PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON CYATHULA PROSTRATA (LINN) BLUME AND HELIOTROPIUM INDICUM (LINN)

Thesis submitted to

The Tamilnadu Dr.M.G.R Medical University, Chennai,

For the award of

DOCTOR OF PHILOSOPHY

In Pharmacy

Submitted by

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Under the Guidance of

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2010

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CERTIFICATE

This to certify that Mr. S. Janardhanan, Research Scholar, Department of Pharmacognosy, JSS College of Pharmacy, Udhagamandalam. He has given plant for identification and the same has been identified by me as ***Cyathula prostrata*** (L.) Blume belong to the family Amaranthaceae and ***Heliotropium indicum*** L. belong to the family Boraginaceae.

(Dr. S. RAJAN)

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Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA)
Institutional Animal Ethics committee (IAEC).

CERTIFICATE

Title of the Project: **Phytochemicals and Pharmacological Studies on Cyathula Prostrata and Heliotropium Indicum**

Proposal Number: JSSCP/IAEC/PH.D/PH.COLOGY/02/2007-08.

Date received after modification (if any):

Date received after second modification: 12.07.07

Approval date: 05.09.07

Animals: Wistar rats/ Albino mice

Rabbits / Guinea pigs

No. of animals sanctioned: 84 + 54 (138)

Male/Female

Expiry date (Termination of the Project): 15DAYS.

Name of IAEC/CPCSEA chairperson: **Prof. K. Elango**

Signature of Chairperson
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MEDICINAL PLANTS—an overview:

"You, herbs born at the birth of time
More ancient than the gods themselves
You, who have a thousand powers,
Free this patient from disease"
"When restoring vanished strength
I hold you herbs within my hand
And the spirit of Disease departs,
Cheated of another death"
Nature cures, not the physician

--- Hippocratus

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security[1].

Herbal medicines have been used since time immemorial and plants form the basic raw material for the preparation of drugs used in the Indian system of medicine. Over the centuries, Societies around the world have developed their own tradition to make sense of medicinal plants and their uses. Some of these traditional medicinal practices may be seen strange and magical, others may appear rational and sensible but all of them are attempts to overcome illness and suffering and to enhance quality of life. The efficacy of herbal medicine heritage of China, India and early American ancestors is now being looked at with renewed interest by today' doctors[2].

HERBAL MEDICINE-CURRENT STATUS

The art of herbal medicine is extremely ancient, probably predates the modern Homo *sapiens.* In ancient cultures, people methodically and scientifically collected information on herbs and developed well defined herbal pharmacopoeias. The earliest recorded evidence of such efforts in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts were Charak Samhita and Sushruta Samhita[2].

Irrespective of the decline in use of herbal medicines, the importance of botanicals in the evolution of medicine remains unchallenged. Many drugs are developed with phytochemicals or taking phytochemicals as lead molecules[3]. Presently, approximately 25% of the drugs prescribed by the modern medicine are plant based which are either directly isolated or synthetic derivatives of the bioactive molecules isolated from plants. While plant derived medicines such as opium, aspirin, digitalis and quinine have a fairly long, history of use, are the new generation of phytomedicines include a large number of life saving drugs like taxol, artimisine, gossypol, vincristine and vinblastine and other[4] such as anti ulcer plant drugs like carbenoxolone, nimbidin, catechin, suphordin, magnolol and plaunotol etc

The growing preference on herbal medicines world over could be attributed to increasing faith in traditional systems of medicines and the common disillusionment towards allopathic medicines, especially due to the toxicity and side effects of synthetic drugs used. For a variety of reason, more individuals now a days prefer to take personal control over their health with the use of herbal medicines, not only to prevent diseases but also to treat them. This is particularly true for a wide variety of illnesses readily treated at home (common cold, etc-Kincheloe, 1997). Herbal products are also commonly used by patients with certain chronic medical conditions, including breast cancer (12%-Burstein, 1999), liver disease (21%-Strader, 2002), human immunodeficiency virus (22%-Kassler, 1991) asthma (24%-Blanc, 2001) and rheumatologic disorders (26%_Rao, 1999)[5].

World Health Organisation (WHO) – views

WHO has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, almost for several hundreds of years, before the development and spread of modern medicine and are still in use today. The traditional preparations comprise medicinal plants, minerals, organic matters, etc. Herbal drugs constitute only those traditional medicines, which primarily use medicinal plant preparations for therapy. WHO estimates that about three quarters of the world's population currently use herbs and other forms of traditional medicines to treat their diseases.[6]

From time immemorial, plants have been an indispensable source of both preventive and curative medicinal preparation for human being. Many indigenous plants have been scientifically tested and found to have medicinal properties that can be used in western style medicine. Such cures are particularly suitable for use in rural communities as inexpensive, sustainable and culturally appropriate alternative to more expensive conventional treatments. As a result, ethnomedicne is being promoted and supported as a way of providing effective medicines for people in less developed areas[7]

Folk medicine is a significant source of Ayurvedic, Unani, Traditional Chinese Medicine (TCM) and Medical herbalism. It incorporates crude medicinal herbs, decoctions and infusions and syrups. Folk medicine is still practiced by some vendors, hakims and vaids in remote areas and some folk preparations are of surprising high curative value[8].The WHO estimates that up to 80% of the world's population use traditional medicines as their primary form of healthcare. The use of herbal medicine, the dominant form of medicinal treatment in developing countries, has been increasing in developed countries in recent years[7].

Scientific interest in medicinal plants has burgeoned due to increased efficiency of new plant derived drugs, growing interest in natural products and rising concerns about the side effects of conventional medicines. Based on current research and financial investments into medicinal plants. It seems that they will continue to play important roles in human health. Herbal medicine is also called botanical medicine or phytomedicine. However, some countries such as Germany is starting to regulate herbal remedies as if they were drugs. The German Commission E conducting some research on safety and effectiveness of medicinal plant use.

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Challenges in improving safety of herbal remedy

Consistency in the quality of herbal drugs can be maintained by

1. Adopting Good Agricultural Practices and Good Field Collection Practices for the cultivation and collection of herbal drugs.
2. Standard practices for preservation and presentation have to be developed and practiced similarly.
3. Standardization techniques with respect to authentification, purity profile and assay have to be developed and put into practice for quality control and quality assurance of herbal drugs[5].

There are two main reasons for interest in natural products. Firstly, the natural products continue to remain as an important source of new drug discovery. This segment is growing rapidly; secondly, the medicinal plants are used as phytomedicine, dietary supplements, food and beverage ingredients and traditional medicines. It is now increasingly accepted worldwide that screening of natural products is a more effective strategy for discovering new chemical entities[9].

The growing preference for herbal medicines world over could be attributed to increasing faith in traditional system of medicine and the common disillusionment towards allopathic medicines especially due to the toxicity and side effects.

Herbal drugs constitute a major share in the Indian Systems of Medicine viz. Ayurveda, Yoga, Unani, Siddha and Homeopathy. More than 70% of the Indian population still use these non allopathic system of medicine. Currently, there is no separate category of herbal drugs or dietary supplements as per the Indian Drugs Act. However there are vast experimental evidences for Observational Therapeautics and Reverse Pharmacology of herbal based medicines. Evidence based herbals are widely used in the diverse systems and manufactured as per the pharmacopoeial guidelines by well organized industries. Significant basic and clinical research is being carried out on the medicinal plants and their formulation

with the state of the art technology in a number of Institute/Universities. There are some good examples that the medicinal plants of India also provide a rich source of anti ulcer drugs such as carbenoxolone, a hemi succinate sodium salt of glycyrhicinic acid from liquorice, Nimbidin from *Azadirachta indica*, Catechin from *Acacia catechu*, Suphordin from *Sophora subprstrata* and Magnolol from *Magnolia* bark[3.] Plaunotol is a potent ulcer-healing drug isolated from *Croton sublyratus*[10]

Many of herbal therapies fall under one single title "Complementary and Alternative Medicines (CAM). The Global Herbal remedy is constantly growing at 15-17% in value per year. Billion of dollars are spent on herbal medicines. According to a recent survey conducted by WHO, almost every person in developed world has visited at least once in his/her life a CAM practioner[11]

Herbal Medicine-Worldwide Current scenario[12]

In Germany and France, many herbs and herbals extracts are used as prescription drugs and their sales in the countries of European Union were around $ 6 billion in 1991 and may be over $ 20 billion now. In U.S.A herbal drugs are sold in health food stores with a turnover of about $ 4 billion in 1996 which is anticipated to double by the turn of the century. In India, the herbal drug market is about $ one billion and the export of plant base crude drugs is about $ 80 billion

Herbal Remedies importance on Indian Scenario[13]

India is having of immense biodiversity in which two out of eighteen hot spots of the world are located. India is also one of the twelve mega biodiversity countries in the world. The total number of plant species of all groups recorded from India is 45,000 (the total number may be even close to 60,000, as several parts of India are yet to be botanically explored). Of these, seed bearing plants account for nearly 15,000-18,000. India enjoys the benefits of varied climate, from alpine in the Himalaya to tropical in the south and arid in Rajasthan. Such climatic conditions have given rise to rich and varied flora in the Indian subcontinent.

Herbal medicine is still the mainstay of about 75-80% of the population, mainly in the developing countries, for primary health care because of their cultural acceptability, better compatibility with human body and lesser side effect. The plant kingdom is by far the most

efficient ‘factory’ of novel compounds to combat against various diseases, but many of them are still unexplored or confined in the various ethnic communities which remain to be scientifically validated. The vast and hitherto untapped wild medicinal plant generic sources together with vital ethno medicinal leads offer grater scope for developing latest therapeutic agents.

Tribal Medicines act as new source[14]

Tribal healer around the Globe where ethno medical treatment is frequently used to treat cut wounds, skin infection, swelling, aging, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal diseases, snakebite and gastric ulcer provide instructions to local people as how to prepare medicine from herbal. They keep no records and the information is mainly conveyed orally from generation to generation. WHO has shown great interest in documenting the use of medicinal plants used by tribal from different parts of the world. Many developing countries have intensified their efforts in documenting the ethno medical data on medicinal plants. Research added skills to explore the scientific claims for diseases by tribal healers on Indian herbs have been intensified. Once these local ethno medical preparations are scientifically evaluated and disseminated properly, people will be better informed concerned efficacious drug treatment and improved health status.

The Development of phytomedicines and the ethanomedicinal approach[15]

The first generation of plant drugs were usually simple botanicals employed in more or less in their crude form. Several effective medicines used in their natural state such as cinchona, opium, belladonna and aloe were selected as therapeutic agents based on empirical evidence of their clinical application by traditional societies from different parts of the world. Following the industrial revolution, a second generation of plant based drugs emerged based on scientific processing of the plant extracts to isolate "their active constituents." The second-generation phytopharmaceutical agents were pure molecules and some of the compounds were even more pharmacologically active than their synthetic counterparts. Notable examples were quinine from *Cinchona*, reserpine from *Rauwvolfia*, and more recently taxol from *Taxus* species. These compounds differed from the synthetic therapeutic agents only in their origin.

They followed the same method of development and evaluation as other pharmaceutical agents.

The sequence for development of pharmaceuticals usually begins with the identification of active lead molecules, detailed biological assays, formulation of dosage forms in that order, and followed by several phases of clinical studies designed to established safety, efficacy and pharmacokinetic profile of the new drug. Possible interaction with food and other medications may be discerned from the clinical trials.

In the development of "Third Generation" phytotherapeutic agents a top-bottom approach is usually adopted. This consist of first conducting a clinical evaluation of the treatment modalities and therapy as administered by traditional doctors or as used by the community as folk medicine. This evaluation is then followed by acute and chronic toxicity studies in animals. Studies should, when applicable, include cytotoxicity studies. It is only if the substance has an acceptable safety index would it be necessary to conduct detailed pharmacological/ biochemical studies.

PEPTIC ULCER

Definition[16]

Peptic ulcers are chronic, most often, solitary lesion that occur in any portion of the gastrointestinal tract exposed to the aggressive action of acid-peptic juices. It can be defined as a breach in the mucosa of the alimentary tract which extends through the muscular mucosa into the sub mucosa or deeper which leads to erosion. Peptic ulcer occurs only in stomach and duodenum whereas ulcer may occur anywhere in the G.I tract.[17]

Ulcer causes disintegration, loss and death of tissue as they erode the layers of the wall of the stomach or duodenum. These crater like lesion cause growing or burning pain and may ultimately result in hemorrhage, perforation, widespread inflammation, scaring and other very serious medical complications.[18]

Types of ulcers

Peptic ulcer disease (PUD) is a serious gastrointestional disorder, which occur due to excessive acid secretion in the stomach..[43] Peptic ulcers are break or holes in the protective

lining of stomach (gastric ulcers), duodenum (duodenal ulcers) and esophagus (esophageal ulcers).[44]

Acute peptic (stress) ulcers

These are multiple, small mucosal erosions, seen most commonly in stomach occasionally involving duodenum.

Chronic peptic ulcers (gastric and duodenal ulcers)

These are not specified but mean gastric and duodenal ulcers, which are major forms of peptic ulcer disease of the upper gastrointestinal tract in which the acid pepsin secretions are implicated in their pathogenesis.[18]

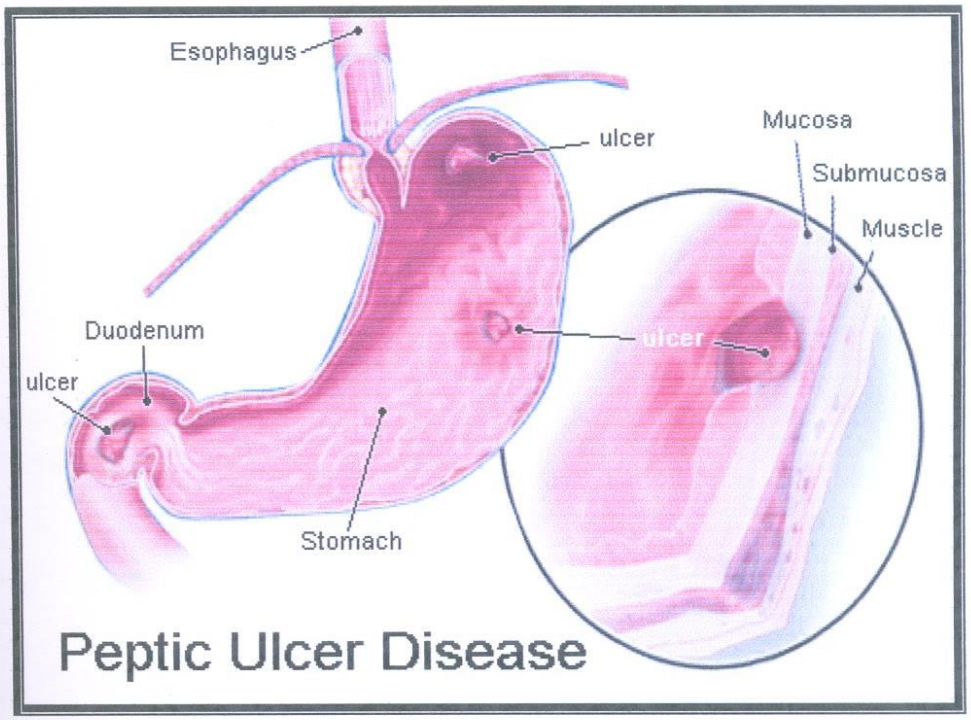

Fig: 1.a. Anatomy of peptic ulcer [5]

Epidemiology[16,19,20]

Peptic ulcer is one of the common diseases prevailing in human population and its incidence is increasing day by day due to rapid development and civilization constraints. The estimates of incidence of peptic ulcer vary ranging between 3-10%.

