"EVALUATION OF ANTI-ULCER ACTIVITY OF

Pithecellobium dulce seeds IN ALBINO WISTAR STRAIN RATS"

DISSERTATION

Submitted to

The Tamilnadu Dr. M.G.R Medical University

Chennai – 32

In Partial fulfillment for the award of the Degree of

MASTER OF PHARMACY

Department of Pharmacology

Under the guidance and supervision of

Prof. Dr. R. ANANDAN, M. Pharm., PhD.,

DEPARTMENT OF PHARMACOLOGY

April 2017

Submitted by

Reg.no: 261325752

DEPARTMENT OF PHARMACOLOGY

PADMAVATHI COLLEGE OF PHARMACY & RESEARCH INSTITUTE

PERIYANAHALLI, DHARMAPURI– 635 205, TAMILNADU

CERTIFICATE

This is to certify that this dissertation work entitled **"EVALUATION OF ANTI ULCER ACTIVITY OF *Pithecellobium dulce* seeds IN ALBINO WISTAR STRAIN RATS"** Constitutes the original work Carried out by **Reg . No: 261325752**, under the guidance and supervision of **Prof. Dr. R. Anandan, M. Pharm., PhD., Professor & Head, Department of Pharmacology,** Padmavathi college of pharmacy and Research Institute, Periyanahalli, Dharmapuri, Tamilnadu - 635205.

Place:Dharmapuri
Date:

PRINCIPAL
Dr. D.C.Premanand, M.Pharm, Ph.D.,
Padmavathi college of pharmacy and Research Institute, Periyanahalli, Dharmapuri, Tamilnadu- 635205.

CERTIFICATE

This Is To Certify That This Dissertation Work Entitled **"Evaluation of Anti Ulcer Activity of *Pithecellobium Dulce* seeds In Albino Wistar Strain Rats"** constitutes the original work carried out by **Reg . No: 261325752**, for the Partial Fulfillment of the requirements for the award of degree of **Master of Pharmacy in Pharmacology,** was carried out in Department of Pharmacology, under the guidance and supervision of **Prof. Dr. R. Anandan, M. Pharm., PhD., Professor & Head, Department of Pharmacology,** Padmavathi college of pharmacy and Research Institute, Periyanahalli, Dharmapuri, Tamilnadu - 635205.

HOD

Prof. Dr. R.Anandan, M.Pharm., Ph.D.,
Professor & Head,
Department of Pharmacology,
Padmavathi college of Pharmacy
and Research Institute,
Dharmapuri, Tamilnadu-635205.

Place: Dharmapuri
Date:

CERTIFICATE

This is to certify that this dissertation work entitled **"EVALUATION OF ANTI ULCER ACTIVITY of *Pithecellobium dulce* IN ALBINO WISTAR STRAIN RATS"** Constitutes the original work carried out by **Reg . No: 261325752**, for the partial fulfillment of the requirements for the award of degree of **Master of Pharmacy in Pharmacology,** was carried out in Department of Pharmacology, under the guidance and supervision of **Prof. Dr. R. Anandan, M. Pharm.,PhD., Professor & Head,** Department of pharmacology, Padmavathi college of pharmacy and Research Institute, Periyanahalli, Dharmapuri,Tamilnadu - 635205. Under my guidance and supervision.

GUIDE

Prof. Dr. R. Anandan, M.Pharm., Ph.D.,
Professor & HOD,
Department of Pharmacology,
Padmavathi college of Pharmacy
and Research Institute,
Dharmapuri, Tamilnadu-635205.

Place: Dharmapuri
Date:

DECLARATION

I Hereby I declare that this thesis work **"EVALUATION OF ANTI-ULCER ACTIVITY OF *Pithecellobium dulce* seeds IN ALBINO WISTER RATS"** has been originally carried out by myself under the supervision and guidance of **Prof. Dr. R.Anandan, M.Pharm., PhD., Professor & Head,** Department of Pharmacology, Padmavathi college of Pharmacy and Research Institute, Dharmapuri, Tamilnadu-635205. This work has not been submitted for any degree at any University.

Place: Dharmapuri

Date: **Reg . No: 261325752**

EVALUATION CERTIFICATE

This is to certify that dissertation entitled **"EVALUATION OF ANTI-ULCER ACTIVITY OF *Pithecellobium dulce* seeds IN ALBINO WISTER RATS"** constitutes the original work carried out by **Mr. K.Muhammed Jaseem, Reg . No: 261325752,** under the guidance and supervision of **Prof. Dr. R.Anandan, M.Pharm., PhD., Professor & Head,** Padmavathi college of Pharmacy and research Institute, Periyanahalli,Dharmapuri-635205, has been evaluated on _____

Evaluators:

1. 2.

ACKNOWLEDGEMENT

The task of preparing this dissertation has been fascinating experience and it is really a movement of great pleasure for me to express my hearty gratitude to those who have helped me in success full completion of this dissertation.

I would like to express our sincere thanks to **Kalvi Kodai Vallal. Mr. M.G. Sekar, B.A.B.L., and Ex**. **M.P and M.L.A.,** Chairman of Sapthagiri Padmavathi and Pee Gee Group of institution and industries for providing all facilities during my post graduate studies.

I would like to thank **Dr. D.C.Premanand, M.Pharm, Ph.D.,** Padmavathi college of pharmacy and Research Institute, Principal, Padmavathi college of pharmacy and Research Institute, for permitting to carry out the work in college.

With a sincere note of gratitude, I wish to express my deepest thanks, indebtedness, and regards to my respected Guide **Prof. Dr. R.Anandan, M.Pharm., PhD., Professor & Head,** Department of Pharmacology, Padmavathi College of Pharmacy and Research Institute. With his illuminating guidance, constructive criticism and invaluable suggestions I have been successful in keeping my spirit high throught the pursuance of my project from the college.

I would like to express my special thanks **to Mr. Vimalan M. Pharm,** Department of Pharmacology for his kind cooperation, valuable suggestions and affection during my project.

I am elated to place on record my profound sense of gratitude to **Dr. R. P. Ezilmuthu M. Pharm, PhD., Head Department of Pharmaceutics, Mr.vasanthan M. Pharm.**

I would like to express my special thanks **to Dr.M. Rajkumar M. Pharm, PhD.,** Head of the Department of Pharmacognosy, **Mrs. T. Karthiyayini , M. Pharm.**

I would like to express my sincere thanks to **Mr. M. Saravanan M. Pharm ., (PhD).,Head Department of pharmaceutical Analysis**

I would like to express my special thanks to Office Administrative Mr Loganathan, Mr.Murali, Mr.Ramesh **Librarian** and non teaching staff for their valuable suggestions.

.

With deep veneration and gratitude, I dedicate all my work to my beloved **Family** who are my ideals and the constant source of moral support I my life. Without their encouragement and blessings no achievement would have been possible.

At last but most importantly I am thankful to God who is the creator and director of all that initial and final modes to destiny.

K.Muhammed Jaseem

(Reg. No: 261325752)

INDEX

S. NO	CONTENTS	PAGE NO.
1.	INTRODUCTION	1
2.	LITERATURE REVIEW	21
3.	PLANT PROFILE	23
4.	AIM & OBJECTIVES	26
5.	PLAN OF WORK	27
6.	MATERIALS & METHODS	28
7.	RESULT & DISCUSSION	40
8.	CONCLUSION	52
9.	BIBLIOGRAPHY	53

LIST OF ABBREVIATIONS

ANOVA	Analysis Of Varience
AEPD	Aqeous extract of *Pithecellobium dulce*
$AL(OH)_2$	Aluminium Hydroxide
CNS	Central Nervous System
CCK	Cholecystokinin
CPCSEA	Committee For The Purpose Of Control And Supervision On Experiments And Animals
ECL	Entero Chromaffin Like
EEPD	Ethanolic Extracts Of *Pethicelloium Dulce*
FIG	Figure
GPSR	G- Protein Coupled Receptor
GERD	Gastro Oesophageal Reflux Disease
GIT	Gastro Intestinal Tract
H.PYLORI	*Helicoacter Pylori*
H_2	Histamine Receptor
H_2SO_4	Sulphuric Acid
IAEC	Institutional Animalethical Committe
IUPAC	International Union Of Pure And Applied Chemistry
M_3	Muscarinic Receptor
MM^2	Milli Meter
ML	Millilitre
MIN	Minutes
NSAID	Nonsteroidal Anti-Inflammatory Drugs
OECD	Organization For Economic Cooperation And Development
PUD	Peptic Ulcer Disease
P.O	Per Oral
RPM	Rotationper Minute
SRMO	Stress Related Mucosal Damage
SST	Somatostatin
SEM	Standard Mean Error
V/V	Volume / Volume
WHO	World Health Organisation
ZES	Zollinger-Ellison Syndrome

1. INTRODUCTION

Medicinal plants are of great value in the field of treatment and cure of diseases. Over the years, scientific research has explained our knowledge of the chemical effects and composition of the active constituents. It has now been universally accepted fact that the plant drugs and remedies are for safer than that of synthetic medicines for curing the complex diseases. Natural product is a source for bioactive compounds and has been a growing interest in drugs of plant origin and such drugs formed an important class for disease control.

History of herbal medicine:

Plants have been used as a source of medicine by man from ancient times. Initially, these formed the bulk of folk or ethno medicines, practiced in India and some other parts of the world like China, Africa and South America. Later a considerable part of this indigenous knowledge was formulated, documented and eventually passed into the organized system of medicine such as Ayurveda, Unani, Siddha and some other outside India. Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served human well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise that plant contains natural substances that can promote health and alleviate illness. Herbs are prime medicinal agents in traditional and holistic therapies. Particularly in India and China an extensive science has been developed. Now a days the medicinal plants play a major roll and constitute the back bone of the traditional medicine. Mainly in Indian materia medica include about 2000 drugs of natural origin folklore practice. In India, plants have been traditionally used for human and veterinary health care. India has a special position in area of herbal medicine. Since it is one of the countries which are capable of cultivating most of the important plants used both in modern and tradition system of medicine.

The traditional heritage of India includes many true tested medicinal plants/drugs for various diseases and to which there is no answer in modern medicine till today. Indian traditional medicine is based on various system including Ayurveda, Siddha, Unani and Homeopathy. The Indian traditional medicines can be classified into two groups. In first group are the medicinal preparations which are generally of plants, minerals, or animal origin or mixtures of two or three of them and have well laid down procedure for their preparations. While the folk medicines

belong to the second group, which are herbal house hold remedies and have no systematic approach for processing raw materials and are mostly used as family traditions. The evaluation of these drugs is primarily based on phytochemical, pharmacological and allied approaches such as chromatography, microscopy and other.

Ayurveda is the ancient system of treatment of a disease consists of salubrious use of drugs, diets, and certain practices. Ayurveda is based on hypothesis that everything in the universe is composed of five basic elements viz. space, air, energy, liquid and solid. The concept of ethno pharmacology has evolved from the requirement for studies in light of modern science on the drugs used in the traditional medicine. In 1981, Bruhn and Holmstedt defined ethno pharmacology as the interdisciplinary scientific exploration of biologically active agents traditionally observed by man. “In its entirety, pharmacology embraces the knowledge of the History, source, chemical and physical properties, compounding biochemical and physiological effects, mechanism of action, absorption, distribution, biotransformation, excretion and therapeutic and other uses of drugs”. A drug is broadly defined as any substance (chemical agent) that affects processes of living. Therefore, briefly, the main component of ethno pharmacology may be defined as pharmacology of drugs used in ethno medicine.

Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Herbalism is also known as botanical medicine, medicinal botany. Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen substituted derivatives such as tannins. Many of the herbs and spices used by humans to season food yield useful medicinal compounds. Many of the pharmaceutical currently available to physicians have a long history of use as herbal medicines, including opium, aspirin, digitalis, and quinine.

The World Health Organization (WHO) estimated that about 80% of the population living in the developing countries relies almost exclusively on tradition medicine, the medicine for their primary health care needs. In almost all the traditional medicine the medicinal plants play a major role in cure of disease. The plant kingdom still holds many species of plants containing substance of medicinal value which have yet to be discovered large no of plants are

constantly being screened for their possible pharmacological value. All plants possess hundreds of characteristics of a morphological, histological, embryological, chemical and genetic nature. Currently, photo chemistries have significant development. The technology involves the isolation, extraction, Purification and characterization of active constituents from natural origin. The isolated compounds are mainly used as therapeutic agent in chronic disease.

According to the WHO, 74% of 119 modern plant- derived pharmaceutical medicines are used in ways that are similar to their traditional uses. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rainforests and other places for possible new pharmaceuticals. Nature can be considered as the ultimate chemist as natural products offer us with an abundant source of novel chemo-types, pharmacophores or lead structures, which could be directly used or derived into ready-made drugs.

