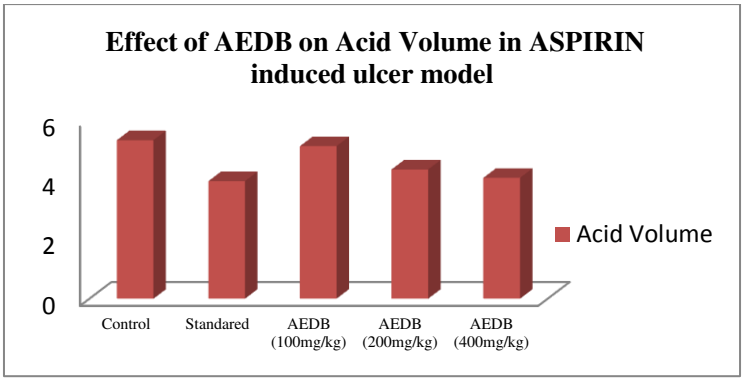
Table No: 14: Effect of AEDB on Acid Volume on NSAID induced Ulcer model

S.No	Treatment	Dose	Acid Volume (ml)
1	Control (water)	-	5.30±0.21
2	Famotidine	3mg/kg	3.93±0.33**
3	AEDB	100mg/Kg	5.10±0.11*
4	AEDB	200mg/Kg	4.32±0.09*
5	AEDB	400mg/Kg	4.04±0.04**

AEDB - *Alcoholic Extract ofDesmostachyabipinnata*

**P<0.001, *P<0.05, compared with control

Figure No : 9 : Effect of AEDB on Acid Volume on NSAID induced Ulcer model



Effect of AEDB on pH in NSAID induced Ulcer model

pH of gastric secretion of group I animals which served as control(water) was 2.15±0.10 and in group III (100mg/kg), group IV (200mg/kg), group V (400mg/kg) was 3.49±3.07, 3.22±4.77, 4.02±3.33 observed respectively. Group II standard (Famotidine) treated had pH 4.88±0.16, as shown in Table 15.

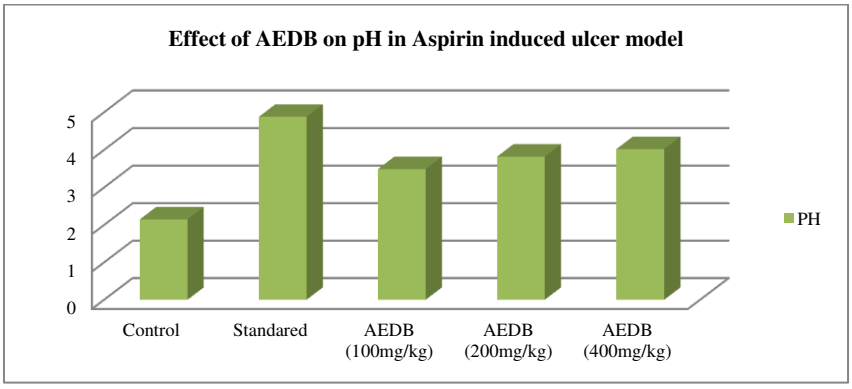
Table No : 15 : Effect of AEDB on pH in NSAID induced Ulcer model

S.No	Treatment	Dose	PH
1	Control (water)	-	2.15±0.10
2	Famotidine	3mg/kg	4.88±0.16**
3	AEDB	100mg/Kg	3.49±3.05
4	AEDB	200mg/Kg	3.82±4.77**
5	AEDB	400mg/Kg	4.02±3.33**

AEDB - *Alcoholic Extract ofDesmostachyabipinnata*

**P<0.001, *P<0.05, compared with control

Fig. No : 10 : Effect of AEDB on p H on NSAID induced Ulcer model



Effect of AEDB on Glutathione in NSAID induced Ulcer model

Since glutathione is intimately associated with the prevention of gastric erosions. It was thought worthwhile to measure glutathione level in control and treated rats. As shown in table 9, group I animals which served as control had glutathione level 0.93±0.012. In group III (100mg/kg), group IV (200mg/kg), group V (400mg/kg) the glutathione level was 0.88±0.012, 1.22±0.094, 1.02±0.024 respectively, as shown in Table 16. The level of glutathione of Group II standard (Famotidine) treated was 1.14±0.19.