It is estimated that 15,000 deaths occur every year as of peptic ulcer disease (PUD). In United States approximately 4 million people have ulcer (gastric & duodenal) and 3,50,000 new cases are diagnosed every year. Around 10,00,000 patients are hospitalized yearly and 3000 people die each year as a result of peptic ulcer. The male/female ratio for duodenal ulcer is 3:1 and gastric ulcer is about 2:1. There has been a significant decrease in the prevalence of duodenal ulcers over the past decades but little change in the prevalence in gastric ulcer. In U.S.A, peptic ulcer disease affects 10% men and 4% women whereas this disease affects the Britain population 5-15% of the adult population at some time during their lives and the prevalence of active disease is 1-2%. Peptic ulcer deaths in people over the age of 75 years account for much of the observed increase in mortality. The disease is uncommon amongst children and juveniles. About 2% of gastric ulcer is malignant whereas duodenal ulcers are never cancerous.[21] The morbidity and mortality rates are higher for gastric ulcer than for duodenal ulcer in Japan.[22]

Ulcer is common in Britain,Norway,Germany, Assam,Kashmir and the south of India and wet parts of Africa such as Ghana, Uganda and in peru. The incidence of ulcer occurs in various parts of our country is Ludhiana (Punjab) 0.2 % Srinagar (Kashmir) 2.5 % and Vellore 1.6 % (south India). The male and female ratio for ulcer found is 1.7 to 1 in a population of 10096 urban dwellers. The men are affected 18 times more commonly than women, in India.[23a,b,c]

Etiology

Anatomical causes of ulcer[24]

It emphasis that portion of the organs which ma y be pinched, rubbed, or pressed between the posterior surface of the liver and the ventral surface of the spine and prevertebral structures. Pressure thus applied to the stomach and duodenum in the hepatavertebral angle results in necrosis and thus may be considered the primary cause of ulceration. All other etiologies become secondary.(Avery D.Weisman,1950)[23].

The time honored dictum “no acid”, “no ulcer” is still broadly correct although gastro duodenal ulceration in the absence of acid is known to occur in some situations. The mucosa of the upper gastrointestinal tract is protected from auto digestion by means of a complex series of physical and chemical mechanism which is known collectively as the mucosal barrier. These include gastric mucus, gastric bicarbonate secretion and rapid generation of mucosal cells, high local blood flow and local prostaglandins activity. The etiology of peptic ulcers possibly may not be explained on the basis of a single factor but is multifactor.

Gastric ulcers arise due to various factors, even though the etiology of gastric ulcers is still debated, it is accepted that ulcers are caused due to net imbalances in mucosal offensive and defensive factors. Ulcer therapy is now mainly focused on limiting the deleterious effects of offensive acid secretion, but the search for new safer alternative drugs has rekindled the interest in cytoprotective drugs, which protect the gastric mucosa from damaging agent without influencing acid secretion or neutralizing intragastric acidity. In the area of a gastric or duodenal peptic ulcer, the mucosa has been attacked by digestive juices to such an extent as to expose the subjacent connective tissue layer (submucosa). This self digestion occurs when the equilibrium between the corrosive hydrochloric acid and acid neutralizing mucus, which forms a protective cover on the mucosal surface, is shifted in favour of hydrochloric acid.

Mucosal damage can be promoted by *Helicobacter pylori* bacteria that colonize the gastric mucus. The apparent role of *Helicobacter pylori* in peptic ulceration cannot be overemphasized. *Helicobacter pylori* infection is present in virtually all patients with duodenal ulcers and about 70% of those with gastric ulcers. Furthermore, antibiotic treatment of Helicobacter pyloric infection promotes healing of ulcer and tends to prevent their recurrences. *H.pylori* produce an enzyme called urease, which splits urea into ammonia and carbon dioxide. While shielding the bacterium from the acidity of the stomach, the ammonia also damages the protective mucous layer of the stomach, and the underlying cells. *H.pylori* also produces catalase, an enzyme that protect the microbes from phagocytosis by neutrophils, plus several adhesion proteins that allows the bacterium to attach itself to gastric cells[24,25,26]

Table No:1 Summary of cells and activity of secretions in the stomach.[27]

Structure	Activity	Result

Parietal cells	Secrete HCl	Kills microbes in food, denature of protein, covert pepsinogen into pepsin
	Secrete intrinsic factor	Binds with vitamin B_{12} for ready absorption which is used in DNA synthesis and in red blood cells formation (erythopoiesis)
Peptic cells	Secrete pepsinogen	Pepsin, the activated form; breaks down to peptides
	Secrete gastric lipase	Split triglycerides into acids and monoglycerides
G cells	Secrete gastrin	Stimulate parietal cells to secrete HCl and chief cells to secrete pepsinogen; contracts lower esophageal sphincter; increase motility of the stomach and relaxes pyloric sphincter

Locally produced prostaglandins E_2 and B_{I2} stimulate the secretion of both the mucus and bicarbonate for protection of the gastric mucosa from acid in the stomach which also directly inhibits gastric acid secretion by parietal cells of stomach

Table No:2 showing the aggravating and defensive factors

Aggravating factors	Defensive factors
Gastric acidity (Acid)	Gastric mucosal secretion
Peptic enzymes (Pepsin), histamines, acetylcholine, serotonin	Bicarbonate secretion into mucus

Refluexed bile salts	*Apical surface membrane transport*
Alcohol, Smoking	Epithelial regenerative capacity
Rapid gastric emptying	Elaboration of prostaglandins
Duodenal gastric reflux	Ischemia shock
Helicobacter pylori	Delayed gastric emptying

Fig: 1.b. Physiological and pharmacological regulation of gastric secretion: The basis for therapy of acid-peptic disorders.[11]

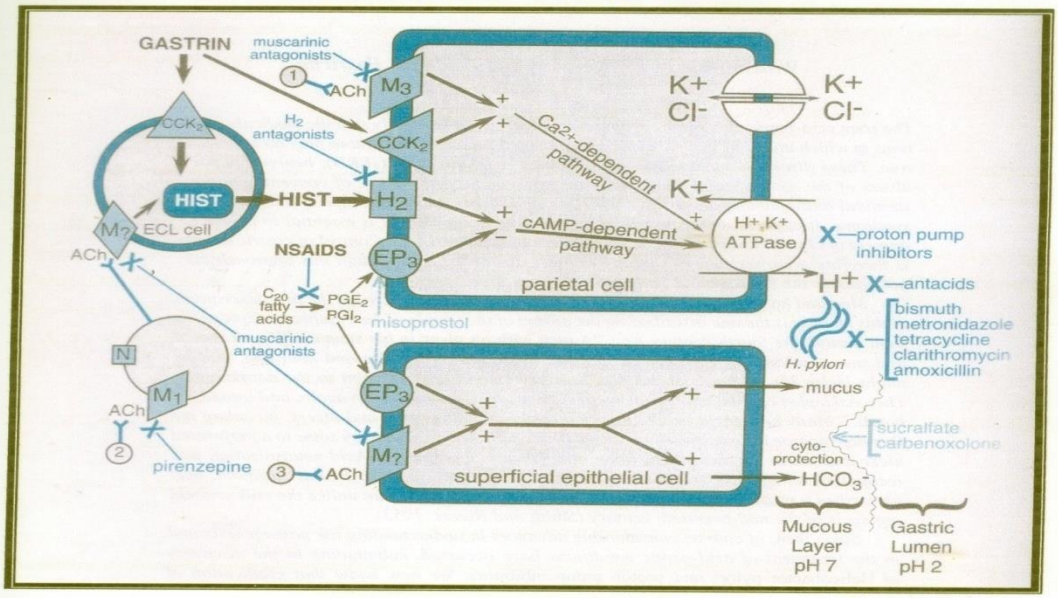

Figure is showing the interactions among an enterochromaffin-like (ECL) cell that secretes histamine, a parietal cell that secretes acid, and a superficial epithelial cell that secretes cytoprotective mucus and bicarbonate. Physiological pathways, shown in solid black, may be stimulatory (+) or inhibitory (-). 1 and 3 indicate possible inputs from postganglionic cholinergic fibers, while 2 shows neural input from the vagus nerve. Physiological agonists and their respective membrane receptors include: acetylcholine (ACh), muscarinic (M), and nicotinic (N) receptors; gastrin, cholecystokinin receptor 2 (CCK_2); histamine (HIST), H_2 receptor; and prostaglandin E_2 (PGE_2), EP_3 receptor. Drug actions are indicated by dashed lines. A blue X indicates targets of pharmacological antagonism. A light blue dashed arrow indicates a drug action that mimics or enhances a physiological pathway. Shown in blue are drugs used to treat acid-peptic disorders. NSAIDs are nonsteroidal antiinflammatory drugs and are ulcerogenic.

The regulation of acid secretion by parietal cells [28],[29]

The regulation of acid secretion by parietal cells is especially important and constitutes a particular target for drug acton. The secretion of the parietal cells is an isotonic solution of

HCl (150 mmol/lit) with a pH less than 1., the concentration of H^+ being more than million times higher than that of the plasma. At the parietal cells lining of the stomach
Carbonic anhydrase catalyses the reaction combines with carbon dioxide and water to give carbonic acid, which dissociate into H^+ and bicarbonate ions.

The ions exchange across the basal membrane in parietal cell is by three mechanisms.

i) Antiport mechanism (A): (Cl^-/HCO_3^-ATPase) In which two different ions move in opposite direction. The Cl- ions actively transported from plasma to canaliculi in parietal cells accompanied by bicarbonate ions ($HCO3^-$).

ii) Symport mechanism(C): (Cl/K^{+-}ATPase) in which two different ions move in same direction. The Cl^- ions actively transported from parietal cells to the lumen(stomach) accompanied by K^+ ions

iii) Proton pump(P): (K^+H^+-ATPase) in which two different ions move in opposite direction. The K^+ ions exchanged for H^+ ions. The K^+ ions move from lumen into parietal cells and the H+ ions from the cell move +into the lumen (stomach) causes secretion of gastric acid (HCl) into the GIT.

Three main stimuli act on the parietal cells:

i) **Gastrin : (** a hormone)

It is a peptide hormones synthesized by endocrine cells of mucosa of gastric antrum and duodenum and secreted into portal blood. It stimulates secretion of acid by parietal cells. Action of gastrin receptor on parietal cells causes increase pepsinogen secretion and stimulates blood flow and gastric motility. Control of gastrin releases involved both neuronal transmitter and blood borne mediators. Stomach contents also directly affect gastrin release, amino acids, peptides, milk and solution of calcium salts stimulate gastrin release. Hence calcium containing antacids should not be used.

ii) **Acetlycholine** (a neurotransmitter)

Acetylcholine is released from neurons and stimulates specific muscarinic M2 receptor on the surface of parietal cells and on the surface of histamine containing cells.

iii) **Histamine** (a local hormone)

The parietal cells are also stimulated by histamine action on H_2 receptors. They respond to amounts that are below the threshold concentration that acts on H_2 receptors in blood vessels. The histamine is derived from mast cells that lie close to the parietal cells. There is a steady basal release of histamine, which is increased by gastrin and acetylcholine

Role of acetylcholine, histamine, gastrin in acid secretion[28]

i) **Single cell hypothesis**:

By this theory, parietal cell has H_2 receptors for histamine, muscainic M_2 receptors for acetylcholine and also gastrin receptors. Stimulation of H_2 receptors increases cAMP and stimulation of the M_2 receptors and gastrin recptors increases cytosolic Ca^{2+} and these messengers synergise to produce acid secretion. All these secretagogues act directly on the parietal cell.

ii) Two cell hypothesis:

According to this hypothesis, gastrin and acetylcholine act either only by releasing histamine or partly by releasing histamine and partly by direct action on their respective receptors on the parietal cell.

Factors affecting ulcers

i) Acid and Pepsin[21]

Increased acid secretion may play an important role in some patients and the high levels of gastric acid secretion seen in patients with gastrin secreting tumours (Zollinger-Ellison syndrome) are invariably associated with ulceration. There is a diurnal variation in acid secretion and it may be that disturbances to this pattern which result in prolonged exposures of the mucosa to the acid/pepsin mixture are more important than absolute quantities of acid and the mixture is highly proteolytic (Roger Walker,1994)

ii) Helicobacter pylori[29]

The evidence for an etiologic link between *Heliobacter pylori* and peptic ulcer disease is overwhelming and universally accepted. It infects only gastric type epithelium but causes ulcers in the duodenum, which usually contains ectopic gastric epithelium.

H.Pylori can be found in the gastric mucous, adheres to gastric epithelium and lives within mucous gel layer overlying gastric epithelium, Penetrates intercellular junctions invades gastric glands and canaliculi of parietal cells. In addition to damaging gastric epithelial cells, *H.Pylori* infection may increases the permeability of the mucous layer to hydrogen ions and combine with gastric acid and pepsin to promote ulcer formation. *H.Pylori* urease generates ammonia from urea, protecting the organism from the highly acidic of the stomach. The pathogen invariably damages the gastric mucosa, resulting in both structural and functional abnormalities. It causes histological gastritis namely, gastric ulcer, duodenal ulcer, gastric adrenocarcinoma, and primary gastric lymphoma. Elimination of the infection results in healing of gastritis and cure of peptic ulcer disease.

iii)Non steroidal anti inflammatory drugs (NSAIDS)

These drugs are known to induce peptic ulcer not only by denaturing mucous glycoproteins but also direct action on gastric epithelial cells and neutrophils by free radicals produced lipid peroxidation, leading to membrane fluidity which in turn increases the influx of Ca^{2+} ions and results in the reduced membrane integrity of surface epithelial cells, there by generating gastric ulcers[30].