"Herbal renaissance" is happening all over the globe as herbal products are the symbol of safety as compare to synthetic medicine, which could be regarded unsafe to human and environment. From many centuries herbs are used for a medicinal, flavoring and aromatic properties but synthetic products of modern age has decreased their importance for a movement. But hopefully blind dependence on synthetic drug is over and now day's peoples are adopting herbalism with hope of security and safety to health.

Traditional system of medicine is found to have utilities as many accounts. Due to population rise adequate supply of drug and high cost of treatment and side effect along with drug resistance has been encountered in synthetic drugs, which has lead to an elevated emphasis for the use of plants to treat human diseases. The affordability of herbs has also drawn the attraction towards their use.

India is one of the oldest civilizations which is known for rich repository of medicinal. The forest of India is Pandora's Box being having rich collection of medicinal and aromatic plants which could be utilize to prepare drugs and perfumes. Ayurveda, the bible of Indian medicinal science has codified about 8000 herbal remedies used for various therapeutic purposes. The other ancient epical health books like, The Rig-Veda, Yajurveda, Atharvaveda, Charaka Samhita and Sushrut samhita has described the use of various medicinal plants which

are still found in many of Ayurveda formulations. But unfortunately a lot of valuable ancient knowledge is being lost in alarming rate.

Green plants biosynthesize and preserve large number of biochemical products, many of which are extractable and used for various scientific investigations. Secondary metabolites of plants also found a number of roles in modern medicine. It is the potential of ancient herbal medicinal systems which provide base to synthesis the lead structures for the development of modified derivatives with increased efficiency and/or reduced toxicity.

Some miraculous useful chemical from plants include vincristine, vinblastine, taxolpodophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine,codeine, aspirin, atropine, pilocarpine, capsaicin, allicin, curcumin, artemesinin and ephedrine. The crude extract from medicinal plants could be used as medicament. In the other side, the isolation and identification of active principle along with elucidation of their mechanism of action of drug extreme importance.

In industrialized nations at the present time, some fifty percent of all prescribed drugs are derived or synthesized from natural products, the available sources for which are animals, marine, plants, and micro-organisms. It is considered that because of the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable source.

1. Peptic Ulcer Disease

Peptic ulcer disease refers to pathological lesions and ulcers of any portion of gastrointestinal tract exposed to acid activated pepsin. Peptic ulcer disease (PUD) differs from gastritis and erosions in that ulcers typically extend deeper into the muscular is mucosa. There are three common forms of peptic ulcers: Helicobacter pylorus (H. pylori) associated Non-steroidal anti-inflammatory drug (NSADI) Induced, and stress ulcers. The term “Stress-related mucosal damage” (SRMD) is preferred to stress ulcer or stress gastritis, because the mucosal lesions range from superficial gastritis and erosions to deep ulcers. Gastric ulcer refers to ulcer in the stomach where as duodenal ulcer is an ulcer found in duodenum of small intestine.

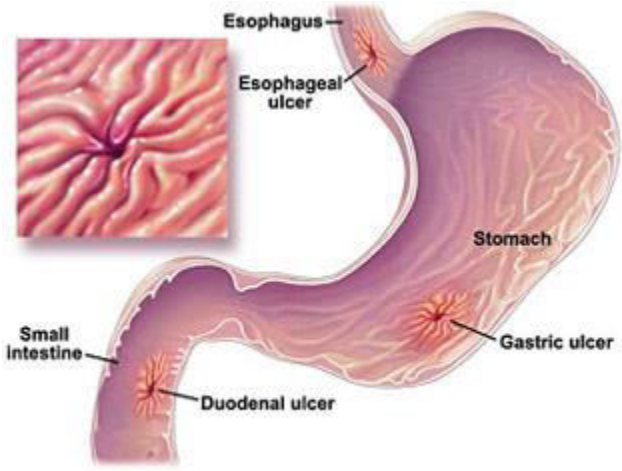
Peptic ulcer is one of the major health problem both in terms of morbidity and in terms of mortality. Research advances have offered new sights in the the therapy and prevention of gastro duodenal ulcerations by measures directed at strengthening the mucosal defense systems rather than by attenuating aggressive acid pepsin factors held responsible for the induction of ulcers. Most of the peptic ulcers are duodenal in nature. The cause of ulceration in patients is mainly due to hyper-secretion of gastric juice and pepsin. The other forms of peptic ulcers are Zollinger-Elllison syndrome, drug associated ulcers and stress ulcers.

Treatment options available are use of muco protective agents, antacids, alginates, motility stimulants and acid suppressants. Anti reflux surgery is done in severe cases. In spite of established anti-ulcer drugs, a rational therapy for peptic ulcer remains elusive and search for safer potential drugs is being carried out.

1.1 Definitions

- An ulcer is a local defect, or excretion of the surface of an organ or tissue that is produced by the sloughing (shedding) of inflammatory necrotic tissue.
- Ulcers are local defect on the surface of an organ produced by inflammation.
- An Ulcer is a discontinuity of the skin exhibiting complete loss of the epidermis and often portions of the dermis.
- Ulceration is the process of ulcer formation and the process of becoming ulcerated. An open sore on the skin surface or on the stomach lining. An open sore on the skin or mucous membrane characterized by the disintegration of the tissue and often the discharge of pus.

Fig1. Peptic ulcer disease



Peptic ulcer is a breach in the gastric and duodenal epithelium associated with acute or chronic inflammation. Peptic ulcer is the most common gastro intestinal disorder in clinical practice. Gastric ulcer refers to ulcer in the stomach whereas duodenal ulcer is an ulcer found in duodenum of small intestine (Jain et al .,1994).

1.2 Types of peptic ulcer

- **Acute (Stress) peptic ulcer**

Acute peptic ulcer are multiple, small mucosal erosion, seen commonly in the stomach but occasionally involving the duodenum.

Etiology-

The Acute ulcers occur due to severe stress. The causes are as follows:

- Psychological stress.
- Physiological stress.

Physiological stress as in the following:

- Shock and severe trauma
- Septicemia
- Expensive burns
- Drug intake (Aspirin, Steroids and Indomethacin).
- Local irritants (Alcohol, Smoking, Coffee).

➢ **Chronic Peptic Ulcer**

Chronic ulcer are also two types either Gastric or duodenal.

Etiology-

Chronic ulcer may be caused by

- *Helicobacter pylori*
- About 15-20% cases infected with *H.Pylori* develops duodena ulcer in their life time while gastric colonization by *H.Pylori* never develops ulceration and remain asymptomatic.
- Aspirin and other salicylates damage the gastric mucosa precipitates

Upper gastro intestinal bleeding and cause gastric ulcer.

Numerous mechanisms whereby aspirin and salicylates damage the mucosa have been suggested the most important of which is their ability to inhibit prostaglandins synthesis.

- Acid-Pepsin in secretion

  There is convulsive evidence that some level of acid-pepsin secretion is essential for the development of duodenal as well as gastric ulcer.

- Mucous secretion

  Any condition that decreases the quantity of normal protective mucous “barrier” predisposes to the development of peptic ulcer.

- Dietary factor nutritional deficiencies have been regarded as etiological factor in peptic ulcer.

- Psychological factor

  Psychological stress, anxiety, fatigue, and ulcer type personality may exacerbate as well as predispose to peptic ulcer disease.

- Local irritants

Some of the local irritating substances implicated in the etiology of peptic ulcer are:

1. Smoking: Cigarette smoking can increase a person's chance of getting an ulcer. Smoking also slows the healing of existing ulcers and contributes to ulcer recurrence.
2. Caffeine: Beverages and foods that contain caffeine can stimulate acid secretion in the stomach. This can aggravate an existing ulcer, but the stimulation of stomach acid can't be attributed solely to caffeine.
3. Alcohol: Alcohol consumption increases the risk of peptic ulcer (Harsh Mohan,2005).

1.3 Complications of peptic ulcer

Some initial complications or symptoms of peptic ulcer disease.

- ❖ Bleeding: Bleeding may be the first and only symptom of an ulcer. Bleeding ulcers can cause vomiting of acidified blood that looks like "old coffee grounds"or bowel movements can become tarry black or even bloody. When an ulcer bleeds and continues to bleed without treatment, a person may become anemic.
- ❖ Perforation: When ulcers are untreated, gastric juices can literally eat a hole in (perforate) the stomach lining, requiring surgery.
- ❖ Obstruction: chronic inflammation from an ulcer can cause swelling and scarring. Over time, this scarring may close (obstruct) the outlet of the stomach, preventing the passage of food and causing vomiting and weight loss. Surgery is required to repair obstructions (Harsh Mohan, 2005; Al-Mofleh et al.,2006).

1.4 Most common types of Ulcers

- **Peptic Ulcer:** Any ulcer that is exposed to pepsin is referred to as peptic ulcers. Peptic ulcers are found in the lining of your stomach or duodenum. Pepsin is normally present along with hydrochloric acid in the stomach lining.
- Gastric Ulcer : When a peptic ulcer is in stomach, it is called a gastric ulcer.
- **Doudenal Ulcer:** When a peptic ulcer is in duodenum, it is called a duodenal ulcer. This type of peptic ulcer develops in the first part of the small intestine.

Lesser types of ulcers

- **Esopohageal Ulcer:** This type of ulcer occurs in the lower and of your esophagus. Esophageal ulcersare often associated with an acid reflux, or GERD (Gastro Esophageal Reflux Disease).
- **Bleeding Ulcer:** Internal bleeding is caused by apeptic ulcer which has been left untreated. When this happens, it is now refered to as a bleeding ulcer – this is the most dangerous type of ulcer.
- **Refractory Ulcer:** Refractory ulcers are simply peptic ulcers that have not healded after at least 3 months of treatment.
- **Stress ulcer:** Stress ulcers are a group of lesions (or lacerations) found in the esophagus, stomach or duodenum.

1.5 Causes of ulcer

The direct cause of peptic ulcers is the destruction of the gastric or intestinal mucosal lining of the stomach by hydrochloric acid, an acid normally present in the digestive juices of the stomach.

The causes by which ulcer from are following:-

- Helicobacter pylori infection, a spiral shaped type of bacteria, is present in more than 90% of patients who have intestinal ulcers and more than 80% of patients with stomach ulcers.
- Helicobacter pylorus weakens the protective mucous coating of the stomach and duodenum which allows acid to get through to the sensitive lining beneath. Both the acid and the bacteria irritate the lining and cause a sore, or ulcer.
- Long term use of anti-inflammatory drugs (NSAIDs) or pain relievers such as aspirin, ibuprofen, and naproxen sodium. NSAIDs lower the stomach's resistance to the harmful effects of acid.
- Smoking.
- Physical Stress such as surgery or extreme injury. Emotional stress is no longer thought to be a cause of ulcers.
- Alcohol.
- Stress and diet can irritate an ulcer, they do not cause it.
- Ulcers can be cured with a one or two week course of antibiotics, even for people who have had ulcers for years.
- Excess alcohol consumption.
- Improper diet, irregular or skipped meals.
- Zollinger-Ellison syndrome (hyper-secretory state).
- Chronic disorders such as liver disease, emphysema, rheumatoid arthritis may increase ulcers.
- Abdominal discomfort is the most common symptom.
- Chronic disorders such as liver disease, emplysema, rheumatoid arthritis may increase ulcers.

1.6 Symptoms of ulcer

The major symptom of an ulcer is a burning or gnawing feeling in the stomach. This pain is often interpreted as heartburn, indigestion or hunger. The pain usually occurs in the upper abdomen, but sometimes it may occur below the breastbone. In some individuals the pain occurs immediately after eating. Symptoms are:

- Weight loss
- Poor appetite
- Bloating
- Burping
- Nausea
- Vomiting
- Sharp, Sudden, persistent stomach pain
- Bloody or black stools
- Bloody vomit or vomit that looks like coffee grounds
- Bleeding, when acid or the ulcer breaks a blood vessel

1.7. Epidemiology

Approximately 10% of Americans develop chronic PUD during their lifetime. The incidence varies with ulcer type, age, gender, and geographic location. Race, occupation, genetic predisposition, and societal factors may play a minor role in ulcer pathogenesis, but are attenuated by the importance of H. pylori infection and NSAID use. The prevalence of PUD in the United States has shifted from predominance in men to nearly comparable prevalence in men and women. Recent trends suggest a declining rate for younger men and an increasing rate for younger men and an increasing rate for older women. Factors influencing these trends include the declining smoking rates in younger men and the increased use of NSAIDs in older adults.

1.8 Risk Factor

Most peptic ulcers occur in the presence of acid and pepsin when Pylori, NSAIDs, or other factors disrupt normal mucosal defense and healing mechanisms. Hyper secretion of acid is the primary pathogenic mechanism in hyper secretary states such as ZES. Ulcer locations are related to a number of etiologic factors. Benign gastric ulcers can occur anywhere in the stomach although most are located on the lesser curvature, just distal to the junction of the astral and acid-secreting mucosa. Most duodenal ulcers occur in the first part of the duodenum.