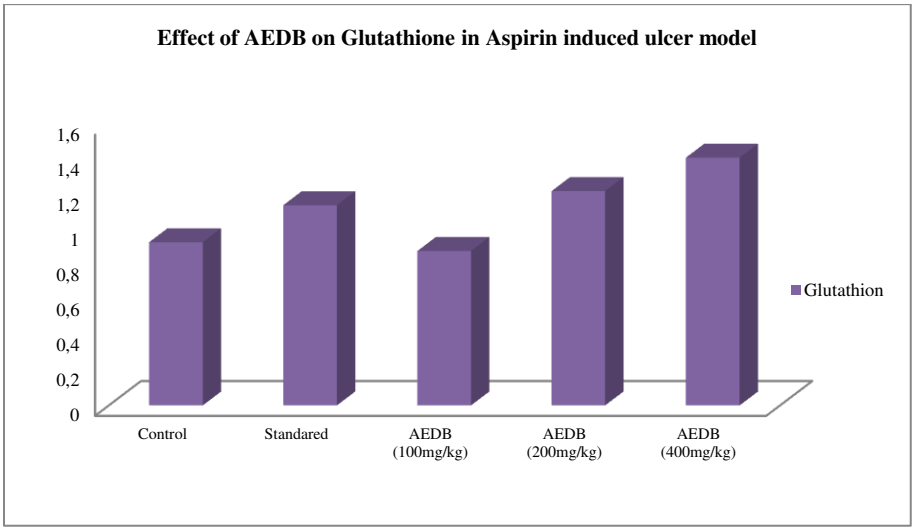
Table No : 16 : Effect of AEDB on Glutathione in NSAID induced Ulcer model

S.No	Treatment	Dose	Glutathione (mcg/gm)
1	Control (water)	-	0.93±0.012
2	Famotidine	3mg/kg	1.14±0.19*
3	AEDB	100mg/kg	0.88±0.065
4	AEDB	200mg/kg	1.22±0.094
5	AEDB	400mg/kg	1.41±0.024*

AEDB - *Alcoholic Extract ofDesmostachyabipinnata*

**P<0.001, *P<0.05, compared with control

Fig. No :11 : Effect of AEDB on Glutathione in NSAID induced Ulcer model



Effect of AEDB on Total Protein in NSAID induced Ulcer

AEDB was also studied for its effect on total protein content of the gastric juice. It showed rise in protein content in the control group whereas pre-treatment with AEDB at different dose levels was observed as declining the protein content. Simultaneously, there was a fall in the protein content in Famotidine treated group. The total protein content of the group I which served control was 0.842±0.02. Group III (100mg/kg), group IV (200mg/kg), group V (400mg/kg) was having the total protein content 0.740±0.09, 0.672±0.05 and 0.650±0.02 respectively. Famotidine which was used as standard (Group II) had total protein content 0.700±0.03, as shown in Table 17. Dose has been increased the ulcer index significantly reduced which showing the table no:17.

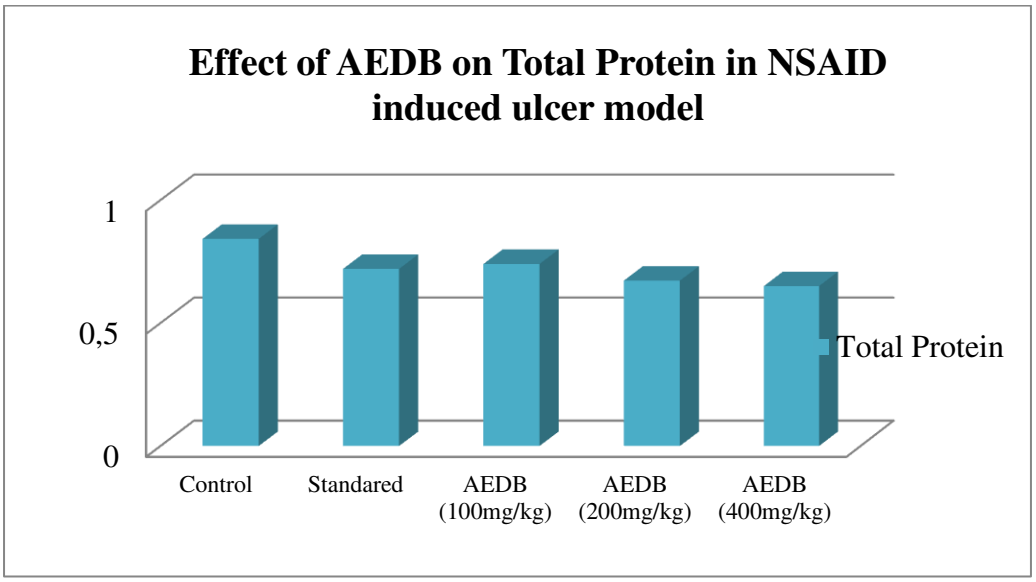
Table No : 17 : Effect of AEDB on Total Protein in NSAID induced Ulcer