NSAIDs induce peptic ulcer by increased secretion of hydrochloric acid and reduction of bicarbonate and mucin production. Loss of mucin degrades the mucosal barrier that noramally prevents acid from reaching the epithelium . NSAID also reduce synthesis of glutathione which causes reduction of free radical scavenger activity (Kumar, 2003)[18].

The growth factor that is directly involved in cell proliferation is regulated by prostaglandins (PGs) which are induced by cycloxgenase 2 (COX-2).

A high incidence of gastric mucosal injuries, such as erosions, small submucosal hemorrhages, and gastric ulcers, is reported in patients administered non-steroidal anti-inflammatory drugs (NSAIDS). The drug induce gastric lesions has been attributed to various causes such as inhibit COX-2 pathway, inhibit the production of endogenous prostaglandins (PGs).[31]

iv) Alcohol

Ethanol rapidly penetrates the gastrointestinal mucosa causing increase in mucosal permeability together with the release of vasoactive products like histamine from mast cells,

macrophages and blood cells may lead to vascular injury, membrane damage, exfoliation of cells and erosion, finally ulcer formation. The metabolism of ethanol depression of glutathione and generates superoxide radicals which can promote lipid per oxidation mediated free radicals, is an important cause of the destruction and damage to cell membrane, which has been demonstrated to play an important role in the pathogenesis of gastric mucosal injury.[32]

Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contribute to the development of the hemorrhage and necrotic aspect of tissue injury.[33]

V) Cigarette Smoking

Cigrette smoking is clearly associated with a higher incidence of peptic ulcer disease (PUD) by impairing mucosal blood flow, following activation of parasympathetic and sympathetic nervous system resulting in the constriction of the smooth muscles of blood vessels of gastric mucosa causing ischemic condition.[34]

vi) Local irritants

Mainly pyloric antrum and lesser curvature of the stomach are the sites which are most exposed for longer periods to local irritants for occurrence of gastric ulcers.[35]

vii) Hormonal factor

Secretion of certain hormone by tumors is associated with ulceration for example elaboration of gastrin by islet cell tumor in Zollinger-Ellison syndrome, endocrine secretions in hyperplasia and adenomas of parathyroid glands, adrenal cortex and anterior pituitary.[35]

viii) Psychological factors

Psychological stress, anxiety, fatigue and ulcer-type personality may exacerbate as well as predispose to peptic ulcer disease. In various stress conditions ulcer is developed mainly due to oxidative damage as indicated by derangements of the antioxidant enzymes creates favourable condition for the accumulation of endogenous hydrogen peroxide (H_2O_2).[35]

Stress causes ischemic condition in the gastric mucosa by reducing blood flow followed by activation of parasympathetic and sympathetic nervous system, resulting in the constriction of the smooth muscles of blood vessels of gastric mucosa.[34]

ix) Genetic factors

People with blood group 'O' appear to be more prone to develop peptic ulcers than those with other blood groups. Genetic influences appear to have greater role in duodenal ulcers as evidenced by their occurrence in families and monozygotic twins. (Harsh Mohan, 1998)[35]

x) Miscellaneous[17,21]

Duodenal ulcers have been observed to occur in association with various other condition such as chronic pancreatitis, chronic obstructive pulmonary disease, alcoholic cirrhosis, chronic renal failure and hyper parathyroidism, which, stimulates gastrin production and therefore, acid secretion occurs.

Bile reflux may play a role in some patients, particularly if the pyloric spincter is incompetent or if the patient has undergone a partial gastrectomy.

Free radicals and ulcers

Free radicals are small, highly charged molecular particles and they possess strong oxidizing power .[36] A free radical exists with one or more unpaired electron in atomic or molecular orbital. Free radicals are generally unstable, highly reactive and energized molecules. The molecule, which loses an electron, also becomes a free radical giving rise to a self – perpetuating chain system. Free radical often attacks DNA, protein molecules, enzymes, and cell leading to alterations in genetic material and cell proliferation.[37]

Sources of free radicals

Free radicals and other reactive oxygen species are derived either from normal essential metabolic process in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoke, air pollutants and chemotherapeutic agents. Even over-exercising generates extra free radicals. Free radicals formation occurs continuously in the cells as a consequence of enzymatic and non-enzymatic reactions. Enzymatic reactions, which serve as

source of free radicals, include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis and in the cytochrome p-450 system. Free radicals are also arised in non enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations.[38]

Benefits of free radicals

The biggest source of free radicals are some very good reasons that generally white blood cells use free radicals to destroy bacteria and virus infected cells, these free radicals prevent immediate death from infection. In addition, with the help of other free radicals, the liver's cytochrome (CYP) P-450 enzymes detoxify harmful chemicals, again protecting us from a quick death. Free radicals are also a normal by-product of everday respiration-breathing in which our body use oxygen and generate energy called "bioenergetics" or Oxidative phosphorylation.[39]

Defense systems against free radicals

The human body although continuously produces free radicals, it possess several defense systems, which are constituted of enzymes and radical scavengers. These are "first –line antioxidant defense system" but are not completely efficient because almost all components of living bodies, tissues, cells and genes undergo free radical destructions.[40]

Excess free radical formation can cause tissue damage. However, tissue damage due to free radicals itself cause more free radical generation. This may contribute to a worsening of the injury. It is known that much of the damage to DNA is done by free radical of oxygen, hydrogen peroxide and superoxide. Free radical will produce the damage through oxidative stress which is defined as the state in which the level of toxic reactive oxygen intermediates (ROI) over come the free radicals, which can react with cell membrane leading to local injury and eventual organ dysfunction.

Oxygen derived free radicals such as the superoxide anion and the hydroxyl radical are cytotoxic and promote tissue injury. These radicals damage cellular membrane and cross link

lipids, proteins and nucleic acids. In addition, they cause degradation of hyaluronic acid, the principle component of the epithelial basement membrane and this promote mucosal injury.[41]

Anti oxidant:

The body has a defense system of antioxidants to prevent the free radical damage.

i**)SODs** are metal containing enzymes that depend on bound manganese, copper or zinc for their antioxidant activity. In mammals, the manganese- containing enzymes is most abundant in mitochondria, while the zinc or copper forms predominant in cytoplasm. Interestingly, SODs are inducible enzymes- exposure of bacteria or vertebrate cells to higher concentrations of oxygen results in rapid increases in the concentration of SOD.

ii)Catalase is found in peroxisomes of eukaryotic cells. It degrades hydrogen peroxide to water and oxygen, and hence, finishes the detoxification reaction started by SOD.

iii)Glutathione peroxidase is a group of enzyme, the most abundant of which contain selenium. 'These enzymes, like catalase, degrade hydrogen peroxide. They also reduce organic peroxides to alcohols, providing another route of eliminating toxic oxidants.[42]

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. The principle micronutrient (vitamin) antioxidnants are vitamin E, C, and beta carotene. Additionally, selenium, a trace metal is required for proper function of one's body antioxidant enzyme systems included in this category. The body cannot manufacture these micronutrients so they must be supplied in the diet.

iv)Vitamin E

d-alpha tocopherol , is a fat soluble vitamin present in nuts, seeds, vegetables and fish oils, whole grains (esp. wheat germ oil) fortified cereals and apricots. Currently recommended daily allowance (RDA) is 15 IU and 12 IU per day for men and women respectively.

v)Vitamin C Ascorbic acid is a water soluble vitamin present in citrus fruits and juices, green peppers, cabbage, spinach, broccoli, kale, cantaloupe, kiwi and strawberries. The RDA is 60mg per day. Intake above 2000mg may be associated with adverse side effects in some individuals.

Vitamin E does not work in isolation from other antioxidants; rather it is part of an interlinking set of redox antioxidant cycles which has been termed the "antioxidant network". It is hypothesized that vitamin E acts catalytically, i.e., efficiently reduced from its free radical (chromanoxyl) form, which arises after quenching lipid radicals, to return back to its reduced native state.

Table No:3 showing the distinction between duodenal and gastric ulcers

Feature	**Duodenal ulcer**	**Gastric ulcer**
Incidence	Four times more than gastric ulcers, Age 25-50years, Male : Female:4:1	Less than duodenal ulcers. Age beyond 60years.Male : Female : 3:1
Etiology	Result of *H.Pylori* infection other factors Hyper secretion of acid–pepsin, association with alcoholiccirrhosis, tobacco, hyperparathyroidism,chronic pancreatitis,blood group O, genetic factors.	Gastric colonization with *H.Pylori* a symptomatic but higher chances of development of duodenal ulcer. Disruption of mucus barrier most important factor. Association with gastritis, bile reflux drugs, alcohol, tobacco
Pathogenesis	Mucosal digestion from hyperacidity most significant factor. protective gastric mucus barrier may be damaged	Usually normal to low acid levels hyperacidity if present is due to high serum gastrin. Damage to mucous barrier significant factor.

Mechanism of ulcer healing

Ulcer healing, a genetically programmed repair process, includes inflammation, cell proliferation, re-epithelialization, formation of granulation tissue. Angiogenesis, interaction between various cell, matrix and tissue remodeling, all resulting in scar formation.

Conventional drugs in the treatment of peptic ulcer and its limitations:

Peptic ulcer patients usually are treated with antacids, histamine antagonists, or both; other drugs are added as necessary. Drug regimens that suppress nocturnal acid secretion are found to result in the highest duodenal ulcer healing rates. During therapy typically provides prompt symptomatic relief and promotes ulcer healing within 4-6weeks

Table No:4 Limitation – Treatment of Peptic Ulcer

Drug Type	Selected Side Effects	Comments
Antacids Aluminium hydroxide Calcium carbonate Magnesium hydroxide sodium carbonate	Nausea, headache, weakness, loss of appetite, constipation (aluminium hydroxide) or diarrhoea (magnesium hydroxide)	Used mainly to relieve symptoms, not as a cure
Proton pump inhibitors Lansoprazole Omeprazole Pantoprazole Rabeprazole Esoprazole	Diarrhea, constipation, headache	Usually well tolerated; most effective means of reducing stomach acid
Antibiotics Amoxicillin Clarithromycin Metronidazole Tetracycline	Diarrohea (amoxicillin, clarithromycin, tetracylcline, altered taste, nausea	Effective for treating peptic ulcers caused by the *H. Pylori* infection
Micellaneous Bismuthsubsalicylate, Misoprostol, Sucralfate	Diarrohea,(bismuthsubsalicylate, misorostol),darkening of the tongue and stool (bismuthsubsalicylate)spontaneous abortion (misoprostol), constipation (bismuthsubsalicylate) may reduce effectiveness of other drugs (sucralfate)	Bismuth subsalicylate is Used in combination with antibiotics to cure *H.Pylori* infection

(ref: www.merck.com)

To conclude this anti ulcer concept, globally, a wide range of allopathic drugs have been used in suppressing as well as treating the peptic ulcer diseases. So these drugs are flooded the market which are effectively heals the ulcer.[45]

In spite the these advancement of highly effective, H2 blockers and gastric proton pump inhibitors, have to yet discover an effective anti-ulcer drugs which not only heals peptic ulcers but also prevents their reoccurrence effectively.[46] because, with the new potent anti-ulcer drugs, healing of a peptic ulcer usually achieved within 6 to 8 weeks in most patients.[47] and 89% of gastric ulcer patients experience ulcer reoccurrence with one year of successful healing with conventional anti ulcer therapy.[48] All allopathic drugs causes all sorts of adverse effects, toxic effects and produce drug interaction with other drugs on chronic administration besides their availability at high cost.

Scope and Plan of work

Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently Infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments.

Many indigenous plants have been scientifically tested and found to have medicinal properties that can be used in western style medicine. Such cures are particularly suitable for use in rural communities as inexpensive, sustainable and culturally appropriate alternatives to more expensive conventional treatments. As s result, ethno medicines is being promoted and supported as a way of providing efficacious medicines for people in less developed areas.

Most of the herbal preparations used for gastrointestinal and other conditions have no side effects, or far fewer side effects than conventional medicines. However, scientists working on the study noticed that local communities are progressively losing their traditional knowledge of indigenous plant medicines. In many cases, older generations are no longer transmitting their knowledge and experience to younger ones.

The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Or traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicines. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. . The herbal medicines/traditional medicaments have, therefore, have derived from rich traditions of ancient civilizations and scientific heritage. The World Health Organization also has recognized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for botanical medicines.

The folkloric Ayurvedic informations encourage scientists to do research on herbal plants. The efficacies of the herbal drug preparations reported by tradition practitioners are not scientifically validated. Modern science helps to isolate, characterize and standardize the constituents present in the herbal drug preparations. The combination of traditional and modern knowledge can produce a better activity or efficacy with lesser side effects.

Gastric ulcer is a common disorder where discontinuity in the gastric mucosa is observed.(anoop).Peptic ulcer is the most common disorder of the GIT has multi-factorial cause in its pathophysiology and cannot achieve complete eradication with a single drug (Heamamalini). The conventional treatment of ulcer comprises as regular feeds, adequate rest, antacid, various drugs and avoidance of ulcergenic agents such as coffee, alcohol and tobacco. The drugs used in the treatment of ulcer include receptor blockers, proton pump inhibitors, drugs affecting the mucosal barrier and act on the central nervous system.[128] Even though a wide range of drugs are available for the treatment of ulcer, many of these do not fulfill all the requirements and causes side effects such as arrhythmias, impotence and haematopoietic changes are noted.[129a,b]

Even though the etiology of gastric ulcers is debated, it is accepted that ulcers are caused due to net imbalances in mucosal offensive and defensive factors.[148] Ulcer therapy is now mainly focused on limiting the deleterious effects of offensive acid secretion, but the search for new safer alternative drugs have rekindled the interesting cytoprotective drugs, which protect the gastric mucosa from damaging agents without influencing acid secretion or neutralizing intra gastric acidity.[154] Although few drugs like sucralfate and prostaglandin analogs, i.e misoprostol, are recognized as cytoprotective agents, many natural drugs have been reported to possess anti ulcer activity.[70,105]

Phytochemcial investigation will be a useful tool for the identification and authentification of the plants for industrial and further research purpose. Hence, phyto chemical tests were carried out on the selected plants. Total phenol and flavanol contents of a tested material are related to the antioxidant activity. Antioxidants, which can scavenge free radicals, have an important role in biological systems. Antioxidants are

Emerging as prophylactic and therapeutic .consequently antioxidant activity was evaluated for the potent extracts. As on today, the herbal medicines are flourishing the markets of

advanced countries and the consumer's world over have shown very good interest on the preference for herbal based remedies or formulations.