1.9 Pathophysiology

A physiologic imbalance between aggressive factors (gastric acid and pepsin) and protective factors (mucosal defense and repair) remain important issues in the Pathophysiology of gastric and duodenal ulcers. Gastric acid is secreted by the parietal cells, which contain receptors for histamine, gastrin, and acetylcholine. Acid (as well as H. pylori infection and NSAID use) is an independent factor that contributes to the disruption of mucosal integrity. Increased acid secretion has been observed in patients with duodenal ulcers and may be a consequence of H. pylori infection. Patients with ZES have profound gastric acid hyper secretion resulting from a gastrin producing tumor.

Patients with gastric ulcer usually have normal or reduced rates of acid secretion (hypochlorhydria). Pepsin is an important cofactor that plays a role in the proteolytic activity involved in ulcer formation.

Pepsinogen, the inactive precursor of pepsin, is secreted by the chief cells located in the gastric fundus. Pepsin is activated by acid pH (optimal pH of 1.8 to 3.5), inactivated reversibly at pH, and irreversibly destroyed at pH7. Mucosal defense and repair mechanisms (mucus and bicarbonate secretion, intrinsic epithelial cell defense, and mucosal blood flow) protect the gastro duodenal mucosa from noxious endogenous and exogenous substances.

The viscous nature and near-neutral pH of the mucus-bicarbonate barrier protect the stomach from the acidic contents in the gastric lumen. Mucosal repair after injury is related to epithelial cell restitution, growth, and regeneration. The maintenance of mucosal integrity and repair is mediated by the production of endogenous prostaglandins. The term cytoprotection is

often used to describe this process, but mucosal defense and mucosal protection are more accurate terms, as prostaglandins prevent deep mucosal injury and not superficial damage to individual cells. Gastric hyperemia and increased prostaglandin synthesis characterize adaptive cytoprotection, the short-term adaptation of mucosal cells to mild topical irritants. This phenomenon enables the stomach to initially withstand the damaging effects of irritants. Alterations in mucosal defense that are induced by *H. pylori* or NSAIDs and the most important co factors in the formation of peptic ulcers.

1.10 Physiology of Ulcer

Gastric acid secretion is a complex, continuous process in which multiple central and peripheral factors contribute to a common end point: the secretion of H+ by parietal cells. Neuronal (acetylcholine, Ach), paracrine (histamine), and endocrine (gastrin) factors all regulate acid secretion. Their specific receptors (M_3, H_2, and CCK_2 receptors, respectively) are on the basolateral membrane of parietal cells in the body and fundus of the stomach. The H_2 receptor is a GPCR that activates the G_s- adenylylcyclase-cyclic AMP-PKA pathway. ACh and gastrin signal through GPCRs that couple to the G_q-PLC-IP_3-Ca^{2+} pathway in parietal cells. In parietal cells, the cyclic AMP and the Ca^{2+} - dependent pathways activate H^+,K^+-ATP ase (the proton pump), which exchanges hydrogen and potassium ions across the parietal cell membrane.

This pump generates the largest known ion gradient in vertebrates; with an intracellular pH of about 7.3 and an intracanalicular pH of about 0.8. The most important structures for CSN stimulation of gastric acid secretion are the dorsal motor nucleus of the vagal nerve, the hypothalamus, and the solitary tract nucleus. Efferent fibers originating in the dorsal motor nuclei descend to the stomach via the vagus nerve and synapse with ganglion cells of the enteric nervous system. ACh release from postganglionic vagal fibers directly stimulates gastric acid secretion through muscarinic M_3 receptors on the basolateral membrane of parietal cells.

The CNS predominantly modulates the activity of the enteric nervous system via ACh, stimulating gastric acid secretion in response to the sight, smell, taste, or anticipation of food (the "cephalic" phase of acid secretion). ACh also indirectly affects parietal cells by increasing the

release of histamine from the enterochromaffin-like (ECL) cells in the fundus of the stomach and of gastrin from G cells in the gastric antrum.

ECL cells, the source of gastric histamine secretion, usually are in close proximity to parietal cells. Histamine acts as a paracrine mediator, diffusiong frim its site of release to nearby parietal cells, where it activates H_2 receptors. The critical role of histamine in gastric acid secretion is dramatically demonstrated by the efficacy of H_2-receptor antagonists in decreasing gastric acid secretion.

Gastrin, which is produced by antral G cells, is the most potent inducer of acid secretion. Multiple pathways stimulate gastrin release, including CNS activation, local distension, and chemical components of the gastric contents. Gastrin stimulates acid secretion indirectly by inducing the release of histamine by ECL cells; a direct effect on parietal cells also plays a lesser role.

Somatostation (SST), which is produced by antral D cells, inhibits gastric acid secretion. Acidification of the gastric luminal pH to <3 stimulates SST release, which in turn suppresses gastrin release in a negative feedback loop. SST-producting cells are decreased in patients with H. pylori infection, and the consequent reduction of SST' inhibitory effect may contribute to excess gastrin production.

1.11 Regulation of Gastric Acid Secretion (Goodman, Gillman, 2006)

Gastric acid secretion by parietal cells of the gastric mucosa is stimulated by acetylcholine, histamine, and gastrin. The receptor-mediated binding of acetylcholine, histamine, or gastrin results in the activation of protein kinases, which in turn stimulates the H^+/K^+ adenosine triphosphatase (ATPase) proton pump to secrete hydrogen ions in exchange for K^+ into the lumen of the stomach. A Cl^- channel couples chloride efflux to the release of H^+. In contrast, receptor binding of prostaglandin E_2 and somatostatin diminish gastric acid production. [Note: Histamine binding causes activation of adenylylcyclase, whereas binding of prostaglandin E_2 inhibits this enzyme. Gastrin and acetylcholine act by inducing an increase in intracellular calcium levels.

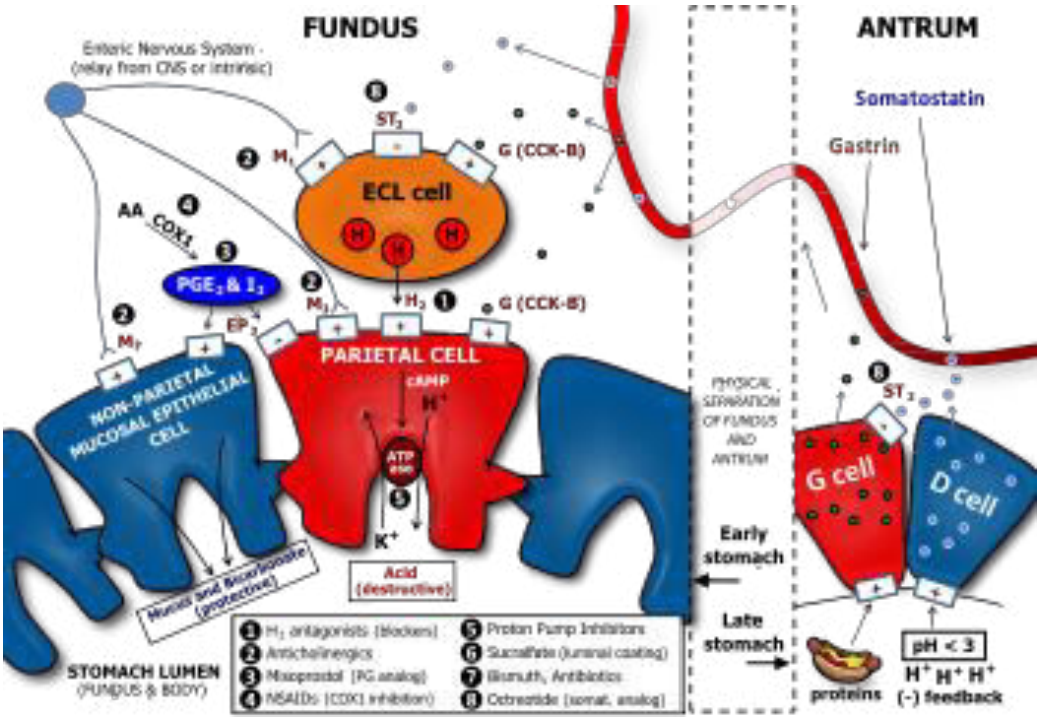


Fig 2. Regulation of Gastric Acid Secretions

1.12 Gastric Defense against Acid

The extremely high concentration of H^+ in the gastric lumen requires robust defense mechanisms to protect the esophagus and the stomach. The primary esophageal defense is the lower esophageal sphincter, which prevents reflux of acidic gastric contents into the esophagus. The stomach protects itself from acid damage by a number of mechanisms that require adequate mucosal blood flow, perhaps because of the high metabolic activity and oxygen requirements of the gastric mucosa. One key defense is the secretion of a mucus layer that protects gastric epithelial cells. Gastric mucus is soluble when secreted but quickly forms an insoluble gel that coats the mucosal surface of the stomach, slows ion diffusion, and prevents mucosal damage by macromolecules such as pepsin.

Mucus production is stimulated by prostaglandins E_2 and I_2, which also directly inhibit gastric acid secretion by parietal cells. Thus, alcohol, aspirin, and other drugs that inhibit prostaglandin formation decrease mucus secretion and predispose to the development of acid-peptic disease. A second important part of the normal mucosal defense is the secretion of bicarbonate ions by superficial gastric epithelial cells. Bicarbonate neutralizes the acid in the region of the mucosal cells, thereby raising pH and preventing acid-mediated damage. (Goodman Gillman-2006).

2. Treatment

2.1 Proton Pump Inhibitors

Chemistry, Mechanism of Action & Pharmacology

The most potent suppressor of gastric acid secretion are inhibitors of the gastric H^+, K^+-ATP ase (proton pump). In typical doses, these drugs diminish the daily production of acid (basal and stimulated) by 80% to 95%. Five proton pump inhibitors are available for clinical use: omeprazole (PRILOSEC, RAPINEX and ZEGERID), and its S-isomer, esomeprazole (NEXIUM), lansoprazole (PREVACID), rabeprazole (ACIPHEX), and pantoprazole (PROTONIX). These drugs have different substitutions on their pyridine and/or benzimidazole groups but are remarkably similar in their pharmacological properties. Omeprazole is a racemic mixture of R- and S- isomers; the S-isomer, esomeprazole (S-omeprazole), is eliminated less rapidly than R-omeprazole, which theoretically provides a therapeutic advantage because of the increased half-life. Despite claims to the contrary, all proton pump inhibitors have equivalent efficacy at comparable doses.

Proton pump inhibitors are pro drugs that require activation in an acid environment. After absorption into the systemic circulation, the pro drug diffuses into the parietal cells of the stomach and accumulates in the acidic secretary canaliculi. Here, it is activated by proton-catalyzed formation of a tetracyclic sulphonamide, trapping the drug so that it cannot diffuse back across the canalicular membrane.

The activated from then binds covalently with sulfhydry1 groups of cysteine's in the H^+,K^+ - ATP ase, irreversibly inactivating the pump molecule. Acid secretion resumes only after new pump molecules are synthesized and inserted into the luminal membrane, providing a prolonged(up to 24 to 48 hour) suppression of acid secretion, despite the much shorter plasma half-lives (0.5to 2 hours) of the parent compounds. Because they block the final step in acid production, the proton pump inhibitors are effective in acid suppression regardless of other stimulating factors.

To prevent degradation of proton pump inhibitors by acid in the gastric lumen, oral dosage forms are supplied in different formulations;

1. enteric-coated drugs contained inside gelatin capsules (omeprazole, esomeoprazole, and lansoprazole)

2. enteric-coated granules supplied as a power for susopension (lansoprazole)

3. Enteric-coated tables (pantoprazole, rabeprazole, and omeprazole)

4. Powdered drug combined with sodium bicarbonate (omeprazole)

The delayed-released and enteric-coated tablets dissolve only at alkaline pH, while admixture of omeprazole with sodium bicarbonate simply neutralizes stomach acid; both strategies substantially improve the oral

Bioavailability of these acid -labile drugs. Until recently, the requirement for enteric coating posed a challenge to the administration of proto pump inhibitors in patients for whom the oral route of administration is not available. These patients and those requiring immediate acid suppression now can be treated parent rally with pantoprazole or lansoprazole, both of which are approved from intravenous administration in the United States.

A single intravenous bolus of 80 mg of pantoprazole inhibits acid production by 80% to 90% within an hour, and this inhibition persists for up to 21 hours, permitting once-daily dosing to achieve the desired degree of hypochlorhydria. The FDA approved does of intravenous pantoprazole for gastroesophageal reflux disease in 40mg daily for up to 10 days Higher doses

(e.g. 160 to 240 mg in divided does) are used to manage hyper secretary conditions such as the Zollinge r-Ellison syndrome.