S.NO	Treatment	Dose	Totel ptotein gm/dl
1	Control (water)	-	0.842±0.02
2	Famotidine	3mg/kg	0.720±0.03*
3	AEDB	100mg/kg	0.740±0.09
4	AEDB	200mg/kg	0.672±0.05*
5	AEDB	400mg/kg	0.650±0.02*

AEDB - *Alcoholic Extract ofDesmostachyabipinnata*

**P<0.001, *P<0.05, compared with control

Fig. No : 12 : Effect of AEDB on Total Protein in NSAID induced Ulcer Model



CHAPTER VIII

8. SUMMARY AND CONCLUSION

The present study is evaluated the antioxidant potential and antiulcer effect of AEDB. The results analysed from the present study have indicate that AEDB possesses antioxidant and antiulcer effect on aspirin induced ulcers.

The preliminary phytochemical screening of whole plant extracts indicate in presence of flavonoid, alkaloid, proteins,amino acids and terpenoids, fixed oil and glycosides

Pre-treatment with AEDB particularly at a dose of 400mg/kg in a single schedule and 400mg/Kg treatment significantly reduce the ulcer index value, total acidity concentration, total volume of acid release and total protein and increase value pH and glutathione content as compared with 100 mg/kg,200mg/kg and control groups.

The antioxidant screening shows that it showed reducing power to DPPH radicals. But the efficiency showed that far below from Vitamin C. The concentration of the AEDB needed to scavenge 50% superoxide anion (IC_{50}) equal to that of standard hence the plant extract have the significant antioxidant activity

The antiulcer effect is screened in ethanol extract of *Desmostachyabipinnata* on NSAID induced anti-ulcer study. The results get from these study have been shown that ethanol extract of *Desmostachyabipinnata* produce antiulcer effect.In aspirin induced model, there is reduction in ulcer index, total acidity, total volume of gastric contents, total protein concentration and higher concentration of glutathione content and pH of gastric secretion they compared with control group. Famotidine used as a standard comparison agents. Famotidine used as H_2 receptor blocker, is significantly reduce about 90% of basal, food induced and hormonal mediated gastric acid, which again induced by histamine, gastrin, parasympathomimetic drugs and vagal stimulation. Famotidine mediate its suppression of gastric secretion by inhibiting the histamine mediated c-AMP dependent pathway

Famotidine encouraged certain type mucus analogues of gastric mucus for patients with duodenal ulcer.

Total acidity responsible quantification acid is present in the gastric secretion. It has a important aggressive factor which induced the ulcer. Gastric release is maintained by vagal control and higher activity of vagus stimulation also contributes to ulcer formation. On ethanol administration, the mucosal mast cells mediate to secretion of vasoactive mediators containing histamine. Histamine is mediated to stimulate the synthesis of cyclic AMP through activation of the enzyme adenylcyclase which mediate the activation of gastric proton pump and secrete of hydrogen ions. The treatment of extracts showed reduce the total acidity of the gastric contents.

Serum protein including albumin and globulin. In the peptic ulcer the total protein concentration of serum or gastric secretion are increased. This may be due to leakage of plasma protein in to the gastric secretion or serum with lower mucosal resistance/barrier of the gastric mucosal layer.After treatment with AEDB there was a significant reduction in protein concentration of gastric juice which enhancing leakage of plasma proteins.

Acid volume is amount (in ml) of acid release in the gastric content release contain HCl, pepsinogen enzyme, mucus secretion, bicarbonates concentration, intrinsic factor and proteins. Amount of acid release is an important factor responsible for the production of ulcer mediated by exposure of the unprotected lumen of stomach by concentrated acids. AEDB treatment showed decrease in the acid volume of the gastric secretion.

Increased pH shows a lower concentration of the hydrogen ion. The hydrogen ion is a major triggering factor responsible for the etiologic factor for ulcer and gastric damage. AEDBtreatment indicate higher concentration of pH of the gastric juices. This values directly shown the AEDB reduce possibility of ulcer and has a protective effect of surface of the gastric mucosa.

In gastric ulcer tissues, Glutathione (g-glutamylcysteinylglycine, GSH) levels were found to be decreased[112]. aspirin-induced genesis of free radical concentration reduces the cysteine concentration which mediated for GSH

released. Values from this study responsible for depletion of gastric GSH is related with induction of gastric lesion in the rats. GSH is a tripeptide and having a superoxide radical scavenger and it protect thiol protein contents essential for release the integrity of tissue against oxidation reaction. In my present study, AEDB treatment showed increase in the glutathione content.

All these data indicate that the AEDB could be regarded as a favourable antioxidant and anti ulcer effect. Mechanism of action and therapeutic need to establish in future research

CHAPTER IX

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