Until the discovery of carbenoxolon in 1965, there were only limited alternatives for the treatment of peptic ulcers in orthodox medicine, except surgical operation. Hence plant remedies have widespread been used in the treatment this disease since thousands of years. In spite of the recent progress by the invention of H2 receptor antagonists and proton pump inhibitors, peptic ulcer still continues to become the problem of people. However, as a consequence of the progress in chemical and biological methods in the last two decades, increasing number of studies are carried out in order to evaluate the potential of plant remedies and to discover leading molecule for the treatment of peptic ulcer disease.

The present study is undertaken on two selected medicinal plants, namely ***Cyathula prostrata*** and ***Heliotropium indicum*** to evaluate the possible phytoconstituents and their quantification. The herbs have been used for the treatment of gastrointestinal disorders, namely for ulcers in traditional system of medicine. And so we had shown interest in carrying out a systematic phytochemical and pharmacological evaluation of both plants used traditionally for the treatment of ulcers

OBJECTIVES OF THE WORK

To select the plants based on ethno medical uses and preparation of their extracts.

To screen the extracts and identify the phytoconstituents

To estimate the phytosonstituents

To quantify the known phytoconstituent present in the plant

To evaluate the pharmacological activity, the extracts were subjected to anti ulcer evaluation on ulcer induced rat models.

Plan of the work

Materials and Methods

The selected two herbs are widely used in traditional medicines i.e in Ayurveda, Siddha, and Unani systems. The plants namely are *Cyathula prostrata* (Linn) and *Heliotropium indicum* (Linn)

The present study is undertaken to explore the anti ulcer preparation based on their efficacy with emphasis to their mechanism of action. The validation of plant extracts for anti ulcer property is correlated with various biochemical parameters involved therein

I 1. Collection of plant material and authentication

2.Pharmacognostical standards[50].

a) organoleptic character

b) total ash

c) acid insoluble ash

d) water soluble ash

e) sulphated ash

f) loss on drying

3. Preparation of extracts[49] by continuous hot extraction process (Soxhlet apparatus) using solvents of decreasing polarity viz. petroleum ether (60-80˚C) chloroform, ethyl acetate, alcohol and water etc.The extracts were subjected for phytochemical analysis

Phytochemical studies of the extracts

4. Qualitative phytochemicals studies. [51]

5. Behavioral pattern of powdered sample with different reagents. [52]

6. Florescent analysis of different extracts.[53]

7. Quantification of lupeol by HPTLC studies. [54a,b]

8. Estimation of total phenolic content by Folin catechin method.[55]

9. Estimation of total flavonol content.[56]

II Pharmacological Screening

1. Acute toxicity and gross behavioral study.[57]

2. Sub-acute toxicity (Loomis,1996 and Hayes,1994).[58]

The hematological parameters and biochemical parameters observed in the treated animals are compared with normal on the 29th day.

I)Hematological parameters investigated.[59]

Blood sample collected from various group of animals were used to study the following parameters.

a) Hemoglobin (Hb) concentration

b) Red Blood Corpuscles count

c) WBC (Total Leucocytes count-TLC)

d) Differential Leucocytes count (DLC)

II) Biochemical parameters investigated

Serum separated from blood samples collected from various group of animals were used for the analysis of the following biochemical parameters.

a) Serum Aspartate Amino Transferase s(ASAT/GOT)

b) Serum Alanine Amino Transferase (ASAT/GPT)

c) Alkaline Phosphate (ALT)

III)Histopathological Observation

For this studies, the animals were sacrificed on 29th day and autopsy was performed to observe any morphological change in the vital organs like liver, kidney, spleen and stomach. Organs were removed and preserved in 10% buffered formalin solution for histopathological studies.

1.Evaluation of Anti-ulcer activity of *Cyathula prostrata and Heliotropium indicum*

Anti-ulcer activity models

a)Anti secretary evaluation

i) Modified pylorus ligated (shay) rat model. [60]

ii)Aspirin plus pylorus ligated (shay) rat model.[60]

b) Drug induced Gastric Mucosal Damage.

i) Acetic acid induced Gastric mucosal damage.[61]

c) Cysteamine induced duodenal ulcer model .[62]

d) Biochemical evaluation.[60]

The following biochemical parameter are analyzed to correlates the anti-ulcer property

a)Secretion and Enzyme Evaluation[63,64,65,66]

i) Gastric volume

ii) pH

iii) Free & Total acidity

iv)Pepsin

v)Total Proteins

b) Carbohydrate content,[67,68,69,70]

i) Total Hexoses

ii) Hexosamine

iii) Fucose

iv) Sialic Acid

v) Total carbohydrate

c) Sodium & Potassium ion concentration,[71]

d) Gastric Mucosal defensive factors

i) Mucos Barrier.[72]

ii) Non-Protein thiol (NP-SH) group.[73]

e)Anti-oxidant parameters

i) Catalase .[74]

ii) Superoxide dismutase (SOD).[75]

iii)Lipid peroxidation products (LPO).[76]

f) Statistical Analysis

i) One way ANOVA followed by (Graph pad version 5.01)

ii) Turkey's Multiple comparison test

Cyathula prostrata (Linn) Blume[77]

Synonyms : *Achyranthes prostrate* (basionym)

Desmochaeta prostate (L) DC

Pupalia prostrate

Taxonomy : Kingdom : plantae-plants

Sub kingdom : Tracheobionta-Vascular plants

Super division: Spermatophyta-Seed plants

Division : Magnoliophyta-Flowering plants

Class : Magnoliosida-Dicotyledons

Sub class : Caryophyllidae

Order : Caryophyllales

Genus : Cyathula Blume-cyathula

Species : *Cyathula prostrate* (L) Blume-pastureweed

Family : Amaranthaceae - Amaranth family

Vernacular Names:

English : Small prickly chaff-flower plant

Hindi : Lal circita

Kannada : Uttarni

Malayalam : Cerukatalati, Cuvannakatalati

Sanskrit : Raktapamargah

Tamil : Cirukatalati, Civappu nayuruvi

Fig No: 3 Exomorphic features of Cyathula prostrata

Occurrence

Cyathula prostrate is small prickly chaff flower plant growing throughout India, in shady moist localities belonging to the family Amaranthaceae., distributed widely in Kerala, Southern parts of Tamilnadu, U.S.A, and Sri lanka.

Habit: A plant is a small sized weed, reaching height upto 10-20 cm height, much branched slender prostrate or decumbent herbaceous annual or perennial rooting at the nodes and the ascending branches, ending with inflorescence.

Stems and Branches

Stems are violetish red in colour and the leaves are simple, oppsosite, exstipulate, short-petioled, rhomboid or ovate, sub-acute, upto 7cm long and 3.5 cm broad,

Flowers: Flowers are reddish violet or violet in terminal spikes or racemes,

Fruits: Ovoid membranous utricles enclosed in the perinath.

Seed : single, oblong, inverse.

Chemical constituents:

The plant contains phytosterol, tannins, flavonoids, and trace of carbohydrates. The phytosterol namely are ecdysterols, amarasterones. and cyasterone. The whole plant is used in vitiated condition of pitta, cough, branchoitis, ulcers and G.I tract abnormalities.

Cyasterone showed moulting hormone activity in calliphore bioassay. The whole plant is used as medicine and its important formulation using the drug as Surasadi tailam, Aviltoladi bhasmam, Svarnamuktadi gulika and Jatyadi tailam and Ardhavilam kashayam etc.

Ethnomedical information

This plant (roots) is used in abdominal disorders, dyspepsia, ulcers, diarrhea and for many more ailments such as constipation, expectorant in folklore medicine.

Uttula et al studied the weed control in oil palm nursery with different herbicide mixture

Phytoconstituents information[78]

Shah,V.C (1971) isolated ecdysterone from *Cyathula prostrata*

Pharmacological review[79]

Furestieri, et al (1996) reported that the plant extract exhibited the anti inflammatory, analgesic & antipyretic activity in rodents used in African medicine.

Heliotropium indicum (linn)[80]

Synonyms : Indian Turnsole

Tiaridium indicum (L)

Taxonomy Kingdom - Plantae-plants

Sub kingdom – Tracheobionta-vascular plants

Super division- Spermatophyta-seed plants

Division - Magnoliophyta-Flowering plants

Class - Magnoliopsida-Dicotyledons

Sub class - Asteridae

Order - Lamiales

Genus - Heliotropium L -heliotrope

Species - *Heliotropium indicum* L-Indian heliotrope

Family - Boragenaceae-Borage family

Vernacular Names:

English - Indian turnsole

Hindi - Siriyari

Kannada - Celeubaladagidha

Malayalam – Telkkata

Tamil - Telkudhai

Telugu - Koddiki

Fig No:4 Exomorphic features of *Heliotropium indicum*

Occurrence

Heliotropium inducm (Linn) is a small weed, road side plant, widely distributed in both hemispheres. About 16 species of *heliotropium indicum* occurs in India, mostly on road sides and waste places of Tamil Nadu, Kerala and Bihar, also in America and Myamnar.

Distribution

Heliotropium indicum (Linn) is a small size weed. A course, somewhat succulent foetid berbaceous annual commonly found on road side and waste places. 15-60 cm in height with densely hirsute ascending branches. Under favourable conditions, it is distributed in throughout India, native to Asia or perhaps South America. Established in all tropical and subtropical countries.

Morphological charaters

Habit: A plant is a small sized weed, reaching height upto 15-60 cm and somewhat succulent foetid herbaceous annualm with densely hirsute ascending branches, leaves simple alternate or sub opposite, ovate, obtuse, narrowed at base. Pale violet flowers, bristly cymes. Fruits nutles. In clay (black, peaty), wet soil-occupying river banks, intertidal flats, lake edges, wetlands, growing in distributed natural vegetation.

Leaves: Alternate, spiral, simple, petiolate, petiole 16-97 mm long. Leaf blade 74-140 mm long, 36-80 mm wide, undissected, elliptic or ovate or triangular, base tapering (narrow attenuate) or oblique, margins entire or crenate, apex –acuminate or acute or obtuse. Blade with indumentums: indumentums hairy (on upper and lower surfaces), hairs are simple.

Flowers: Arranged inflorescences, in spikes or in cymes: predominantly purple or blue color, pedicle 1 mm long, perinath 2-whorled. Calyx 1.5-3 mm long. Corolla 4-7 mm long, 5 petals, all petals joined. Ovary syncarpous, superior, 4-celled. Ovules 1 per cell.

Styles: 1 simple.

Fruits: Schizocarps, of mericaps, non-fleshy, 3-7 mm long, 2.5-7 mm wide.

Flowring Period : May, July and October.

Chemical Contituents[82] Helitropium indicum (Linn) was reported to contain the chemical constituents as

Leaves contain : B sitosterol, acacic acid lactone, leupiol acetate, ursolic acid, Oleo-Nollic acid, dihydroxy sterol and triterpeniol (14 alpha methyl-5 al Pha-cholest-9,24 diene-3beta 7, alpha diol, 14 alpha methyl-24-, methylene, 5 alpha cholestra 9, 24-diene, 3 alpha, 7alpha dial, Acaci acid lactone).

Whole Plant Pyrrolyzidine alkaloids, (such as like indicine, indicinine, acetylindicine, indicine-N-oxide, heliotrine, lasiocarpine, echinatine, supinine and heleurine have been reported from the aerial parts of the full grown plants) Flavaonoids, sterol, geranyl aromatic derivatives. Acacid acid lactone, leupiol acetate, ursolic acid, oleonollic acid, dihydroxy sterol and triterpeniol.

Lietrature survey-*Heliotropium indicum*

Ehtanomedical information[81]

In Indian medicine the leaves are used in fever, urticaria, ulcer, wounds, localized inflammation, gonorrhea, ringworm, rheumatism.

Pharmacognostical studies [83]

Dattagupta et al., studied the pharmacognostic features of the leaf of *H.indiucm.*

Phytochemical review [85]

Pantey, et al l (1982) reported that heliotrine alkaloid was isolated form *H.indicum* seeds and in subsequent year, revealed the presence of heliotric acid a& lasiocarpine from seeds and leaves respectively.

Wirz, et al explained about the organ specific distribution of pyrrolidine alkaloids and sites of their formation in *H.indicum* in 1993.

Rajagam observed the presence of phenol and simple phenolic acids in fresh leaves than in stem & roots of *H.indicum*

Srinivas,et al exhibited that the leaves contains two new dihydroxy sterol and a triterpenoid compound in 2002.

Deraniyagala et al (2002) isolated Rosemarinic acid and methyl rosemarinate were from *H.indicum*

Aniruddha et al has studied the property of enhance production in callus culture of *H.indiucm*[90]

Pharmacological review

Kugelman, et al (1976) evolved the antitumor principle of *H.indicum* (Indicine-N-oxide)[84]

Srinivas et al (2000) investigated the anti-inflammatory property of *H.indicum* in Carragenan induced hindpaw oedema & cotton pellet induced granuloma in rats. [88]

Bournel et al.,(1995) and Reddy, et,al. (2002) experimented the ethanolic extract of *H.indicum* was evaluated its wound healing acivitiy in rats[86],[87]

Rao, et al (2002)studied the anti microbial acitity of *H.indicum.* [89]

Akinlolu Abdulazeez Adellaja (2006) studied gastroprotective effects of *H.indium* on gastric ulcerated mucosa.[91]

Table no: 5 CHEMICALS used FOR ESTIMATION OF ANTI ULCER PARAMETERS

Chemical	preparation
0.1N sodium hydroxide (NaOH)	0.04gm of Sodium hydroxide in 100ml of dist.water
Stock solution of Sodium chloride (NaCl)	2.542 gm of Sodium chloride in 1 liter of dist.water
Stock solution of Potassium chloride(KCl)	1.909 gm Potassium chloride in 1 liter of dist.water
90% Alcohol	Absolute alcohol is 99.9%, to produce 90%alcohol, 90 ml of absolute alcohol is mixed with 10ml of dist.water
0.1N Sodium hydroxide (NaOH)	0.4 gm of Sodium hydroxide in 100 ml of dist.water
6N Hydrochloric acid (HCl)	51 ml of conc.HCl in 100 ml of dist.water
5N Sodium hydroxide (NaOH)	20 gm of Sodium hydroxide in 100 ml of dist.water
0.1N Sulphuric acid (H_2SO_4)	0.54 ml of conc. Sulphuric acid in 100 ml of dist.water
8 % w/v Sucrose	8 gm of sucrose in 100 ml of dist.water
0.2%w/v Sodium chloride (NaCl)	200 mg of NaCl in 100 ml of dist.water
0.1% Alcian blue 8 GX	100mg of alcian blue 8 GX in 100 ml of dist.water
0.16 M of Sucrose	5.4768 gm of sucrose in 100 ml of dist.water
0.5 M Magnesium chloride ($MgCl_2$)	10.165 gm of $MgCl_2$ in 100 ml of dist.water
0.02 M Ethylene diamine tetra acetic acid	0.744 gm of EDTA in 100 ml of dist.water
50 % Trichloro acetic acid (TCA)	25 gm of TCA in 50 ml of dist.water
Tris Buffer	3.6 gm of tris buffer in 50 ml of dist.water and pH (8.8)(HCl). Make up to 100 ml with dist.water
5,5 Dithio-bis-(2-nitrobenzoic acid)	39.6 gm of 5.5 di thio-bis- (2-nitrobenzoic acid) in 100 ml of dist.water
Phosphate buffer	Dissolve 0.05 gm of anhydrous di sodium hydrogen phosphate and 0.0301 gm of potassium di hydrogen phosphate in dist. Water to produce 100 ml
Hydrogen peroxide solution (H_2O_2)	0.9 gm NaCl+2.92 gm EDTA in 100 ml of dist.water and add 1ml of H_2O_2
Sodium carbonate buffer (Na_2CO_3)	0.53 gm of Na_2CO_3 in 100 ml of dist.water 0.42 gm of $NaHCO_3$ in 100 ml of dist.water and pH adjusted to 10.2
Adrenaline solution	30 mg of Adrenaline in 10 ml Dist.water and pH adjusted by one drop of HCl
0.375% Thiobarbituric acid	0.375 gm of TBA in 100 ml of dist.water
Trichloacetic acid (TCA)	15 gm of TCA in 50 ml of dist.water

EXPERIMENTAL PART

The selected two medicinal plants are widely used in traditional medicine like Ayurveda, Siddha and Unani. The plants are namely Cyathula *prostrata* and *Heliotropium indicum*.