2.2 H_2-Receptor Antagonists

Chemistry, Mechanism of Action & Pharmacology

The H_2-recptor antagonists inhibit acid production by reversibly competing with histamine for binding to H_2 receptors on the basolateral membranes of parietal cells. Four different H_2 receptor antagonists, which differ mainly in their pharmacokinetic s and propensity to cause drug interactions, are available in the United States: cimetidine (TAGAMENT), ranitidine (ZANTAC), famotidine (PEPCID), and nizatidine (AXID). These drugs are less potent than proton pump inhibitors but still suppress 24 hour gastric acid secretion by about 70% the H_2 receptor antagonists predominantly inhibit basal acid secretion, which accounts for their efficacy in suppressing nocturnal acid secretion.

2.3 Prostaglandin analogues:

Misoporostol

Prostaglandin E_2 (PG E_2) and prostacyclin (PG I_2) are the major prostaglandins synthesized by the gastric mucosa. They bind to the EP_3 recepto on parietal cells) and stimulate the G_i pathway, thereby decreasing intracellular cyclic AMP and gastric acid secretion. PG E_2 also can prevent gastric injury by cytoprotective effects that include stimulation of mucin and bicarbonate secretion and increased mucosal blood flow. Although smaller does than those required for acid suppression can protect the gastric mucosa in laboratory animals, this has not been convincingly demonstrated in humans; acid suppression appears to be the most important effect clinically. Since NSAIDs diminish prostaglandin formation by inhibiting cyclooxygenase, synthetic prostaglandin analogues offer a logical approach to reducing NSAID in duced mucosal damage.

Sucralfate :

In the presence of acid-induced damage, pepsin-mediated hydrolysis of mucosal proteins contributes to mucosal erosion and ulcerations. This process can be inhibited by sulphated polysaccharides. Sucralfate (CARAFATE) consists of the octasulfate of surcrose to which A1 $(OH)_3$ has been added. In an acid environment (pH 4), sucralfate undergoes extensive cross-linking to produce a viscous, sticky polymer that adheres to eopithelial cells and ulcer creaters for up to 6 hours after a single does. In addition to inhibiting hydrolysis of mucosal proteins by pepsin, surcralfate may have additional cytoprotective effects, including stimulation of local production of prostaglandins and epidermal growth factor. Surcralfate also binds bile salts; thus, some clinicians use surcralfate to treat individuals with the syndromes of biliary esophagitis or gastritis (the existence of which is controversial).

Antacids:

The antacids largely have been replaced by more effective and convenient drugs. Nevertheless, they continue to be used by patients for a variety of indications, and some knowledge of their pharmacology is important for the medical professional. Many factors, including palatability, determine the effectiveness and choice of antacid. Although sodium bicarbonate effectively neutralizes acid, it is very water-soluble and rapidly absorbed from the stomach, and the alkali and sodium loads may pose a risk for patients with cardiac or renal failure. Depending on particle size and crystal structure, $CaCO_3$ rapidly and effectively neutralizes gastric H^+, but the release of CO_2 from bicarbonate-and carbonate-containing antacids can cause belching, nausea, abdominal distension, and flatulence. Calcium also may induce rebound acid secretion, necessitating more frequent administration. Combinations of Mg^{2+} (rapidly reacting) and $A1^{3+}$ (slowly reacting) hydroxides provide a relatively balanced and sustained neutralizing capacity and are preferred by most experts.

Magaldrate:

Is a hydroxyl magnesium aluminates complex that is converted rapidly in gastric acid to Mg $(OH)_2$ and A1 $(OH)_3$, which are absorbed poorly and thus provide a sustained antacid effect. Although fixed combinations of magnesium and aluminum theoreticlly counteract the adverse effects of each other on the bowel (Al^{3+} can relax gastric smooth muscle, producing delayed gastric emptying and constipation, while Mg^{2+} exerts the opposite effects), such balance is not always achieved in practice.

Simethicone, is a surfactant that may decrease foam and hence oesophageal reflux is included in many antacid preparations. However, other fixed combinations, particularly those with aspirin, that are marketed for "acid indigestion" are irrational choices, are potentially unsafe in patients predisposed to gastro duodenal ulcers, and should not be used.

2.4 Other Acid Suppressants and Cytoprotectants

The M1 muscrainic receptor antagonists pirenzepine and telenzepine can reduce basal acid production by 40% to 50% an long have been used to treat patients with peptic ulcer disease in countries other than the United State. The Ach receptor on the parietal cell itself is of the M3 subtype, and these drugs are believed to suppress neural stimulation of acid production via actions on M1 receptors of intramural ganglia. Because of their relatively poor efficacy, Significant and undesirable anticholinergic side effects, and risk of blood disorder (pirenzepine), they rarely are used today.

2. LITERATURE REVIEW

J. Megalal, A. Geetha et al, 2010 evaluated the gastroprotective effect of hydroalcoholic fruit Extract of *Pithecellobium dulce* (HAEPD) in the injury of rat gastric mucosa induced by absolute ethanol and as well as to elucidate the role of reactive oxygen species.

M. Sugumaran et al, 2008 studied the study Locomotor activity of aqueous and alcoholic extracts of leaves of *Pithecellobium dulce* Benth.

M. Sugumaran et al, 2006 studied the macroscopical characters of the leaves, leaf constants, physico-chemical constants, extractive values, colour, consistency, pH, extractive values with different solvents.

P. Ponmozhi et al, 2011 investigated the beneficial role of anthocyanin extracted from *Pithecellobiumdulce* fruit percarp.

V.S. Mule et al, 2011 were undertaken to evaluate several neuropharmacological activities of the aqueous and ethanolic extracts of *Pithecellobium dulce* Benth (Leguminosae) Leaves in swiss albino mice.

N. Ramam et al, 2012 reported a green chemistry approach for the biological synthesis of silver nanoparticles using the aqueous leaf extract of *Pithecellobiumdulce*, which acts as a reducing and capping agent.

M. Sugumaran et al, 2009 studied the Ethanolic and aqueous leaf extract of *Pithecellobium dulce* for its antidiabetic activity using streptozotocin-induced diabetic model in rats.

Parasenjit Manna et al, 2001 have carried out to investigate the protective role of the aqueous extract of the fruits of *P. dulce* (AEPD) against CC14-induced hepatic disorders.

J. Ravikumar et al, 2008 were carried out to determine the effect of methanolic extract of *Anisochilus carnosus* leaves for antiulcer, gastric antisecretory and cytoprotective effect in pylorus ligated rats.

V. Tandon et al, 2011 have reviewed This paper reviews the present state of knowledge regarding the use of some traditional medicinal plants in curing worm infections in different regions of the world, with particular reference to north-east India.

M. Sugumaran et al, evaluated the aqueous and alcoholic extract of *Pithecellobium dulce* leaves for radical scavenging activity using reducing power assay method.

3. PLANT PROFILE

Fig:3 *Pithecellobium dulce* fruit

Fig: 4 *Pithecellobium dulce* seeds

The Earlier Reports on the Pharmacognostical, Phytochemical, Pharmacological, and Microbial Studies on *Pithecelloium dulce Listed* Below.

Botanical Information

Synonym(S): Inga Dulcis (Rox B.)Wild

Mimosa Dulcis Rox B.

Taxonomical Classification

Kingdom:	*Plantae*
Sub Kingdom:	*Tracheobionta*
Division:	*Magnoliophyta*
Class:	*Magnoliopsida*
Subclass:	*Rosidae*
Order:	*Fabales*
Family:	*Fabaceae*
Sub Family:	*Mimosoideae*
Tribe:	*Ingeae*
Genus:	*Pithecelloboum*
Species:	*P.dulce*

Vernacular Name: (Agroforestry Tree Database)

Arabic	Tamar Hindi
Bengali	Amil Dekhani Babul
English	Madras Thorn, Manila Tamarind

Hindi	Jangal Jelbi, Dakhani Babul
Indonesian	Asam Koranji
Malay	Asam Kranji
Spanish	Guama Americano
Tamil	Kodukapuli

Plant Descripton

A small to medium – sized semi evergreen shrub or tree reaching 5-20 m. In height, with a short trunk. The tree is broadly crowned and the trunk is often crooked or has low branches.

Seeds:

Pods are 10-15 X 1.5 Cm; The Color Becoming Reddish Brown As They Ripen . Each Pod Contains 5-10 Shiny Black Seeds Up To 2 Cm Long.

Distribution:

Native: Argentina, Bolivia, Brazil, United States Of America, Mexico

Exotic: Cambodia, China, Cuba, India, Indonesia, Jamaica, Philippines.

Phytochemical Constituents:

Seeds have been reported to contain Steroids, Saponins, Lipids, Phospholipids, Glycosides, Glycolipids And Polysaccharides.

Medicinal Uses:

- Frequent Bowel Movements: Decoction of bark taken as tea.
- The Leaves, when applied as plasters, used for pain, venereal sores.
- Salted Decoction of Leaves for indigestion; also used as abortifacient.
- Bark used in dysentery, dermatitis and eye Inflammation.
- In Brazil, *P. avaremotem,* used as a Cancer Elixer.

4. AIM AND OBJECTIVE OF THE STUDY

The aim of present study is to evaluate Anti-ulcer activity of *Pithecellobium dulce* seed extracts by pylorus ligation method in Albino Wistar strain rats.

Many plants showing Anti-ulcer activity is due to the presence of antioxidants, *Pithecellobium dulce* seeds has not been explored for the Anti-ulcer Activity but the plant possess antioxidant activity according to the phytochemistry and pharmacological properties of *Pithecellobium dulce* an overview. Hence my objective and my future work were concentrated on the Anti-ulcer activity of *Pithecellobium dulce* seeds.

5. PLAN OF WORK

5.1 preliminary work

- Literature survey
- Plant selection
- Authentication

5.2 Pharmacognostical Studies

- Collection And Authentication of Plant
- Preparation of Extracts
- Qualitative Phytochemical Analysis

5.3 Pharmacological Studies

- Pylorus ligation induced ulcer model
- Anti ulcer activity
- Acute toxicity studies

5.4 Biochemical Parameters

- Ulcer index
- Free acidity
- Total acidity
- Volume of gastric juice
- Percentage protection
- P^H

5.5 Histopathological Studies

6. MATERIALS AND METHODS

6.1 Animals:

- Albino wistar strain rats

6.2 Chemicals:

- Petroleum ether
- Ethanol
- Ranitidine
- Topfer's reagent
- Formalin solution
- 0.01N sodium hydroxide
- Dragendorff's reagent
- Mayer' reagent
- Sodium nitroprusside
- Sodium picrate
- Sulphuric acid
- Alpha- Naphthol
- Chloroform
- Acetic anhydride
- Sodium hydroxide
- Copper sulphate
- Sodium carbonate
- Ferric chloride
- Hydrochloric acid

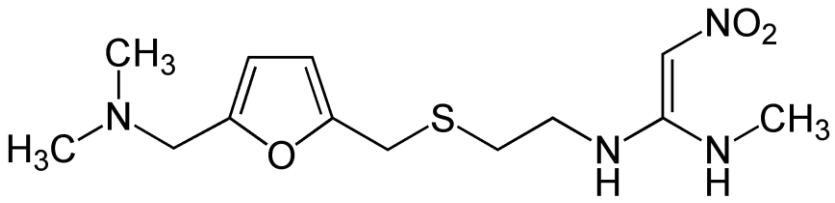
6.3 Instruments:

- Soxhlet appararatus
- Heating mantle
- Round bottom flask
- Centrifuge

6.4 Drug information

Ranitidine

Srructure :



N-2 (5 dimethy lamino) methy 1-2 fur any / methy / thioethy1-N'-methy1-2 nitro-1, 1-3thene diamine

Description:

A non-imidazole blocker of those histamine receptors that mediate gastric secretion (H_2 receptors).It is used to treat gastro intestinal ulcers.

Mechanism of action:

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

Common brand names:

Zantac, Zantac150 maximum strength, Zantac75.

6.5 Collection, Authentication & Drying of Plants

6.5.1 Preliminary work

From various articles and journals it was concluded that antioxidants can be used in the treatment of various diseases including peptic ulcer. *Pithecellobium dulce* contains antioxidant property so it was selected for the evaluation of Anti ulcer activity.

6.5.2 Drying and size reduction of seeds

The seeds of *Pithecellobium dulce* was dried and pulverized to coarse powder with the help of mixer grinder. The coarse powder was passed through sieve to maintain uniformity and packed into air tight container and stored in cool and dry place. This material was used for the further study.