1 Collection of plant material and authentification

The plant materials were collected from Kottakkal area, Kerala which were properly identified and authenticated by comparing with the voucher specimen available at the Survey of Medicinal Plants, Kottakkal, Kerala and also they were verified by Dr. Rajan, Botanist, Botanical Survey of India, Emarald, Ootagamund. A voucher specimen is deposited in the department of pharmacognosy for further reference

2. Preparation of extracts[46] by cold maceration process using solvents of decreasing polarity viz, petroleum ether (60-80˚ C), chloroform, ethyl acetate, alcohol and water etc. The extracts were subjected for phytochemical analysis.

The plant materials were dried under the shade, mechanically reduced to a coarse powder and the powder was used for extraction by cold maceration process using hydro alcohol (50:50 alcohol and water) for 72 hours then, it was filtered and concentrated under reduced pressure. The dried extracts were used and stored in vacuum desiccators for biological evaluation.

Percentage yield

The percentage yield of the ethanolic extract was calculated using the formula

$$\% \text{ yield} = \frac{\text{Weight of the extract}}{\text{Weight of the crude drug}} \times 100$$

SUCCESSIVE EXTRACTION;

The various solvents were distilled, purified and used for extraction

Petroleum ether extraction

The shade dried material (100g) was extracted with previously purified petroleum ether (60-80˚C) by cold maceration process for 7 days. After completion of the extraction, the solvent was removed under reduced pressure and the extractive value was determined.

Chloroform extract

The marc left after petroleum ether extraction was dried and extracted with chloroform by cold maceration process for 7 days. After completion of extraction, the solvent was removed under reduced pressure and the extractive value was determined.

Ethyl acetate extract

The marc left after chloroform extraction was dried and extracted with ethyl acetate by cold maceration process for 7 days. After completion of extraction the solvent was removed under reduced pressure and the extractive value was determined

Ethanol (95%) extract

The marc left after ethyl acetate extraction was dried and extracted with ethanol (95%) by cold maceration process for 7 days. After completion of extraction, the solvent was removed under reduced pressure and the extractive value was determined.

Aqueous extraction

The marc left after ethanol extraction was dried and extracted with chloroform water for 7 days by cold maceration process. After completion of extraction, the solvent was removed under reduced pressure and the extractive value was determined.

3. Pharmacognostical standards[47]

a) Organoleptic character

b) Total ash

c) Acid insoluble ash

d) Water soluble ash

e) Sulphated ash

f) Loss on drying

f) Acute toxicity and gross behavioral study[74]

g) Selection and preparation of dose for pharmacological screening

Based on the results of acute toxicity and gross behavioral study, the maximum tolerated dose was found to be 2000mg/kg with no significant behavioral changes and toxicity caused in animals' Consequently, the dose was calculated 1/10, 1/5 for the experimental evaluation.

The hydroethanolic extracts of testing plants were suspended in 0.3% CMC suspension, two dose levels at 200 mg and 400 mg/kg body weight of the animals selected for screening the anti-ulcer activity.

3.1 Pharmacognostical standards

a) Organoleptic characters:

This is one the physical character to identify the crude drug on its inherent properties such as colour, odour and taste in nature. The crude drugs were observed physically on the basis of their properties adhered to them.

b) Physio - chemical constants:

i) Total ash value:

About 3 gm of the powdered was accurately weighed in a silica crucible which was previously ignited and weighed. The powdered drug was separated as a fine even layer on the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450˚C to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get a constant weight. The percentage of a total ash was calculated with reference to the air dried drugs.

ii) Acid insoluble ash value:

The ash described in the determination of total ash was boiled with 25 ml of 2N hydrochloric acid for 5 minutes. The insoluble ash was collected on an ash less filter
paper and washed with hot water. The insoluble ash was transferred to a pre weighed silica crucible, ignited, cooled and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

iii) Water soluble ash value:

The ash described in the determination of total ash was boiled with 25 ml of water for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred to a pre weighed silica crucible, ignited, for 15 minutes at a temperature not exceeding 450˚C cooled and weighed. The procedure was repeated to get a constant weight. The percentage of water soluble ash was calculated with reference to the air dried substance

iv) Loss on drying :

Loss on drying is the loss in weight in % determined by means of the procedure given below. It determine the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (desiccators or hot air oven). If the sample is in the forms of large crystals, then reduced the size by quickly crushing to a powder.

Procedure:

About 1.5 gm of powder drug was weighed accurately in a tarred porcelain dish, which was previously dried at 105˚C in a hot air oven to constant weight and then weighed. From the difference in weight, the percentage of loss on drying with reference to the air dried substance was calculated (Kokata et al 1995)

v) Sulphated ash value:

A silica crucible was heated to redness for about 10 min allowed to cool in a desiccator and weighed. About 2 gm of the powdered drug was accurately weighed to the crucible and ignited gently at first until the drug was thoroughly charred. The crucible was cooled and the residue was moistened with 1 ml of sulphuric acid heated gently until white fumes were no longer evolved and ignited at 800˚C ±25 ˚C until the carbon particles were disappeared. The ignition was allowed to cool, few drops of sulphuric acid was added and again heated. The ignition was carried out to a constant weight. The percentage of sulphuric ash was calculated with reference to the air dried substance and was recorded.

vi) Extractive value:

Extractive values of crude drugs are useful for their evaluation especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

Water soluble extractive values:

5 gm of the powdered drug was macerated with 100ml water in a closed flask for 24 h, shaking frequently during the first 6 h. Thereafter it was filtered through a filter paper, taking precaution against excessive loss of water. 25ml of the filtrate was then evaporated to dryness at 105˚C and weighed. The percentage (w/w) of water soluble extract was then calculated with reference to the air dried substance and the results were recorded (Table No :)

Alcohol soluble extractive value:

5 gm of the air dried coarse powder of the whole plant materials was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 h and allowed to stand for 18 h. Therefore, it was filtered rapidly taking precautions against the loss

of solvent. 25 ml of the filtrate was evaporated to dryness in a tare flat bottomed shallow dish, dried at 45˚C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air dried substance and were recorded (table no :)

vii) Foaming index:

Foaming index is mainly performed to determine the saponin content present in an aqueous decoction of plant material.

Determination of foaming index:

Weigh accurately about 1gm of coarsely powdered drug and transferred to 500 ml conical flask containing 100 ml of boiling water. and maintained at moderate boiling at 80˚C -90 ˚C for about 30 minutes. Then it was cooled and filtered to make up to volume 100ml. Cleaned 10 stoppered test tube of uniform dimensions were taken and marked from 1 to 10. Measured and transferred the successive portion of 1, 2, 3, ml up to 10ml and adjust the volume of the liquid in each tube with water to 10 ml. Stoppard uniformly and allowed to stand for 15 minutes and measure the height of the foam. If the height of the foam in every tube is less than 1cm, the foaming index is less than 100 is considered as not significant. Here, the foam was more than 1cm height after the dilution of plant material in the test tube .The corresponding number of the test tube was the index sought, if the height of the foam in every tube is more than 1cm, the foaming index is more than 1000. In this case, 10 ml of the first decoction of the plant material is measured and transferred to a 100 ml volumetric flask (V2) and volume is made to 100ml and followed the same procedure.

Foaming index = 1000/a (V1)

Foaming index = 1000/a (V2)

Where a=volume (ml) of decoction used for preparing the dilution in the tube where exactly 1cm or more foam was observed

4.1 Qualitative phytochemical screening

The following tests were carried out on the herbal extracts to detect various phytoconstituents present in them.

4.1.1Detection of alkaloids

About 50mg of solvent free extract was stirred with little quantity of dil HCl and filtered. The filtrate was tested carefully with various chemical reagents

S.No	Test details	Observation	Inference
1	Mayer's test: Few ml of filterate + 2 drops of Mayer's reagent along the sides of tube	White or creamy precipitate	Presence of alkaloids
2	Wagner's test Few ml of filterate + few drops of Wagner'reagent along the sides of tube	Reddish brown precipitate	Presence of alkaloids
3	Hager's test: Few ml of filtrate + 2 ml of Hager's reagent	Prominent yellow precipitate	Presence of alkaloids
4	Dragondroff's test Few ml of filtrate + 2 ml of the reagent	Prominent reddish brown precipitate	Presence of alkaloids

4.1.2. Detection of carbohydrates

About 100 mg of the extract was dissolved in 5 ml of distilled water and filtered. The filterat was subjected to the following tests.

S.No	Test details	Observation	Inference
1	Molish'test 2 ml of filtrate + 2 drops of alcoholic soln of -α- naphthol. The mixture was shaken well and 1 ml of conc.H_2S0_4 added slowly along the sides of the tube, cooling the tube in ice water and allowed to stand	A violet ring was developed at the junction of two layers	Presence of carbohydrates
2	Fehling' test: 1 ml of filtrate +1 ml each of Fehling's soln A &B and heated on	Formation of red precipitate	Presence of sugar

	water bath.		
3	Barfoed's test 1 ml of filtrate +1 ml Barfoed's reagent and heated on water bath for 2 minutes	Formation of red precipitate	Presence of sugar
4	Benedict's test 0.5 ml of filtrate +0.5 ml of benedict's reagent and heated on water bath for 2 minutes	A characteristics colored precipitate	Presence of sugar

4.1.3 Detection of glycosidess

About 50 mg of the extract was hydrolyzed with conc.HCl for 2 hours on a water bath and filtered. The hydrolyzed extract was subjected to the following test

S.No	Test details	Observation	Inference
1	Bontrager's test 2ml of filtrate hydrolysate +3 ml of chloroform and shaken. The chloroform layer was separated and add 10% NH_3 soln to it.	Formation of pink color	Presence of glycosides
2	Legal 's test About 50 mg extract dissolved in pyridine. Sodium nitropruside soln was added and made alkaline using 10% NaOH soln	Characteristic pink color	Presence of glycosides

4.1.4 Detection of Saponins

Foam or broth test:

A small quantity of extract was diluted with distilled water to 20 ml, The suspension was shaken in a graduated cylinder for 15 min. A 2 cm layer of foam or froth which is stable for 10 min. indicates the presence of saponins.

4.1.5 Detection of proteins and amino acids

About 100 mg of extract was dissolved in 10 ml of distilled water and filtered through whatman no 1 filter paper and the filtrate was subjected to tests for proteins and amino acids.

S.No:	Test details	Observation	Inference
1	Millons's test: Add 2 ml of the filterate + few drops of Millons's reagent.	White precipitate	Presence of proteins
2	Biuret test: 2ml of filtrate +1ml of 2% $CuS0_4$+1ml of 95% ethanol+ excess of KOH pellets.	Pink color in the ethanolic layer	Presence of proteins
3	Ninhydrin test: 2 drops of ninhydrin soln +2 ml of aqueous filterate	Characteristic purple color	Presence of amino acids

4.1.6 Detection of Phytosterols:

Libermann Burchard's test

The extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1ml of conc.H_2SO_4 was added along the sides of the test tube. Red, pink or violet at the junction of the liquids indicates the presence of steroids/triterpenoidds and their glycosides.

4.1.7Detection of phenolic compounds and tannins

S.NO	Test details	Observation	Inference
1	Ferric chloride test: About 50 mg extract dissolved in dist. water + few drops of neutral $FeCl_3$ soln.	Formation of the green or violet color	Presence of phenolic compounds
2	Gelatin test: A little quantity of extract dissolved in dist.water +2 ml of 1% gelatin soln. containing 10%	Development of white precipitate	Presence of phenolic compounds

	NaCl		
3	Lead acetate test: A small quantity of the extract dissolved in dist water 3 ml of 10% lead aceate soln	A bulk white precipitate	Presence of phenolic compounds
4	Alkaline reagent test: An aqu. soln of extract + 10% NH_4OH	Yellow fluorescence	Presence of flavanoids
5	Shinodo test or Mg-HCl reduction test A little quantity of extract dissolved in alcohol + few fragments of magnesium turnings and conc.HCl dropwise	Pink or crimson red color	Presence of flavanol glycosides is inferred
6	50 mg of extract + $FeCl_3$ soln	Dark green color	Presence of phenolic compounds confirmed

4.2.1Fluorescence analysis (Chase, 1949)

For fluorescence studies, the powered drug and various extracts were studied under both the day light and UV light , at wave length of 254 nm and 365 nm. The observations were srecorded

4.3.Total phenolic content (TPC) by Folin-Ciocalteu (FC) method

Chemicals and regents

Folin-Ciocalteu Reagent: Folin-Ciocalteu was diluted (1:10) with distilled water and

sodium carbonbate: 202.5 g of sodium carbonate ($Na_2C0_3.10H_2O$) was dissolved in 1litre of distilled water and used.

Methanol: Distilled

Preparation ot Test Solutions

10 mg of the extracts was dissolved in 10 ml of methanol to get 1 mg/ml solution. These solutions were diluted with methanol to obtain lower dilutions

Preparation of standard solution

Gallic acid: 100 mg of Gallic acid monohydrate was dissolved in 100 ml of distilled water to get 1 mg/ml solution. It was serially diluted with distilled water to obtain solution ranging from 25 mg/ml

Procedure

400 µg/ml of the extracts(1 mg/ml to 0.5mg/ml) was separately mixed with 2ml of Folin-Ciocaltew reagent and 1.6 ml of sodium carbonate. After shaking, it was kept for 2 hours reaction time. The absorbance was measured at 750 nm (Shimadzu UV-160A Spectrophotometer, Shimadzu Corporation, Japan). Using gallic acid monohydrate, standard curve was prepared and linearity was obtained in the range of 2.5 to 25 µg/ml. From the standard curve the total phenol content of extracts was obtained. The total phenol content was expressed as Gallic acid equivalent in mg/g or w/w of the extracts.

4.3.1Estimation of total flavonol content

Flavonol are capable of forming complexes with metal ions and act as antioxidants. The flavonol content was measured using a calorimetric assay. (Woisky and Salatino, 1998)

Preparation of test and standard solutions

The plant extracts (50 mg each) were dissolved separately in 50 ml of methanol. These solutions were serially diluted with methanol to obtain lower dilution. Rutin (5mg) was dissolved in 50 ml of methanol. It was serially diluted with methanol to obtain lower dilution.

Procedure

A known volume (0.5ml) of the extract was mixed with 1.5 ml methanol and 0.1ml of 1M potassium acetate. After 5 min, 0.1ml of 10% aluminum chloride was added and finally it was diluted with 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415nm in spectrophotometer. The total flavonol content was expressed as rutin equivalent in mg/g of the extract.

4.3.2Quantification of lupeol by HPTLC method

Estimation of lupeol content in Heliotropium indicum

The content of lupeol was estimated in different extracts of *Heliotropium indicum* such as pet.ether, ethyl acetate, chloroform, alcohol and aqueous extracts respectively. The sample concentrations were made, which represents 10 mg/ml and the standard was 1 mg/ml used for the lupeol estimation by HPTLC

HPTLC fingerprint profile

The high performance thin layer chromatography (HPTLC) finger print profile of the pet ether, ethyl acetate, chloroform, alcohol and aqueous extracts of *Heliotropium indicum* were carried out using pre-coated silica gel plates (0.2mm, Merck 60 F254 and 60 G, Germany) as the stationary phase, 100 mg of each extract was reconstituted separately in 10 ml of ethanol and used for the HPTLC fingerprint analysis. It was spotted as a band of single concentration using a Camag Linomat IV applicator (CAMAG, Switzerland).

The plates were eluted with their respective mobile phases in CAMAG twin trough chambers. The chambers were saturated with the mobile phase for a period of 45 minutes before elution. Plates were then densitometrically scanned with CAMAG TLC scanner III using the Wincats software at multi wavelengths either under UV light using deuterium lamp or tungsten lamp after derivatisation. Photodocumentation was carried out using a Linomat Reprostar unit under UV light at 254 nm and 365 nm wave length.

5.Animals

Healthy albino wister strain rats of either sex weighing 180-220 g, were used from the animal house, J.S.S.college of Pharmacy, Ootacamund, Tamilnadu, India. The animal house was well maintained i.e well ventilated and animals had 12 ± 1 h day and night schedule. The animals were housed in large spacious hygienic cages during the course of the experimental period and room temperature was maintained at 25 ± 1ºC. The animals were fed with standard rat feed and water ad libitum. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (approvalno: JSSCOP/IAE/Ph.D/Ph.Cology/02/2007-2008

5.1 Selection and preparation of dose for pharmacological screening

Based on the results of acute toxicity and gross behavioral study, the maximum tolerated dose was found to be 2000mg/kg with no significant behavioral changes and toxicity caused in animals. Consequently, the dose was calculated 1/10, 1/5 for the experimental evaluation.

The hydroethanolic extracts of testing plants were suspended in 0.3% CMC suspension, two dose levels at 200 mg and 400 mg/kg body weight of the animals selected for screening the anti-ulcer activity.

Pharmacological studies

Toxicological study

Acute Toxicity study was carried out on 14 days experiment by administration of one single dose to animals orally and observed the toxicity behaviors in animals

Number of animals required: 12 rats

Number of group : 4 groups (3 animals each group)

Preparation of dose:

The hydroethanolic extract of *Cyathula prostrate* and *Heliotropium indicum* were suspended in 0.3% CMC in water to prepare a dose level of 2000 mg/kg , administered orally , with respect to the animal weight.

Procedure:

The procedure was divided into two phases, Phase I (observation made on first day), and Phase II (observed the animals next 14 days). Four set of healthy rats (each set of 3 rats) were used for the experiment. The animals were divided and fasted for 18 h deprived from food, water withdrawn before 4 hours of the dosing, body weights were noted before and after dosing with the extract (s) 2000 mg/kg orally. The animals were observed individually for the first 4 hour to show clinical symptoms, any change in behavioural or mortality and again the body weight were recorded after 6 h post dosing.

From the next day onwards, each day an hour the behavioural change, clinical symptoms or mortality was observed in the same animals for next 14 days and animal body weights were recorded on 8^{th} and 14^{th} day. The same procedure was repeated with another set of animals to nullify the errors.

5.1.2 Selection and preparation of dose for pharmacological screening

Based on the results of acute toxicity and gross behavioral study, the maximum tolerated dose was found to be 2000mg/kg with no significant behavioral changes and toxicity caused in animals. Consequently, the dose was calculated 1/10, 1/5 for the experimental evaluation.

The hydroethanolic extracts of testing plants were suspended in 0.3% CMC suspension, two dose levels at 200 mg and 400 mg/kg body weight of the animals selected for screening the anti-ulcer activity.

Selection and preparation of dose for pharmacological screening:

The hydroethanolic extracts of the selected plants , selected the dose 1/10 , and 1/5th of toxicity dose level were suspended in 0.3 % CMC in water to represent two dose level such as 200 mg/kg and 400 mg/kg body weight of the animals.

6. Evaluation of extracts for anti ulcer property

6.1Anti-secretary model

i) Modified Pylorus ligated (Shay) Rat Model: (Anoop et.al., 2003).

The adult albino wister rats of either sex weighing about 180-250 g were selected and divided into seven groups of six animals in each group and placed in cages with grating floor to avoid coprophagy and fasted for 48 hours, allowing free access of water

Group I: animals treated with solvent control (0.3%CMC in water, orally-1ml/kg)

Group II: animals treated with Positive control –Ranitidine 27 mg/kg (suspended in 0.3% CMC)orally

Group III: animals treated with Positive control-Omeprazole 2 mg/kg (suspended in 0.3% CMC) orally

Group IV: animals treated with Test extract- CP 200 mg/kg (suspended in 0.3% CMC) orally

Group V : animals treated with Test extract- CP 400 mg/kg (suspended in 0.3%CMC) orally

Group VI: animals treated with Test extract- HI 200 mg/kg (suspended in 0.3%CMC)

orally

Group VII: animals treated with Test extract – HI 400 mg/kg (suspended in0.3%CMC)

orally

The animals were anaesthetized under ketamine and xylazine anaesthesia, (Jim gourdon, 2002) the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall closed by uninterrupted sutures.

The test extracts were administered twice daily for 3 days and standard drugs were administered once daily orally for 3 days prior to and one hour before to pyloric ligation. The animals were deprived of both food and water during the post-operative period. The animals were sacrificed using excess ether anesthesia, 4 h after the ligation.

The stomach was excised carefully, keeping the oesophagus closed, opened along the greater curvature and collected the gastric juice, centrifuged and then, the gastric juice was subjected to various biochemical analyses. The mucosa was flushed with saline and the stomach pinned on a frog board.

The ulcer index was calculated according to the method (Anoop,et.al.,2003), the lesion were counted with the aid of hand lens (10x) and each given a severity rating as follows:

Ulcer score	Description
0	Normal
1	Scattered haemorragic spots
2	Deeper haemorragicspots and some ulcers
3	Haemorragic spots and ulcers
4	Perforation

The ulcer score was divided by a factor of 10 to get the ulcer index

The gastric juice thus collected was centrifuged. The volume and P^H was recorded and subjected to bio-chemical estimations like free acidity, total acidity, sodium and potassium concentration, total proteins, total hexoses, hexosamine, fucose, pepsin activity, then total carbohydrate content and finally carbohydrate/protein ratio of the gastric juice.

6.1.2 Aspirin plus ligated (Shay) Rat Model: (Anoop et.al., 2003).

The adult albino wister rats of either sex weighing about 180-250 g were selected and divided into seven groups of six animals in each group and placed in cages with grating floor to avoid coprophagy and fasted for 48 hours, allowing free access of water

Group I : animals treated with solvent control (0.3%CMC in water, orally-1ml/kg)
Group II : animals treated with Positive control –Ranitidine 27 mg/kg (suspended in 0.3% CMC)orally
Group III : animals treated with positive control-Omeprazole 2 mg/kg (suspended in 0.3% CMC) orally
Group IV : animals treated with test extract- CP 200mg/kg (suspended in 0.3% CMC) orally
Group V : animals treated with test extract- CP 400mg/kg (suspended in 0.3%CMC) Orally

Group VI : animals treated with test extract- HI 200mg/kg (suspended in 0.3%CMC) orally
Group VII:animals treated with test extract – HI 400mg/kg (suspended in0.3%CMC) orally

The animals were anaesthetized under ketamine and xylazine anaesthesia, (Jim gourdon, 2002), the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall closed by uninterrupted sutures.

The test extracts were administered twice daily for 3 days and standard drugs were administered once daily orally for 3 days prior to and one hour before the administration of aspirin. The aspirin was administrated at a dose of 200mg/kg orally in a suspension prepared in 1% CMC with water, one hour prior to pyloric ligation. (Time interval between reference drugs and aspirin was maintained one hour). The animals were deprived of both food and

water during the post-operative period. The animals were sacrificed using excess ether anesthesia, after 4 h after the ligation.

The stomach was excised carefully, keeping the esophagus closed, opened along the greater curvature and collected the gastric juice, centrifuged and then, the gastric juice was subjected to various biochemical analyses. The mucosa was flushed with saline and the stomach pinned on a frog board.

The ulcer index was calculated according to the method of (Anoop,et.al.,2003) the lesion were counted with the aid of hand lens (10x) and each given a severity rating as follows:

Ulcer score	Description
0	Normal
1	Scattered haemorragic spots
2	Deeper haemorragicspots and some ulcers
3	Haemorragic spots and ulcers
4	Perforation

The ulcer score was divided by a factor of 10 to get the ulcer index. The percentage of ulcer protection was calculated according to the standard formula.

$$\frac{\text{Ulcer index in -control-Ulcer index in test}}{\text{Ulcer index in control}} \times 100$$

The gastric juice thus collected was centrifuged. The volume and pH was recorded and subjected to bio-chemical estimations like free acidity, total acidity, sodium and potassium concentration, total proteins, total hexoses, hexosamine, fucose, pepsin activity, total carbohydrate content and carbohydrate/protein ratio of the gastric juice were calculated.

6.1.3Bio-chemicals estimations in gastric juice

The various biochemical parameters like carbohydrate content viz. fucose, hexosamine, total hexoses and total carbohydrates were evaluated. Secretions and enzymes viz.gastric volume, pH, free and total acidity, total protein and pepsin were evaluated.

Gastric volume

This was measured after centrifuging the gastric fluid, allowed to stand, decaned and poured into the measuring cylinder of graduation 0.01ml.

Determination of pH

The pH of the gastric juice was measured using the pH meter (Cyberscan, India)

Determination of free acidity and total acidity

1ml of gastric juice was pipetted into a 100 ml conical flask. Added 2 or 3 drops of topfer' s reagent and titrated with 0.01N sodium hydroxide (NaOH-which was previously standardized with 0.01 N oxalic acid) until all traces of the red colour disappears and the colour of the solution was yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Then 2 or 3 drops of phenolphthalein was added and titration was continued until a definite red tinge reappears. Again the total volume of alkali added was noted. This volume corresponds to total acidity.

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Actual Normality of NaOH} \times 100}{0.1} \text{ meq/l/100g}$$

Estimation of total proteins

The dissolved protein in gastric juice was estimated in the alcoholic precipitate obtained by adding 90% alcohol with gastric juice in 9:1 ratio. Then 0.1ml of alcoholic precipitate of gastric juice was dissolved in 1ml of 0.1N NaoH, and from this 0.05 ml was taken in another test tube, to this 4 ml of alkaline mixture was added and kept for 10 min then 0.4 ml phenol reagent was added and again 10 min was allowed for colour development. Reading was taken against blank prepared with distilled water at 610 nm in Hitachi spectrophotometer. The protein content calculated from standard curve prepared with bovine albumin and was expressed in term of µg/sml of gastric juice.

Estimation of pepsin

For each determination placed four tubes (1) and (2) containing 5 ml of substrate, (3) and (4) containing 10 ml of trichloroacetic acid. The gastric juice was mixed with an equal volume of HCl at PH 2.1 warmed to 37˚ C and added 1ml of mixture to the tubes (1) and (4). Incubated for 15 minutes , mixed tube (1) with tube (3). Allowed to stand for 4 min. (1) + (3) gives test and (2) + (4) gives blank. Filtered 25 min after the beginning of the filtration, 2 ml of filtrate was pipette into 10 ml of sodium hydroxide. Mixed by gentle rotation. After 30 min, the intensity of colour was measured at 680 nm in Hitachi spectrophotometer. The difference between test and blank gives a measure of peptic activity. As standard mixed 2ml of freshly prepared phenol solution containing 50 µg/ml /ml with 10 ml of sodium hydroxide and 1ml of phenol reagent was added. After 5 to 10 min, the colour intensity was measured at 680 nm

Estimation of total carbohydrates:

The dissolved mucosubstances in gastric juice were estimated in the alcoholic precipitate obtained by adding 1ml of gastric juice to 9 ml of 90 % of alcohol and the mixture was kept for 10 min and the supernatant was discarded. The precipitate separated was dissolved in 0.5 ml of 0.1 N sodium hydroxide. To this 1.8 ml of 6 N HCl was added. This mixture was hydrolyzed in the boiling water bath for 2 hr. The hydrolysate was neutralized by 5 N sodium hydroxide using phenolphthalein as indicator and the volume was made up to 4.5 ml with distilled water and used for the estimation of hexoamine, hexoses,fucose and sialic acid as described below

Estimation of hexoamine:

0.5 ml of the hydrolysate fraction was taken. To this 0.5 ml of acetyl-acetone reagent was added. The mixture was heated in boiling water bath for 20 min. Cooled under running tap water. 1.5 ml of 90 % alcohol was then added followed by an addition of 0.5 ml of Ehrlich"s reagent. The reaction was allowed for 30 min. The colour intensity was measured in Hitachi spectrophotometer at 530 nm against the blank prepared by using water instead of hydorlysate. Hexosamine content of the sample was determined from the standard curve prepared by using D (+) glucosamine hydrochloride and concentration has been expressed in µg /ml of gastric juice.