6.5.3 Extraction Process of *Pithecellobium dulce* seeds

The dried coarse powder of the seeds (250G) was packed well in soxhlet apparatus and was subjected for continuous hot extraction. The powder mass was defatted with Petroleum Ether (60-80c) followed by extraction with Ethanol (90%V/V) and water by soxhelation and maceration respectively. The extracts were filtered and the filtrates were concentrated under reduced pressure to obtain the extract as solid residues.

Pharmacognostical studies

6.5.4 Screening of powdered leaves

Colour - Pale yellow

Odour - Characteristic

Taste - Characteristic

Texture - Rough

6.5.5 Screening of Crude Extracts (Qualitative Phytochemical Analysis)

The crude extracts obtained by solvent extraction were subjected to various qualitative tests to detect the presence of common chemical constituents As:

- ❖ Alkaloids
- ❖ Carbohydrates And Glycosides
- ❖ Phytosterols
- ❖ Fixed Oil And Fats
- ❖ Saponins
- ❖ Tannins And Phenolic Compounds
- ❖ Proteins And Amino Acids
- ❖ Gums And Mucilages
- ❖ Flavonoids

1. Tests For Alkaloids

A Small Portion of the solvent free extracts were stirred separately with a few drops of dilute Hcl and filtered. The filtrates were tested carefully with various alkaloid reagents such as

a) Mayer's Test - Yellow Precipitate
b) Dragondroff''s Reagent - Orange-Brown Precipitate
c) Hager' Test - Yellow Precipitate
d) Wagner' Test - Reddish Brown Precipitate

2. Test For Carbohydrates And Glycosides

A Small quantity of extracts were dissolved in 5ml of distilled water and filtered. The filtrates were subjected to Molisch's Test to detect the presence carbohydrates. Filtrates was treated with 2-3 drops of 1% alcoholic Naphtha solution and 2ml of con.H_2SO_4 was added along the sides of test tube.

Appearance of brown Ring at the Junction of two layers showed the presence of carbohydrates.

Another portion of the extract was hydrolyzed with Hcl for few hours on a water bath and the hydrolysate was subjected to Legal's and Borntrager's Test to detect the presence of different glycosides.

a) **Legal' test:** To the hydrolysate 1ml pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour showed the presence of glycoside and aglycones.

b) **Borntrager's test:** Hydrolysate was tested with chloroform and chloroform layer was separated. To this equal quantity of Dil. Ammonia solution was added. Ammonical layer acquired rose pink colour showed the presence of glycosides.

3**.** **Test for phytosterols**:

a) **Salkowski test:** The extract was dissolved in chloroform and equal volume of con. H_2So_4 was added. Formation of bluish red to cherry colour in acid layer represented the steroidal components in tested extract.

b) **Liebermann-Burchard test:** A small portion from each extract was taken about 1ml of acetic anhydride and dissolved by warming. The contents were cooled and a few drops of Con. H2so4 were added in each case by the sides of the test tube. Appearance of blue colour showed the presence of sterols.

4**.** **Test for Saponins:**

a) A little fraction from the various extracts were boiled with about 1 ml of distilled water and shaken. Appearance of a characteristic foam formation indicated the presence of Saponins.

Aqueous and alcoholic extracts were tested directly.

b) A little fraction from various extracts was taken with about 2ml of distilled water. A small quantity of sodium carbonate was added to each and shaken. The characteristic foam formation indicated the presence of Saponins.

Aqueous and alcoholic extract were tested directly.

c) The extracts were diluted with 20ml of distilled water and it was agitated on a graduated cylinder for 15 mts.

Formation of foam showed the presence of Saponins.

5) **Test for fixed oils and Fats:**

a) Small quanntities of various extracts were separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of Fixed oils.

b) Few drops of 0.5N Alcoholic potassium hydroxide was added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1to 2 hrs. Soap formation of foam shows the presence of fixed oils and fats.

6) **Test for Tannins and Phenolic compounds:** Small quantities of various Extracts were dissolved in water and tested for the presence of phenolic Compounds and Tannins with dilute Ferric chloride solution(5%) - Violet colour , 1% Solution of Gelatin containing 10% sodium chloride – White precipitate, Lead Acetate solution – White precipitate.

7) Test for Flavonoids:

a) **Shinoda test:** To the test solution add few magnesium turnings and con. Hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

b) About 5ml of each portion was boiled with distilled water and then filtered. To 2ml of the filtrate, few drops of 10% ferric chloride solution were then added. A green blue or violet coloration indicated the presence of phenolic hydroxyl group.

c) Small quantities of various extracts were dissolved in few ml of water and treated with aqueous sodium hydroxide solution – Yellow color, With Con.H_2s0_4 - Yellowish orange color indicates Flavones.

8) **Test for Phenolic and free Aminoacids:**

Small quantities of various extracts were dissolved in few ml of water and Treated with

a) **Millon's Reagent** – Appearance of red colour shows the presence of Proteins and Free amino acids.

b) **Ninhydrin reagent** – Appearance of purple colour shows the presence of Proteins and Free Aminoacids.

c) **Biuret test:** Equal volume of 5% sodium hydroxide solution and 1% coper suphate were added – Appearance of Pink to Purple colour showed the presence of Proteins and Free Aminoacids.

9) **Test for Gums and Mucilages:** About 10ml of various extracts were added Separately to 25ml of absolute Alcohol with constant stirring and filtered. The precipitate was dried and examined its swelling properties

6.5.6 Animals

Wistar Albino strain rats weighing between 150- 200gms were procured from the animal house Padmavathi college of Pharmacy. They are maintained at standard housing conditions at a room temperature of 24^0 C, relative humidity of 45-55% with 12 : 12 hour light/ dark cycle. The feeding was done with commercially available rat feed pellets ad water *ad libitum* during the experiment.

Animal model used for investigation (Wistar Albino strain Rats)

Class	:	Mammalia
Family	:	Muridae
Order	:	Rodentia
Genus	:	Rattus
Scientific name	:	*Rattusnorve gicus*

6.6.6 Pharmacological evaluation

Screening of In vivo Antiulcer Activity

Animal care and handling as per CPCSEA guideline

Wistar albino strain rats of weight 150-250 grams were selected. The animals were acclimatized to the standard laboratory conditions in well cross ventilated animal house at temperature 25+ 2^0C relative humidity 44-56% and light and dark cycles of 12 and 12 hours respectively for 1week before and during the experiments. The animals were fed with standard diet ad water ad libitum.

The experiments were approved by CPCSEA and the institutional ethics committee. Food was withdrawn 18 hours before the start of the activity.

Acute oral toxicity study and selection of doses

A safe oral dose of EEPD and AEPD was determined through the acute oral toxic test in rats as described by the Organization of Economic Co-Operation and Development (OECD) as per 423 guidelines (OECD Guidelines for the Testing of Chemicals). The EEPD&AEPD at different doses up to 2000mg/kg,was prepared by dissolving the extract in distilled water and the concentration was adjusted in such a way that it did not exceed 1ml/100g of the rat. The extract was then administered (p.o) and animals were observed for behavioral changes, any toxicity and mortality up to 48 h. The doses (250 mg/kg,p.o) of EEPD and AEPD were later chosen for this study based on the acute toxicity testing.

Experimental Design

6.6.7 Pylorus Ligation Induced Ulcer Model

Wistar albino strain rats weighing 150-20g were selected for pyloric ligation ulcer model. Rats were divided into five groups, each group consisting of six animals. Animals were fasted for 48 hours.

Group-I: Normal

Group-II: Control (Saline 2ml/kg)

Group-III: Treated with Ranitidine50mg/kg

Group-IV: Treated with AEPD (100mg/kg)

Group-V: Treated with EEPD (100mg/kg)

The oral treatment was carried out 1 hour before pyloric ligation, respectively. After 48 hours of fasting, ulcer induction was undertaken according to Shay et al.

The rats were mildly anaesthetized with Anesthetic Ether and the abdomen was cut open through a midline incision. The pylorus was secured and ligated with silk sutures, after which the wound was closed and the animals were allowed to recover from anesthesia. After ligation of the pylorus, drinking water was withheld and the gastric examinations were under taken 18 hours after pylorus ligation. The animals were sacrificed with an overdose of Anesthetic Ether and the stomach were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a magnifier lens (10x) to assess the formation of ulcer as shown in figure 1.

Using transpires surgical tapes assessed ulcer area, which was fixed on a light and transparent sheet and the ulcer area was measured for each stomach. The number of erosions formed on glandular portion of the stomach was counted and each was given a severity rating on a 1-3 scale. The gastric contents were collected and centrifuged at 1000RPM for 10 min. One ml of the supernatant liquid was pipette out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer's reagent as an indicator, to the endpoint when the solution turned to orange colour.The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink color. The volume of NaOH required was noted and was taken as corresponding to the total acidity.

Ulcer index will be then calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach.

Scoring of ulcer will be made as follows

No ulcer (0)

Superficial ulcers (1)

Deep ulcers (2)

Perforation (3)

Mean ulcer score for each animal will be expressed as ulcer index-

$U_I = U_N + U_s + U_P \times 10^{-1}$

Where

U_I= Average of number of ulcers per animal

Us= Average of severity of score

Up= Percentage of animals with ulcers

The percentage of ulcer protection will be determined as follows-

$$\% \text{ Protective} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Reagents for biochemical estimations of free and total acidity

Reagents for estimation of free and total acidity

a) Freshly prepared 0.01N oxalic acid solution was used to standardize sodium hydroxide.

b) Freshly prepared 0.01N Sodium Hydroxide

c) Topfer' reagent. It is dimethyl amino azo benzene 0.5% in absolute ethanol available in 100ml package.

d) Freshly prepared 1% phenolphthalein solution prepared in 50% absolute ethanol.

Methods for biochemical estimation of free and total acidity.

Collection of gastric juice

Gastric contents collected from pylorus ligated rats was centrifuged and the volumes of gastric juice were noted. The gastric juice was subjected to biochemical estimation as follows.

Determination of free and total acidity

1ml of gastric juice was pipette into a100ml conical flask, 2or 3 drops of Topfer's reagent was added and titrated with 0.01N Sodium hydroxide until all traces of red color disappears and the color of the solution turns to yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Then 2or3 drops of phenolphthalein solution were added and titration was continued until a definite red tinge appears. Again the total volume of alkali added was noted now this volume corresponds to total acidity.

Acidity was calculated by using the formula

$$\textbf{Acidity} = \frac{\textbf{Volume of NaoH x Normality of NaoH x 100}}{0.1} \quad \textbf{meq/lt/100g}$$

Statistical Analysis

The Statistical analysis was performed by One Way ANOVA followed by Dunnet's comparison test and Student- New man- keuls test. The values are expressed as Mean +SEM.

7. RESULTS & DISCUSSION

7.1 EXTRACTION

The extraction value of EEPD

Total Amount of crude drug used = 250gm

Amount obtained as Ethanolic Extract = 23gm

% Yield = 23/250 x 100

= 9.2% W/W

The extraction value of AEPD

Total Amount of crude drug used = 230gm

Amount obtained as Ethanolic Extract = 21gm

% Yield = 21/230 x 100

= 9.1% W/W

Table: 2 Percentage Yield

Plant name	Part used	Method of extraction	Percentage yield
Pithecellobium dulce	Seed	Soxhlet extraction	**9.2%w/w**
Pithecellobium dulce	Seed	Maceration	**9.1%w/w**

7.2 PRELIMINARY PHYTOCHEMICAL SCREENING

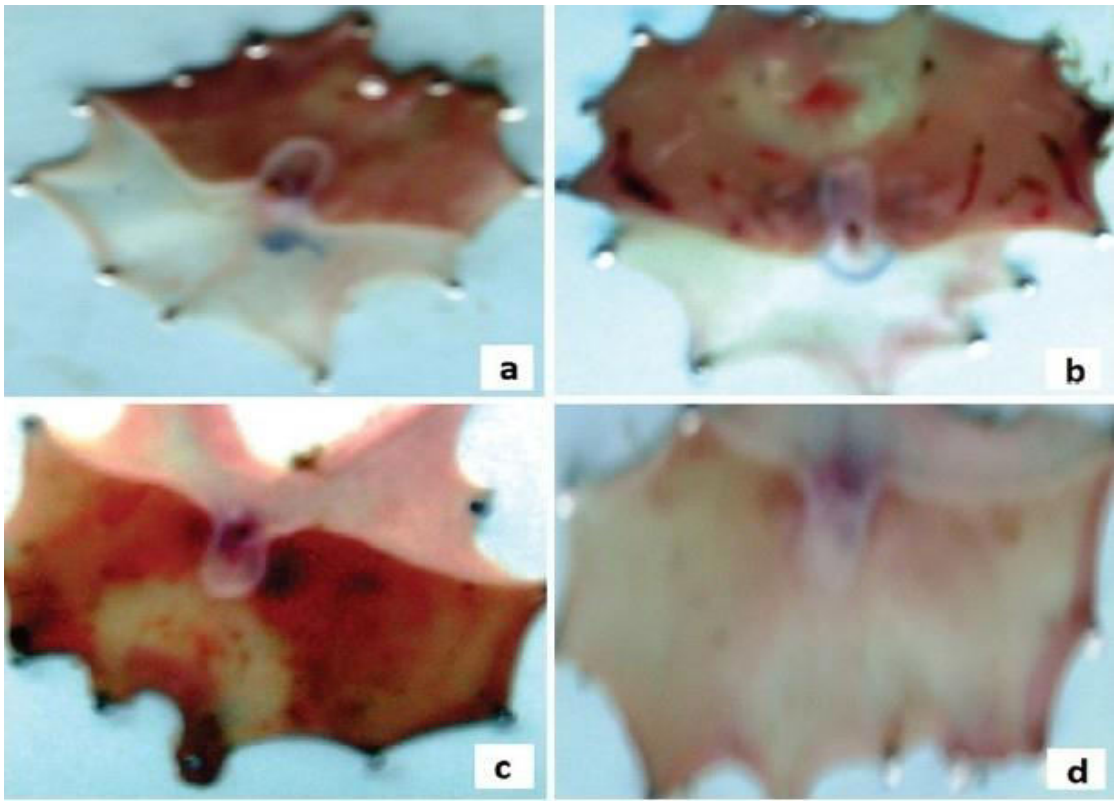
The Extracts of *Pithecellobium dulce* was subjected for phytochemical screening and found that Saponins, Sterols, Glycosides, Flavonoids, Fixed oils and Fats, Gums and Mucilages were present. The results were as follows.