Estimation of total hexoses:

To 0.4 ml of hydrolysate, 3.4 ml of orcinol reagent was added. The mixture was then heated in the boiling water bath for 15 min. This was then cooled under running tap water and intensity of the colour was read in Hitachi-spectrophotometer at 540 nm against the blank by using distilled water instead of hydrolysate. Total hexoses content was determined from the standard curve of D (+)-galactose-mannose and has been expressed in µg /ml of gastric juice.

Estimation of Fucose

In this method, three test tubes were taken. In one tube 0.4 ml of distilled wter was taken to serve as control and in each of the other two tubes 0.4 ml of hydrolysate were taken. To all three tubes 1.8 ml of sulphuric acid:water (6:1) was added by keeping the tubes in ice cold water bath to prevent breakage due to strong exothermic reaction. This mixture was then heated in boiling water bath for exactly 3 min. The tubes were then taken out and cooled. To the blank and to one of the hydrolysate containing tubes (unknown) 0.1 ml of cysteine reagent was added while cysteine reagent was not added to the last test tube containing the hydrolysate (unknown blank) . It was then allowed for 90 min to complete the reaction. The reading was taken in Hitachi-spectrophotometer at 396 and 430 nm setting the zero with the distilled water. The optical density for the fucose in the hydrolysate was calculated from the differences in the reading obtained at 396 and 430 nm and subtracting the values without cysteine. This was read against standard curve prepared with D (+) fucose. The fucose content was expressed in terms of µg/ml of gastric juice.

$$\text{True optical density} = \frac{(OD396 - OD430)\ \text{unknown} - (OD396 - OD430)\ \text{unknown blank}}{(OD396 - OD430)\ \text{water blank}}$$

Estimation of sialic acid

To 0.5 ml of the hydrolysate in 0.1 N H_2S0_4, 0.2 ml of sodium periodiate was added and mixed thoroughly by shaking. A time of 20 min was allowed to elapse before addition of 1 ml of sodium arsenate solution to this mixture. The brown color produced was disappeared after shaking. Then 3 ml of thiobarbituric acid was added and the mixture was heated in boiling water bath for **15** min. After cooling the tubes, 4.5 ml of cyclohexanone was added and thorough shaking was done for 15 sec till all the colour was taken up by the cyclohexanone

supernatant. The mixture was centrifuged to get a clear pink layer of cyclohexanone. This supernatant was pipetted out and the intensity of colour was measured in Hitachi- spectro phtometer at 550 nm. The sialic acid content of the sample was determined from the standard curve of sialic acid and has been expressed in terms of µg/ml of gastric juice.

Estimation of sodium and potassium ion concentration

This was carried out using flame photometer. Stock solution was prepared. Sodium stock solution was prepared by dissolving 2.542 g NaCl in 1L of distilled water. It contains 1 mg Na per ml Stock solution was diluted to give four solutions containing 10, 5, 2.5, 1 ppm of sodium ions. Then Potassium stock solution was prepared by dissolving 1.909 g KCl in 1 L of distilled water. It contains 1 mg potassium per ml Stock solution was diluted to give four solutions containing 20, 10 5, and 2 ppm of potassium ions.

For sodium and potassium, the flame intensity corresponding to the concentration of stock solution was noted by using appropriate filters. The results were plotted in a graph, The flame intensity of the gastric juice was noted. The concentrations of sodium and potassium ions were calculated from the graph. The results are expressed in terms of mg/L

6.2Acetic acid induced chronic ulcer model

Adult Wister albino rats of either sex weighing 180-250 g were divided into nine groups of six animals each and placed in cages with grating floor to avoid coprophagy and fasted for 24 hour allowing free access of water.

Group I: animals treated as control (Non-ulcerated ss)

Group II: animals treated as solvent control (Ulcerated, 0.3% CMC in water orally)

Group II: control sacrificed on 4th day (Ulcerated)

Group IV: animals treated with positive control –Ranitidine 27mg/kg (suspended n 0.3% CMC)orally

Group V: animals treated with positive control-Omeprazole 2mg/kg (suspended in 0.3% CMC) orally

Group VI: animals treated with test extract- CP 200 mg/kg (suspended in 0.3% CMC) orally

Group VII: animals treated with test extract- CP 400 mg/kg (suspended in 0.3%CMC)

orally

Group VIII: animals treated with test extract- HI 200 mg/kg (suspended in 0.3%CMC)
orally

Group IX: animals treated with test extract - HI 400 mg/kg (suspended in0.3%CMC)
orally

Laparotomy was performed under combined ketamine and xylazine anaesthesia on experimental rats that were deprived of food during the preceding 24 hours. Fifty microlitre of 50% glacial acetic acid was administered by placing cylindrical plastic mould (6.5 mm in diameter) was firmly placed upon the wall of the stomach corpus at the region of the lesser curvature for 60 s, and the stomach wall wiped using cotton wool soaked in 0.9% NaCl solution. The abdominal incisions were stitched up and disinfectant (Povidine iodine) applied to the area each day to avoid infection.

The animals then continued to receive their regular diet, with free access to water. Four days after the operation, a control group (group III) of six rats were sacrificed using ether and the stomachs were removed and cut open along the greater curvature in order to establish the degree of ulceration prior to the onset of treatment. .

The remaining rats were divided into seven groups of six rats in each group. An additional group of six healthy non-ulcerated rats (group I) was subjected to the same experimental conditions but neither given text extract nor the standard drug. The other group(s) were administered with respective drug once a day for the fifteen days, group II-received with 0.3% CMC in water orally, groups- IV and V were given the positive control drug Ranitidine 27 mg/kg and Omeprazole 2 mg/kg while the groups VI and VII were with CP-200 & 400 mg/kg and groups VIII and IX were HI 200 & 400 mg/kg administered orally respectively.
On the final day of the experiment, the rats were sacrificed by using excess anesthesia
The ulcer index and mucus production were evaluated and the healing rates of the ulcers were calculated by comparing the ulcer status of the test extracts and the standard drugs with those of the ulcerated untreated controls. The degree of auto healing was evaluated by comparing the untreated control ulcers with those of the rats killed on day 4 post operation.

6.2.1Estimation of antiulcer potential

Gastric mucosal defensive factors

Estimation of mucous barrier

Glandular portion of stomach of 24 hrs fasted rats were everted and soaked for 24 h in 10 ml of 0.1 % alcian blue 8GX dissolved in 0.16M sucrose buffered with 0.05M sodium acetate adjusted to pH 5.8 with HCl . Uncomplexed dye was removed by two successive washes of 15 and 45 minutes in 0.25 N sucrose. Dye complexed with mucous was dilated by immersion in 10 ml aliquots of 0.5 M magnesium chloride for 2 h. The resulting blue solution was shaken with equal volume of diethyl ether and the optical density of aqueous phase was measured at 605 nm. The barrier mucous was expressed in terms of microgram of alcian blue dye/g of wet stomach glandular tissues. Mucous barrier [microgram of alcian blue dye/g of wet stomach glandular tissues (g)].

$$\frac{\text{Absorbance X 105}}{1 \qquad \text{E 1\% X} \qquad \text{Wt of glandular tissues}}$$

E1% 1cm for alcian blue =189

6.2.2. Estismation of non-protein sulfhydryl (NP-SH) group

The glandular part of the stomach was homogenized in ice-cold 0.02 M EDTA. Aliquots (5ml) of the homogenates were mixed in 15 ml test tubes with 4 ml of distilled water and 1 ml of 50 % trichloroacetic acid. The tubes were shaken intermittently for 10 to 15 minutes and centrifuged at 3000 r.p.m. Two ml of supernatant was mixed with 4 ml of tris Buffer pH 8.9, 0.1 ml of 5,5 dithio-bis-(2nitro benzoic acid) was added and the samples were shaken. The absorbance was read within 5 min of addition of 5,5 dithio-bis- (2 nitro benzoic acid) at 412 nm against a reagent with no homogenate.

II In- *Vitro* anti oxidant parameters

6.3.1 Catalase (CAT)

The catalysis of H_2o_2 to H_2O in an incubation mixture adjusted to p^H 7 was recorded at 254 nm. The reaction mixture contained 2.6 ml of 25 mM potassium phosphate buffer p^H 7 and 0.1 ml of tissue homogenate and was incubated at 37 ˚C for 15 min and the reaction was started with the addition of 0.1ml of 10 mM H_2O_2. The time required for the decrease in

absorbance from 0.45 to 0.4 representing the linear portion of the curve was used for the calculation of enzyme activity. One unit of catalase activity was defined as the amount of enzymes causing the decomposition.

6.3.2. Super oxide dismutase (SOD)

Super oxide dismutase measurement was done based on the ability of SOD inhibit spontaneous oxidation of adrenaline to adrenochrome. 2.78 ml of sodium carbonate buffer (0.05 mM) and 100 µl of stomach homogenate or sucrose (blank) a 30 ˚C for 45 min after reaction was initiated by adding 100 µl solution (9.0mM). The change in the absorbance was recorded at 480 nm for 8-12 min; throughout the assay procedure temperature was maintained at 30 ˚C the activity was expressed as units/mg protein.

6.3.3 Estimation of lipid peroxide

The method was used to estimate the total amount of lipid per oxidation product (thiobarbituric acid reacting substance) in the homogenate.

Procedure:

For TBARS, 0.1ml of tissue homogenate (Tris-buffer, PH7.5) was mixed with 2.0 ml of TBA-TCA-HCl reagent (thiobarbituric 0.37%, 0.25N HCl and 15 % TCA mixed in 1:1:1 ratio). The resultant solution was placed in water bath for 5 min and centrifuged at 1000 rpm for 10 min. The absorbance of clear supernatant was measured against reference blank at 535 nm. The results were expressed as nM/min/mg tissue protein.

Duodenal Ulcers

6.4 Cysteamine induced duodenal ulcers in rats (Szabo,et al., 1978)

Wister albino rats of either sex weighing 180-220 g were divided into nine groups of six animals each and duodenal ulcers were induced by the administration of cysteamine hydrochloride 400 mg/kg in 10% aqueous solution orally two times at an interval of 4 h and the animals were fed with food and water throughout the experiment and test extracts were administered 30 min before each dose of cysteamine hydrochloride

Group I: animals treated with solvent control (0.3 %CMC in water, orally-1ml/kg)

Group II: animals treated with positive control –Ranitidine 27 mg/kg (suspended

in 0.3% CMC)orally

Group III: animals treated with positive control-Omeprazole 2 mg/kg (suspended in 0.3% CMC) orally

Group IV: animals treated with test extract-CP 200 mg/kg (suspended in 0.3% CMC) orally

Group V: animals treated with test extract-CP 400 mg/kg (suspended in 0.3% CMC) orally

Group V: animals treated with test extract- HI 200 mg/kg (suspended in 0.3% CMC) orally

Group VII: animals treated with test extract –HI 400 mg/kg (suspended in 0.3% CMC) orally

After 24 hr of the 1st dose of cysteamine, duodenaum was excised carefully and opened. The duodenal ulcers were scored for intensity, using a scale of 0 to 3. The ulcers scores after converting it into ulcer index were subjected to statically analysis by comparing with normal (solvent control).

The duodenal ulcer score was noted by using a scale of 0 to 3

Ulcer score	Descriptive Observation
0	Normal ulcer
1	Superficial mucosa erosin
2	Deep ulcer/Transmural necrosis
3	Perforated ulcers

6.5 Sub acute toxicity studies:

The adult male wister albino rats weighing 160-180 g, were selected and divided into 5 groups comprises six animals in each group. Group I served as normal, Groups II & III and Group IV & V received *Cyathula prostrta* at a dose level of 200, 400 mg /kg body weight orally and *Heliotropium indicum* at a dose level of 200, 400 mg/kg body weight orally once daily for 28 days. On the 29th day the animals were sacrificed by cervical decapitation, blood was collected by cardiac puncture and were used to study various haematological and biochemical parameters. The body was cut open and the vital organs like liver, kidney. Spleen and stomach were excised and stored in 10% buffered formalin for histopathological examination. |

The haematological and biochemical parameters noted and compared with normal on the 29th day.

6.5.1Haematologicla parameters investigated (Ghai, 1993)

The following haematological parameters were estimated.

a) Haemoglobin (Hb)

b) RBC count

c) WBC count

d) Differencial leukocyte count

6.5.2 Bio-chemical parameters investigated

a)Assay of Aspartate amino transferase (ASAT or GOT) (Bergmeyer, 1972, Engelhardi and Norges, 1970)

Aspartate amino transferase in serum was assayed using Ecoline diagnostic kit

ASAT catalyses the following the reaction

2—Oxoglytarate + L-Asparate ASAT→ Glutamate + Oxaloacetate

Oxaloacetate + NADH + H MDH→ Malate + NAD +

as U/L.

The rate of NADH consumption was measured photometrically at 340 nm and was directly proportional to the ASAT activity in the sample. Aspartate amino transferase level in serum was expressed as U/L.

PROCEDURE

Reagents 1 and 2 of Ecoline diagnostic kit for ASAT were mixed at the ratio of 4:1 and the temperature was maintained at 30ºC. To 50 µl of the sample, 0.5% ml of reagent solution was added and mixed. After 1 min, the decrease in absorbance was measured every min for 3 min at 340nm.

$$\text{Enzyme activity [U/L]} = (\Delta A / \text{min}) \times 2143$$

Where, Δ A is the decrease in absorbance per min.

b)Assay of Alanine amino transferase (ALAT) or (GPT): (Bergmeyer,1972, Engehardi and Norges, 1970)

Alanine aminotransferase in serum was assayed by using Ecoline diagnostic kit

ALAT catalyses the following reaction.

2-Oxaglytartarate + L.alanine ALAT→ Gluatamate + Pyruvate

Pyruvate + NADH + H+ LDH→ Lactate + NAD

where, LDH is lactate dehydrogenase. The rate of NADH consumption was measured spectro photometrically at 340 nm and is directly proportional to the ALAT activity in the sample. ALAT level in serum was expressed as U/L.