Table 3: Phytochemical screening tests and results

S.No	Group	Ethanolic Extract	Aqueous Extract
1.	Alkaloids	–	–
2.	Saponins	+	–
3.	Sterols	+	+
4.	Glycosides	+	+
5.	Flavonoids	+	+
6.	Fixed oils and Fats	+	–
7.	Tannins and phenolic compounds	–	–
8.	Proteins and Aminoacids	–	–
9.	Gums and Mucilages	+	+

(+) indicates Presence (-) indicates Absence

7.3 Macroscopical view of pylorus ligation induced ulcer



a) **Normal**
b) **Control**
c) **Standard**
d) **Ethanolic extract of *pithecellobium dulce***
e) **Aqueous extract of *pithecellobium dulce***

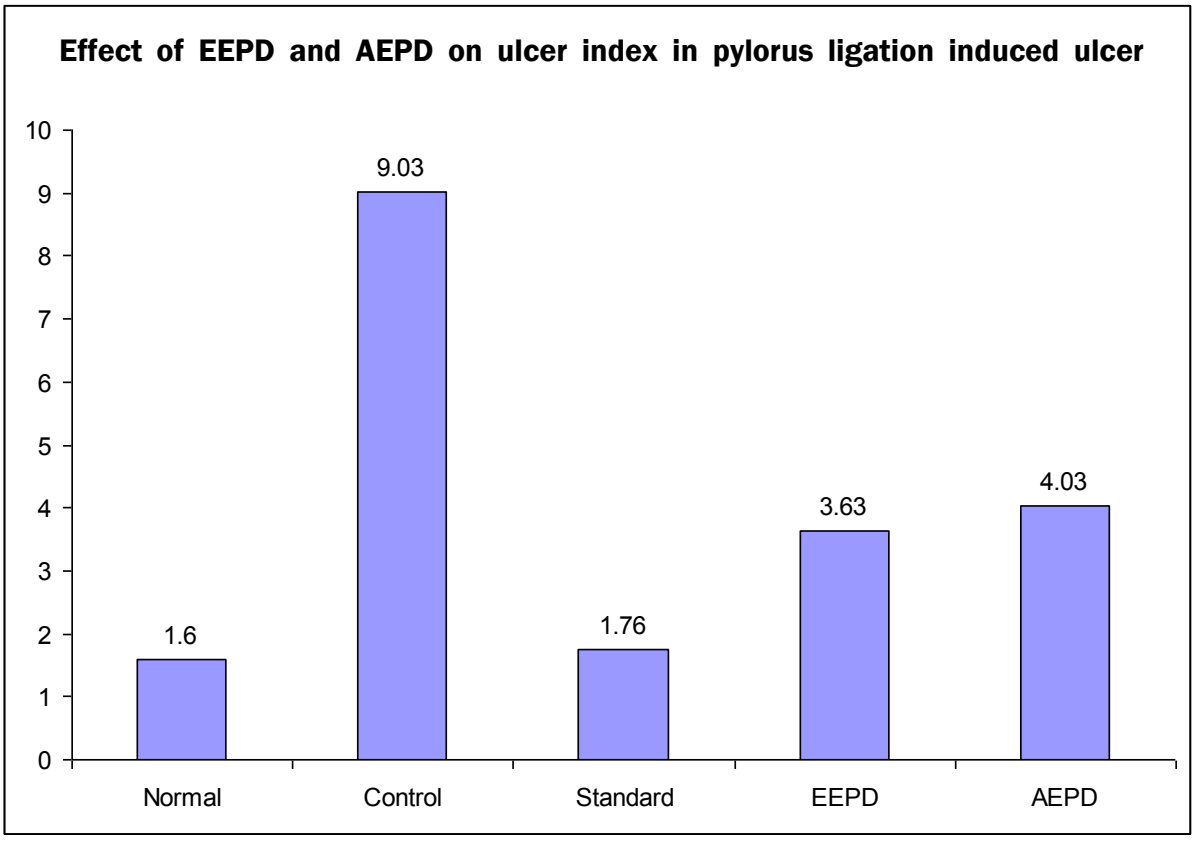
7.3 PHARMACOLOGICAL SCREENING

7.3.1 Pylorus ligation induced ulcer:

Effect of EEPD and AEPD on ulcer index in pylorus ligation induced ulcer (Table no 7.3.1.1)

Group	Treatment	Dose(mg/kg)	Ulcer index (mm2/rat)	% Protection
I.	Normal	2	1.60	--
II.	Control	2	9.03	--
III.	Standard	50	1.76	80.5
IV.	EEPD	100	3.63*	59.8*
V.	AEPD	100	4.03*	60.8*

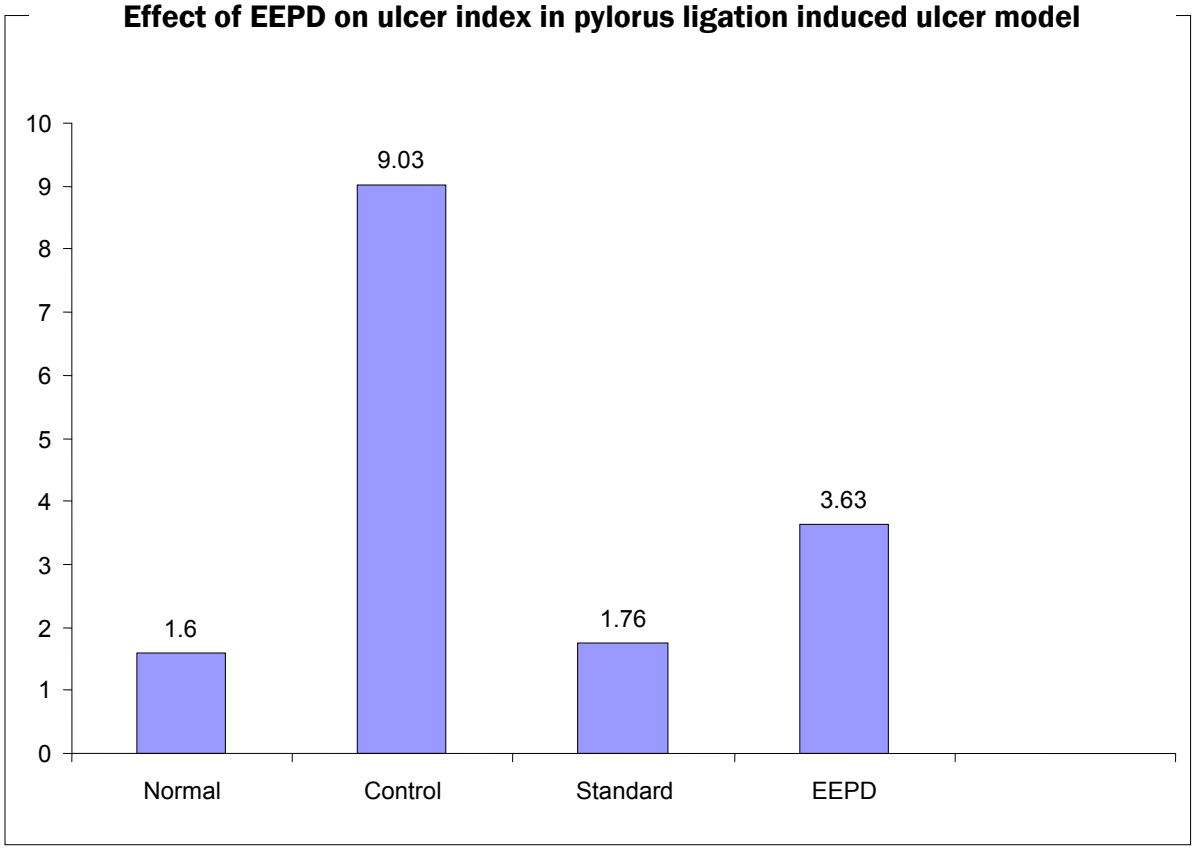
Values are expressed as (Mean ± S.E.M.), n=5, *p< 0.05 when compared with control group. (Statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's t-test.) Results were considered to be statistically significant at *P<0.05*.



Effect of *Pithecellobium dulce* seed Ethanolic extract on volume of gastric juice, Total acidity and Free acidity in Pylorus ligation induced ulcer. (Table no 7.3.1.2)

Group	Treatment	Dose (mg/kg) p.o	Volume of gastric juice	P^H	Free Acidity (mEq/L)	Total Acidity (mEq/L)
I	Normal	2	3.1±0.23	4.1	5.4±0.08	7.6±0.2
II	Control	2	4.2±0.18	2.8	4.6±0.05	22.3±0.3
III	Standard	50	1.9±0.07	7.5	3.1±0.02	6.8±0.16
IV	EEPD	100	2.4±0.11*	6.9	3.4±0.03*	5.9±0.12*

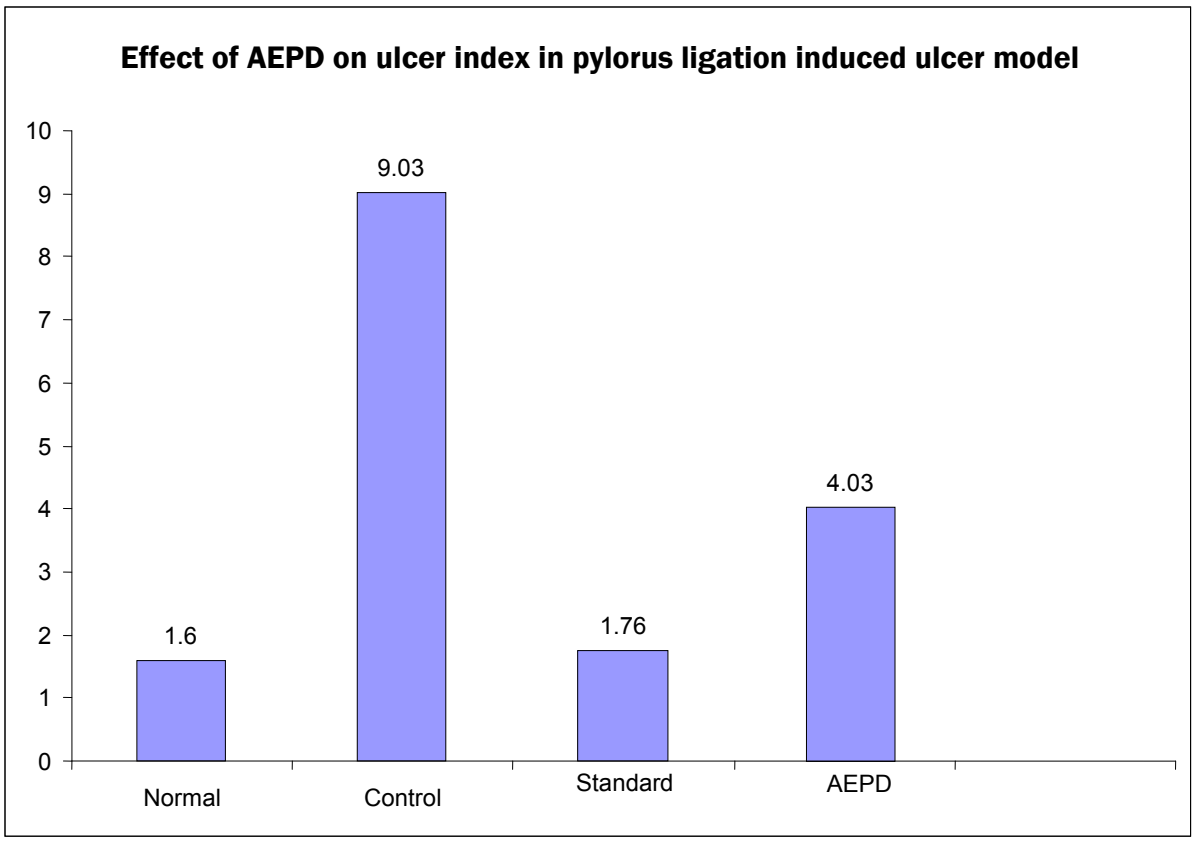
Values are expressed as (Mean ± S.E.M.), n=5, *p< 0.05 when compared with control group. (Statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's t-test.) Results were considered to be statistically significant at *P<0.05*.



Effect of *Pithecellobium dulce* seed Aqueous extract on volume of gastric juice, Total Acidity and Free acidity in Pylorus ligation induced ulcer. (Table no 7.3.1.3)

Group	Treatment	Dose (mg/kg) p.o	Volume of gastric juice	P^H	Free Acidity (mEq/L)	Total Acidity (mEq/L)
I	Normal	2	3.1±0.23	4.1	5.4±0.08	7.6±0.2
II	Control	2	4.2±0.18	2.8	4.6±0.05	22.3±0.3
III	Standard	50	1.9±0.07	7.5	3.1±0.02	6.8±0.16
IV	AEPD	100	1.5±o.28*	6.4	2.1±0.18*	6.3±0.27

Values are expressed as (Mean ± S.E.M.), n=5, *p< 0.05 when compared with control group. (Statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's t-test.) Results were considered to be statistically significant at *P<0.05*.


Effect of AEPD on ulcer index in pylorus ligation induced ulcer model
10
9
8
7
6
5
4
3
2
1
0
1.6
9.03
1.76
4.03
Normal
Control
Standard
AEPD

Histopathological analysis of pylorus ligation induced ulcer model

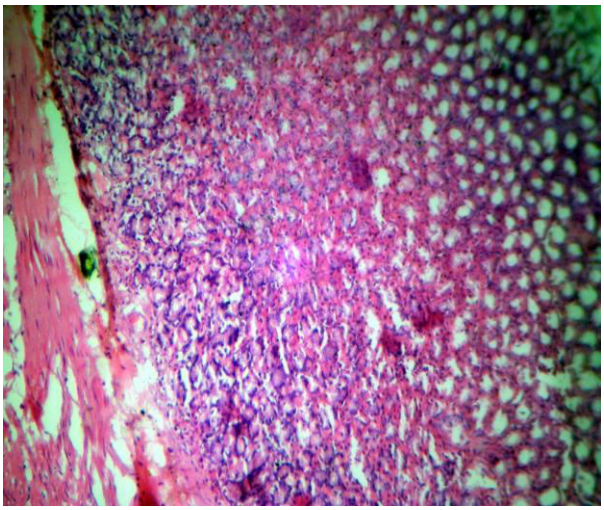


Fig:1 Stomach of normal rat

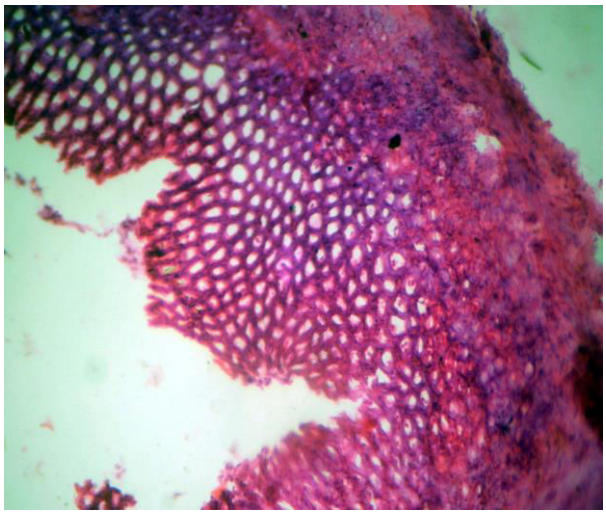


Fig:2 Stomach of control rat showing erosion in the upper part of epithelium with RBCs in Eroded portion

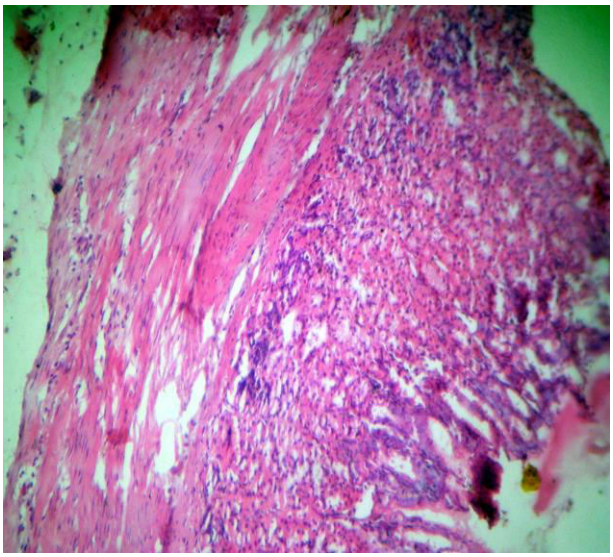


Fig: 3 Stomach of rat treated with ranitidine Showing small erosions with minimum Deviation from normal morphology

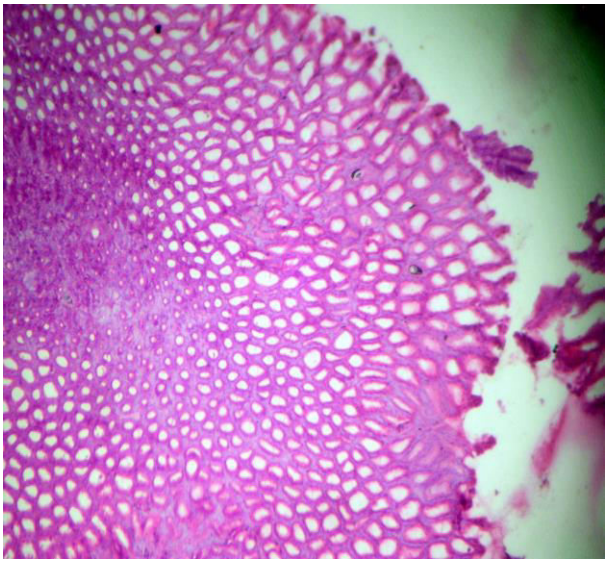


Fig: 4 Stomachs of EEPD treated rats showing small superficial erosion with minimum deviation from normal morphology

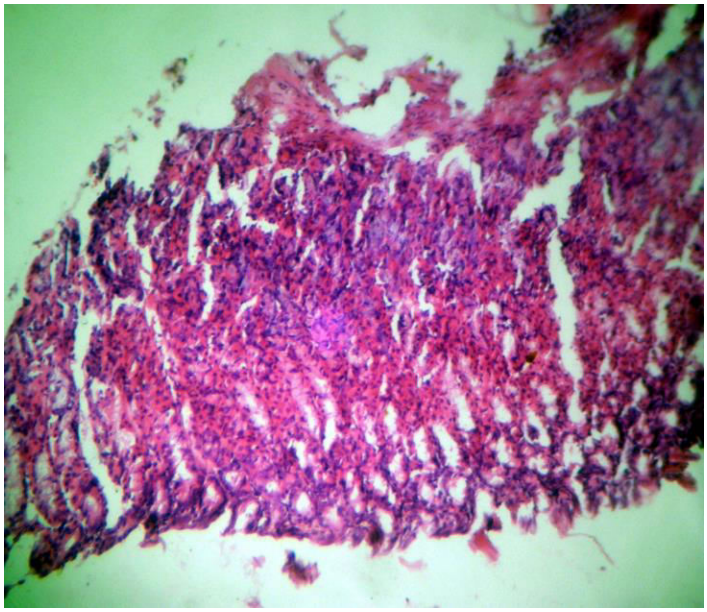


Fig: 5 Stomachs of AEPD treated rats showing small superficial erosion with minimum deviation from normal morphology

DISCUSSION

Peptic ulcer and gastritis have been associated with multi pathogenic factors and could be due to disturbances in natural balances between the aggressive factors (e.g. of acid, bicarbonate, pepsin) and maintenance of the mucosal integrity through the endogenous defense mechanism. Generally various non specific methods are used to restore these imbalances including regular food intake, adequate rest, and avoidance of ulcerogenic agents (e.g. tobacco, alcohol and coffee).Their aims are to attenuate and possibly block the gastric acid secretion or to enhance the mucosal defense mechanisms. The latter can be achieved through increasing mucus production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis. In addition there are also drugs, such as pump inhibitors, histamine (H2)-antagonists, anti cholinergics and antacids, used in the treatment of ulcer.

The H_2 receptor is a G protein coupled receptor (GPCR) that activates the Gs-adenylcyclase-cyclic AMP-PKA pathway. The H2 Receptor antagonists inhibit acid production by reversibly competing with histamine for binding to H2 receptors on the basolateral membrane of parietal cells. Four different H_2 –receptor antagonists, which differ mainly in their pharmacokinetics and propensity to cause drug interactions, are available in the United States: cimetidine (TAGAMET), Ranitidine (ZANTAC), famotidine (PEPCID),and nizatidine(AXID). These drugs are less potent than proton pump inhibitors but still suppress 24-hour gastric acid secretion by about 70%. We evaluated effects of ethanolic and aqueous extracts obtained from *Pithecellobium dulce* seeds in animals using the different standard experimental models of induced gastric ulcers. In case of Pylorus ligation model, The total acidity was decreased. Circular and linear lesions were frequently observed in the stomach of all the control animals. Administration of *Pithecellobium dulce* extracts resulted in a significant reduction in ulcer index in dose dependent manner when compared to control, despite the availability of many pharmaceutical products for the treatment of gastric ulcers in the market as mentioned above, their successes were limited by presence of several adverse effects (e.g. anaphylaxis reactions, gynecomastia, hematopoietic changes, thrombocytopenia, acute interstial nephritis, nephrotoxicity and hepatotoxicity). Due to the reported side effects of available antiulcer drugs, focused have been shifted towards natural products as the new sources of antiulcer agents.

Pithecellobium dulce has been reported to exert several pharmacological properties such as antimicrobial, anti inflammatory, antibacterial, antioxidant, antidiabetic, hepatoprotective, activities. Despite claim of its potential in the treatment of gastric ulcer, this plant so for not been screened for anti-ulcer potential activity. Thus, we take this opportunity to report the preliminary findings on anti ulcer potential of *Pithecellobiumdulce* seed extracts for the first time here. The present study demonstrated the potential of EEPD and AEPD to significantly reduced gastric ulceration as indicated by the reduction in ulcer index in the pylorus induced assays. Based on further findings using the PL assay, the extracts was suggested to act by reducing the volume of gastric juice secreted, gastric free and total acidities. These results suggested that EEPD and AEPD possess anti-secretory potency as well s acid neutralizing effect. It is also possible to suggest that the observed antiulcer activity associated with *P.dulce* is the ability to exhibit antioxidant activity cited above. Oxidative stress, resulting from the increase production of oxygen derived free radicals (e.g. superoxide anion, hydrogen peroxide and hydroxyl radicals), has been known to take part in the pathogenesis of various diseases including gastric ulcer and antioxidants help to protect cells from damage elicited by oxidative stress while enhancing the body's defence systems against degenerative diseases.

P.dulce seed extract had been reported to possess antioxidant activity and to contain various types of compounds such as flavonoids, saponins, sterols, carbohydrates, glycosides, lipids, fixed oils and fats, gums and mucilages. The anti ulcer activity is probably due to the presence of bioactive compound like flavonoids. Statistical analysis revealed that ethanolic and aqueous extracts of *Pithecellobium dulce* contains antiulcer activity due to the presence of flavonoids and sterol.

In the histopathological examination, stomachs of control rat shows erosion in the upper part of epithelium and RBCs are seen in the eroded portion (Fig 2), stomachs of rats treated with standard drug (ranitidine) showed small erosions with a minimal deviation from nor-mal morphology, (Fig 3). Stomachs of rats treated with EEPD extract showed small superficial erosion with minimal deviation from normal morphology, (Fig 4) and stomachs of rats treated with AEPD extract showed superficial erosions with minimum dev-iations from normal morphology (Fig 5).

Effect of EEPD and AEPD in pylorus ligated rats

Pylorus ligation in ulcerated control group had produced ulcer in all animals and the mean ulcer index was 9.03 indicating the ulcerogenic effect. Mean gastric content volume as 4.2±0.18,free acidity as 4.6±0.05,total acidity as 22.3±0.3 indicating the ulcer production in animal. However the ulcer index showed significant dose dependent reduction in the animal pre treated with EEPD 100mg/kg(UI; 3.63) and with AEPD 100mg/kg(UI;4.03). It indicated 59.8% gastro protection with EEPD 100mg/kg and 60.8% with AEPD 100mg/kg as compared with ulcerated control. The results indicate that AEPD 100mg/kg was effective in protecting ulcers in pylorus ligated rats. Pylorus ligation had produce ulcers in all animals pre treated with Ranitidine 50mg/kg. However, ulcer index (1.76) showed significant reduction as compared with ulcerated control and showed 80.5% gastro protection.

8. CONCLUSION

Here present study was carried out to investigate anti-ulcer activity of Ethanolic and Aqueous extracts of *Pithecellobium dulce* seeds in pylorus ligation induced ulcer in the Wistar albino strain rats. It has not produced any lethal effect up to the dose level of 2000mg/kg during acute toxicity testing. The Aqueous extract of *Pithecellobium dulce* showed significant Anti ulcer activity. *Pithecellobium dulce* belongs to the family Mimosoideae and the qualitative photochemical study reveals the presence of saponins, sterols, glycosides, flavonoids, fixed oils and fats, gums and mucilage.

The present study provided preliminary data for the first time that the seeds of *Pithecellobium dulce* possess significant anti ulcer activity in animal models. It has a gastric anti secretary effect that is comparable to reference drug Ranitidine. The anti ulcer activity is probably due to presence of bioactive compounds like Flavonoids and sterol. The observation justifies the ethanomedical uses of the plant as anti ulcer agent and as antacid in addition to its nutritional values. Histopathological studies of the stomach in pylorus ligation models exhibit normal architecture of stomach tissue. This protective effect might have been mediated by both anti-secretary and cytoprotective mechanisms .Standard dose of Ranitidine and Extracts of *Pithecellobium dulce* significantly decreased the gastric volume, total acidity, free acidity and ulcer index. Among the extracts AEPD with 100mg/kg is more effective compared to EEPD 100mg/kg. So I concluded that my plant *Pithecellobium dulce* possessing Anti ulcer activity according to the data and further research work can be carried out to different formulations. However there is a shortage of clinical trial regarding its potency and efficacy.

9.BIBLIOGRAPHY

- Anoop A, Jegadeesan M. Behavioral and sub acute toxicity studies on Cissus Quadraangularis Linn variant I. Amala Res Bull 2002; @2; 104-10.

- Anoop A, Jegadeesan M. Gastric and duodenal antiulcer and Cytoprotective effects of Cissusquadrangularis L. variant II in rats. NigerJ Nat Prod Med

- Anti Ulcerogenic Activity Of Hydroalcoholi Fruit Extract Of P.Dulce In Different Experimental Ulcer Models In Rats (Biomed Search.Com) Medline Citation

- Antiulcer activity of anisochilus carnosus leaf extract in pylorus ligated rats Journal Article Authors : J Ravikumar, H Y Santhosh, M N Nagashruthi, Mohammed Asad*,Undefined, Volume 45, Issue 12, p.979-981 (2008).

- Beradri, R.R and S.L. Welage XXXX. Peptic Ulcer Disease Pharmacotherapy :a Pathopysiologic approach.6ed: McGraw-Hill, PP: 632-648.

- Drug screening methods, second edition,S.K. Gupta,International students Edition.

- Elisabetsky, E, Costa- Campaos, L. Medicinal plant genetic resources and international co-operaration: the Brazilian perspective. J. Ethnopharmacol.1996, 51,111-19.

- Evaluation of antihyperlipidemic and antioxidant activity of albizia amara (Roxb.) Boiv. International Journal of Biological & Pharmaceutical Research Journal homepage : Sartaj Banu Mulapalli*[a], Helen Sheeba D.A. [a], Navitha.
- Extraction of anthocyanic and analyzing its antioxidant properties from *pithecellobium dulce* fruit pericarp Asian Journal of pharmaceutical and clinical research ,Vol.4, suppl 1,2011.P. Ponmozhi[1], M. Geetha[2], Dr. M. Saravana Kumar[3], P. Suganya Devi[4] P.G. Department of biotechnology, Dr. Mahalingam centre for research and development, N.g.m. College, Pollachi.

- Finkel R Cueddu LX, Clark MA. LiPPincott's illustrated review Pharmacology 4th ed. South Asian edition, Firrst Indian Reprint;2009;329-335.

- Free Radical Scavenging Activity of Folklore : Pithecellobium dulce Benth. Leaves, Ethnobotanical Leaflets (http:/opensiuc.lib.siu.edu/ebl) M. Sugumaran, T. Vetrichelvan, S. Darlin Quine,

- Furis B.S.Hannaford AJ Vogel's Text book of Practical organic chemistry 1978, Longman group publication p. 400-402.

- Gastroprotective And Antioxidant Effects Of Hydro Alcoholic Fruit Extract Of *Pithecellobium dulce* On Ethanol Induced Gastric Ulcer In Rats J. Megala1, A. Geetha2*

- Ghosh MN.Fundamentals of pharmacology 3rd editinn, Hilton and Co., Kolkkata,2005,190-7.54. New OECD 425 Guidelines foresting animals. 201,

- Goel RK, Bhattacharya SK.Gastroduodenal mucosal defence and mucosal protective agents. Indian JExp Biol. 1991:29;701-774.

- Goel RK,attacharya SK. Gastroduodenal mucosal defence and mucosal protective agents. Indian J Exp Biol. 1991;29:701-774.

- Hansen K, Nyman U, Smitt UW,Andersen A Gudiksen L, Rajasekharan S,Pushpagadan P, In vitro screening of traditional medicines for anti hypertensive effect bsed on inhibition of the angiotensin converting enzyme

- Indian journal of pharmacology 2003 35,166-1672.

- Indian Pharmacopoeia1996,Government of India 947-949,A53-54,A70-71,A73,A76,A89,A105.

- International Journal Of Pharma And Biosciences, Vol.1 Dec-2010.,Preliminary Phytochemical Analysis Of Some Important Indian Plant Species.

- J.Y Kang, A Wee, M.Tech; The effect of chille ingestion of gastrointestinal mucosal proliferation and azoxymethane- induced cancer in the rat Journal of Gastroentrology Hepatol. 1992: 7(2), 194-198.

- Jabeen S, Shah, MT, Khan S, Hayat MQ, Determination of major and trace Elements in ten important folk therapeutic plants of Haripurbasin, Pakistan,Journal of Medicinal plants Research, 4(7),2010,559-556.

- Jaffery GH, asset J, Mendham J, Denney RC. Vogel's text book of quqntitative Chemical analysis,. 5th edition .Longman Scientific and Technical publications: London ;1991.

- James.A Duke1983.Hand book of energy crops

- Johnson,T.(2001) News.Chinese medicine now part of primary care scene in BC. *Can.med.Assoc*.J., 164,1195.

- Joy P.P, Thomas J. Mathew S, and Skaria,B.P. Medicinal plants. Tropical Horticulture vol.2.(eds.Bose, T.K..,Das, P. and Joy,P.P.).Nayaprakash, Calcutta,2001.

- Kadel C, Jain AA. Folklore claims on snakebite among some tribal communities of Central India, Indian Journal of Traditional Knowledge, 7(2),2008,296-299.

- KG Yeoh, J.Y Kang Yap, R. Gaun C.C TAN Chille protects against aspirin induced pasrto duodenal mucosal injury in humans. Disease Digestive Science.1995;40 (3) : 580-583.

- Kinchen Tantra-Tease Your Palate,A Food Blog For Indian Recipes.

- Kozyrskyj,A.(1997) Herbal products in Canada. How safe are they ? *Can. phys. Med.Famille can.,* 43,697-702.

- Locomotor activity of leaf extracts of *pithecellobium dulce* Journal Article ,M. Sugumaran; T. Vetrichelvan; S. Darline Quine,Ethanobotanical leaflet, Volume 12, p.49-493 (2008).

- Medicinal Plants Of Bangladesh , Dr.Shaikh, Www. Mphd.Info.

- Ministry of Agriculture, Fisheries and Food (MAFF)(1998)*Herb Legislation*, London, Herb society.

- Neuropharmacological Profile of Ethanoloc Extract of Pithecellobit Leaves in Mice,Reseatch J. Pharmacology and Pharmacodynamics : 3 (1) : 2011 : 27-30, Mule V.S.*, Potdar V.H., Jadhav S.D.

- OECD (2000). Guidance document on the Recognition, assessment and use of clinical signs as humane endpoints ofr experimental animals used on safety evaluation. Environmental health and safety monograph series on testing and assessment No19.

- Parmar, N.S. and J.K Desai, 1993, A review of the duodenal antiulcer agents Indian J. Pharmacol.
- Philippine Medicinal Plants,Kamatsile Www. Stuartxchange.Com/Kamatsile.
- Phytochemicals as Cure of Worm Infections in Traditional Medicine Systems V. Tandon, A.K. Yadav, B. Roy and B.Das ,Emerging Trends in Zoology,
- Phytomedicinal Role of Pithecellobium dulce against CC14-mediated Hepatic Oxidative Impairments and Necrotic Cell Death,Evidence-Based Complementary and Alternative Medicine**,**Volume 2011 (2011), Article ID
- Phytomedicinal Role of *Pithecellobium dulce* against CC14-mediated Hepatic Oxidative Impairments and Necrotic Cell Death. Parasenjit Manna, Sudip Bhattacharyya, Joydeep Das, Jyotirmoy Ghosh, and Parames C. SilDivision of Molecular Medicine, Bose Institute, P-1/12, CIT scheme VII M, Evidence –Based Complementary and Alternative, Volume 2011 (2011), Article
- Ravi V1, Saleem TSM1,PatelSS1,Ramamurthy J2,Gauthaman K1* Anti-Inflammatory Effect of Methanolic extract of Solanum nigrum Linn Berries International Journal of Applied Researchin Natural Products Vol. 2(2), pp33-36, June-July 2009.
- Reference Laboratory Notes For Bio1003, 1999,Organic Molecules-Laboratory Tests.
- Russel Green J, Norman Harris D Pathology and therapeutics for pharmacists. A basis for clinical practice.2nd and 3rd edition. Pharmaceutical press publisher's 2008.
- S.K Kulkarni, Handook of experimental pharmacology, VallbahPrakashan,New Delhi, 19999,3, 148-150.
- Samy RP Thwin MM, Gopalakrishnakone P,Ignacimuthu S,Ethnobotanical survey of folk plants for the treatment of snakebites I southern parts of tamilnadu, India.J Ethnopharmacol.,115(2),2008, 302-312.
- Sibbald, B. (1999) New federal office will spend millions to regulate herbal remedies,vitamins. *Can.med.Assoc. J*., 160, 1355-1357.
- Siti fahimah Zahra MA, Mahmood,Hapipah MA, Sujitha MN, Salmah I. Antiulcer activity of Aqueous Ficusdeltoidea against ethanol induced Gastri mucosal Injury in Rats. Journal of Medical sciences. 209: 3(2);42-46.
- Studies on some Pharmacognostic profiles of Pithecell'obium dulce Benth. Leaves (Leguminosae**),** Anc Sci Life 2006 Jan – Jun; 25 (3&4) : 92 – 100 PMCID : PMC3335217,M. Sugumaran, T. Vetrichelvan, and D. Venkapayya
- SzaboS,Trier JS rown,Schnoor J, Hman HD, Bradford JC. A qualitative method for sessing the extend of experimental gastricerosions and ulcers. J Phatmacol Methods 1985: 13;59-66.

- Ten Medicinal plants from Burma, A literature study By. Alirezasesoltani,February-2011

- Tyler, V.E.(2000)Herbal medicine:From the past to the future.Public Health Nutr., 3,447-452 WHO (1993) *Research guidelines for Evaluating the safety and Efficacy of Herbal Medicines.*

- Uma R Khurmal,Anupama Mangotra2Screening of some Leguminosae seeds for nematicidal activity.www.publishcsiro.au.

- V.Palanivel, Divya B. Evaluation of Antiulcer Activity of Ethanolic Leaf Extract of Ficus religiosa in albino Rats.2012, (6)47.

- Wikipedia,the free encyclopedia

- www.medicine.net.

- Yasutada Akiba,Masahiko akamasaishil;Acid sensing mechanism and the hyperemic response in rat gastrointestinal mucosa. In; Y.Kasua, T.Mutto, Y Matsuo editors. Gastrointestinal function regulation and disturbance.Japan; Excerpta Medica 2002; 20; 85-91.