PROCEDURE

Reagents 1 and 2 of Ecoline diagnostic kit for ALAT were mixed at the ratio of 4:1 and the temperature was maintained at 30ºC. To 50 µl of the sample, 0.5 ml of the reagent solution was added and mixed. After 1 min, the decrease in absorbance was measured every min for 3 min at 340 nm.

Enzyme activity [U/L] = (Δ A /min) x 2143

Where, Δ A is the decrease in absorbance per min

c)Assay of alkaline phosphatase (ALP): (Bergmeyer, 1972, Engelhardi and Norges,1970)

Alkaline phosphatase in serum was assayed by using Ecoline diagnostic kit. ALP catalysis the following the reaction.

4-Nitrophenyl Phosphate + H_2O $\xrightarrow{ALP}$ Phosphate + 4-nitrophenolate

The rate of increase in 4-nitrophenolate was determined spectro photometrically at 405nm and is directly proportional to the ALP activity in the sample. Alkaline phosphatase level in serum is expressed as U/L.

PROCEDURE

Reagents 1 and 2 of Ecoline diagnostic kit for ALP were mixed at the ratio of 4:1 and the temperature was maintained at 30ºC. To 20 µl of the sample, 1 ml of the reagent solution was added and mixed. After 1 min, the increase in absorbance was measured every min for 3 min at 405 nm.

Enzyme activity [U/L] = (Δ A /min) x 2754

6.5.3Histopathological Studies:

After the estimation of haematological and biochemical parameters, same animals (rats) on 29th day were sacrificed and autopsy was performed to observe any morphological changes in the vital organs like liver, kidney, stomach and spleen. Then they were removed and preserved in 10% buffered formalin for histopathological studies

Results and Discussions :

These plants i.*e Cyathula prostrate* and *Heliotropium indicum* were collected from Kottakkal area in Kerala which were properly identified and authenticated by comparing with voucher specimen available at the survey of Medicinal plants, Kottakkal, Kerala

Table No: 6 Organoleptic characters of Cyathula prostrata and Heliotrpium indicum
Macroscopic characters

Organoleptic Characters	Observation	Observation
	Cyathula prostrata	***Heliotropium indicum***
Appearance	Leaves-simple, opposite, exstipulate, short petiole Flowers-reddish violet in terminal spikes Fruits-ovoid Seed-single, oblong inverse	Simple, alternate, unequal hispid, pubescent Flowers-pale violet Fruits-nut lets combined in pairs Seed-each lobe 4 ribbed
Odour	No specific odour	No specific odour
Colour	green	green
Taste	No taste	Bitter

Table No:7 Pharmacognostical standardization data

Pharmacognostical Standardization parameters	***Cyathula prostrata***	***Heliotropium indicum***
Total ash	2.1% w/w	2.95% w/w
Acid insoluble ash	0.95% w/w	1.3% w/w
Water soluble ash	1.0 % w/w	1.6 % w/w
Sulphated ash	3.2 % w/w	4.4 % w/w
Loss on drying at 110˚C	8.0 % w/w	11 % w/w

Table No:8 Extracts – Nature & Yield **- *Cyathula prostrata***

S.No	Solvent Extract	Colour	Consistency	Extractive value % w/w
1	Pet.ether (60-80 ˚C)	Dark yellowish	Residue	0.66
2	Chloroform	Dark green	Greenish residue	0.60
3	Ethyl acetate	Dark green	Green residue	1.2
4	Ethanol	Brownish green	Dark greenish residue	3.3
5	Water	Dark brown	Brownish residue	4.24

Table No:9 Extracts –Nature & Yield - ***Heliotropium indicum***

S.No	Solvent Extract	Colour	Consistency	Extractive value % w/w
1	Pet.ether (60-80˚C)	Dark yellowish	residue	0.99
2	Chloroform	Dark green	Dark green residue	1.8
3	Ethyl Acetate	Dark green	Green residue	0.91
4	Ethanol	Brownish green	Dark greenish residue	4.98
5	Water	Dark brown	Brown residue	6.03

Table No: 10 The Fluorescence characters of different extract under both day light and UV-Light -**Cyathula prostrata**

S.No	Name of the extract	Daylight	UV 254nm	UV 365nm
1	Pet.ether (60-80ºC)	Dark yellowish	Yellow	Yellow
2	Chloroform	Dark green	Yellow	Yellow
3	Ethyl acetate	Dark green	Yellow	Yellow
4	Alcohol	Brownish green	Green	Green
5	Aqueous	Dark brown	Bright green	Bright green

Table No: 11 The Fluorescence characters of different extracts under both day light and UV-Light - **Heliotropium indicum**

S.No:	Name of the extract	Daylight	UV 254 nm	UV 365 nm
1	Pet.ether	Dark yellowish	Dim yellow	Bright yellow
2	Chloroform	Dark green	Yellow	Bright yellow
3	Ethyl acetate	Dark green	Bright yellow	Bright yellow
4	Alcohol	Brownish green	Greenish yellow	Bright yellow
5	Aqueous	Dark brown	Greenish yellow	Bright yellow

Table No: 12 Qualitative analysis of Phytochemical evaluation- ***Cyathula prostrata***

Phytochemcial evalution	Pet.ether extract	Ethyl ace.extract	Chloroform extract	Alcoholic extract	Water extract
Alkaloids	--	--	--	--	--
Aminoacids/Proteins	--	--	--	--	--
Carbohydrates	--	--	--	--	--
Fixed oils/Fats	--	--	--	--	--
Phytosterols	+	--	--	--	--
Saponins	+	--	--	--	--
Tannins/Phenolic compd	--	+	+	+	
Flavanoids	--	+	+	+	
Terpenoids	+	--	--	--	--
Gums /Mucilages	--	--	--	--	--
Glycosides	--	--	--	--	--

Table No :13 Qualitative Phytochemical evalution- ***Heliotopium indicum***

Phytochemcial evalution	Pet.ether extract	Ethyl ace.Extract	Chloroform extract	Alcoholic extract	Aqueous extract
Alkaloids	--	+	+	+	--
Aminoacids/Proteins	--	--	--	--	--
Carbohydrates	--	--	--	--	--
Fixed oils/Fats	--	--	--	--	--
Phytosterols	--	--	--	--	--
Saponins	+	--	--	--	--
Tannins/Phenolic compd	--	+	+	+	
Flavonoids	--	+	+	+	
Terpenoids	--	--	--	--	--
Gums /Mucilages	--	--	--	--	--
Glycosides	--	--	--	--	--

Table No: 14 represented the characteristic behavioural changes with different reagents of ***Cyathula prostrata***

S.No	Reagents	Observation	Inference
1	Powder + Picric acid	No stained yellow	Absence of alkaloids
2	Powder + Conc.H_2So_4	Greenish colour	May be tannins or carbohydrates
3	Powder + $FeCl_3$	Slight pink colour	May be phenolic content
4	Powder + Iodine soln	No brown colour	Absence of alkaloids
5	Powder + Ammonia soln	No change	Absence of alkaloids
6	Powder + 5% KOH soln	No change	Absence of alkaloids
7	Powder + NaOH soln	No change	Absence of alkaloids
8	Powder + $AgNo_3$	No change	Absence of proteins

Table No: 15 represented the characteristic behavioural changes with different reagents of ***Heliotropium indicum***

S.No	Reagents	Observation	Inference
1	Powder + Picric acid	Dark yellowish	May be alkaloids
2	Powder + Conc.H_2So_4	Greenish colour	May be carbohydrates
3	Powder + $FeCl_3$	Slight pink colour	May be phenolic content
4	Powder + Iodine soln	Brown colour	May be alkaolids
5	Powder + Ammonia soln	Colour darkened	"
6	Powder + 5% KOH soln	Colour darkened	"
7	Powder + NaOH soln	Colour darkened	"
8	Powder + $AgNo_3$	No change	Absence of proteins

Table No: 16 Extraction, phytochemical analysis, total phenol and total flavonol Estimation of ***Cyathula prostrata***

Extract	Nature	Yield %w/w	Phytochemicl analysis	Total phenol mg/g a,b	Total Flavonol mg/g a,c
Petroleum ether	Dark yellow sticky residue	0.66	Steroids, terpenoids	---	---
Chloroform	Dark green	0.60	Flavanols,phenolic,tannins No alkaloids	40.0 ±0.6	20.0 ±0.81
Ethyl acetate	Dark green	1.2	Flavanols,phenolic,tannins No alkaloids	38.2 ±2.9	184.0 ±0.83
Ethanol	Brownish green	3.3	Flavanols,phenolic,tannins No alkaloids	50.22 ±2.4	26.0 ±1.82

a. Average of six determinations, Mean ± SEM**, b**: gallic acid, **c**: rutin equivalent in mg/g of the extract

Table No: 17 Extraction, phytochemical analysis, total phenol and total flavonol Estimation of ***Heliotropium indicum***

Extract	Nature	Yield % w/w	Phytochemical analysis	Total phenol mg/g a,b	Total Flavonol Mg/g a,c
Petroleum ether	Yellow sticky residue	0.99	--------	-------	-------
Chloroform	Dark green solid	1.8	Flavanols, phenolic comppounds alkaloids and tannin	32.0 ±1.9	43.0 ±1.6
Ethyl acetate	Dark green solid	0.91	Flavanols, phenolic compounds alkaloids and tannin	350.32 ±6.23	54.0 ±1.9
Ethanol	Drak green solid	4.98	Flavanols, phenolic compounds alkaloids and tannin	216.6 ±3.2	58.0 ±1.9

a: Average of three determinations, Mean ± SEM, **b:** gallic acid, **c:** rutin equivalent in mg/g of the extract

Table No: 18 showing the Lupeol content in ***Heliotropium indicum*** determined by using HPTLC technique

S.No	Extract	% w/w Lupeol content
1	Pet.ether	0.213
2	Chloroform	0.28
3	Ethylacetate	0.478
4	Ethanol	0.089

Table No: 19 Foaming Index of ***Cyathula prostrata*** Linn & ***Heliotropium indicum*** Linn

S.No	Test Tube No	Height of foam (in cm) *Cyathula prostrata* (Linn)	*Heliotropium indicum* (Linn
1	1	1.2	0.8
2	2	2.5	1.7
3	3	3.0	1.9
4	4	3.2	2.0
5	5	3.4	2.1
6	6	3.6	2.1
7	7	3.8	2.1
8	8	4.0	2.1
9	9	4.2	2.2
10	10	4.6	2.2

Foaming Index

i) The foamig index of Cyathula prostratass == 1000
ii) The foaming index of heliotropium indicum == 1000

The pharmacognostical features such as macroscopical characters, preliminary phytochemcial studies of *Cyathula prostrate* and *Heliotropium indicum* were carried out and presented. The results revealed that the plant *cyathula prostrate* appeared as leaves-simple, opposite, exstipulate, short petiole, Flowers-reddish violet in terminal spikes, Fruits-ovoid, Seed-single, oblong inverse. Green color, no specific odour and taste.

Heliotropium indicum plant appeared as Leaves-simple, alternate, unequal, hispid, pubescent, Flowers-nut lets, combined in pairs, Seed- each lobe 4 ribbed and no specific odour and taste is bitter (Table no: 6)

The organoleptic characters may authentify the plants some extent but, that required additional information such as the parameters like- physio-chemical constants were done to helpful in determining the quality and purity of crude drugs in the powder form according to the standard procedures. From the results, it was observed on the plants, *Cyathula prostrate and Heliotropium indicum* that the Total ash value, Acid insoluble ash, Water soluble ash , Sulphated ash and Loss on drying at 110c were 2.1%w/w, 0.95%w/w,1.0%w/w,3.2%w/w,8% w/w and 2.95%w/w, 1.3%w/w,1.6%w/w, 4.4%w/w –11 %-respectively. (Table no: 7) These values may help to identify the authentic crude drugs from the closely related species

The results of extraction and phytochemical analysis of *Cyathula prostrate* and *Heliotropium indicum* are given in Table no: 7 Among the five successive extracts, the yield of alcohol and aqueous extracts of both the plants were found to be high (Table no: 9)

The preliminary phytochemical studies of both plants, revealed that the plant extract of *Cyathula prostrate* i.e petroleum ether extract showed the presence of steroids and terpenoids and the successive extraction with chloroform, Ethylacetate , and ethanol & aqueous showed the presence of flavanoids, phenolic compds, and tannins

And the plant *Heliotropium indicum*, petroleum ether extract yielded Dark yellow solid mass showed no steroids and terpenoids but, on the successive extracts gave positive tests for the presence of alkaloids, flavanoids , phenolic compds, and tannins (Table no:10 & 11)

The fluorescence characters of the powdered *Cyathula perostrata and Heliotropium indicum* when treated with various chemical reagents (Table no: 12 & 13) and those of its extracts have been studied. The cortex (CP) showed reddish pink color with $sbcl_2$ indicating the presence of steroids and triterpenoids. And the paraenchymatous tissues showed the brown colour with dragendroff's reagent

indicating (HI) the presence of alkaloids. This study may be helpful to provide supplement information to distinguish the authentic crude drug (s) from the closely related species.

The table No: 14 & 15 showed the characteristic behavioural change with different reagents which may give the basic phyto constitiuents presence in each of the extract that indicate the possible activity claimed with the respect to the folk medicine practice. The table No 9 represents the phytoconstiteunts found in *cyathula prostrata* with various reagents , such as terpenoids, as saponins, tannins, phenolic content and flavanol and in case of *Heliotropium indicum* the phytoconstituents are alkaloids, tannins, phenolic content, flavanol with various chemical reagents.

The phyto chemical analysis as quatification of total phenol, flavanol estimated by spectroscopic method and estimation of lupeol was by HPTLC method.(Table No: 16, & 17). The total phenolic content determined in *cyathula prostrate* comprises the chloroform extract 40.0mg/g, ethyl acetate extract 38.2mg/g, ethanolic extract 50.22mg/g and the flavanol content in chloroform extract 20mg/g, ethyl acetate extract 184.2 mg/g ethanolic extract 26.0mg/g .The total phenolic content determined in *Heliotropium indicum* comprises the chloroform extract 32mg/g, ethyl acetate extract 350.32mg/g, and ethanolic extract 216.6mg/g. The special content as lupeol determined by HPTLC, found that the pet.ether extract 0.213%, chloroform extract 0.28%, ethyl acetate0.478% and ethanolic extract 0.089% respectively. (Table no: 18)

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JSS COLLEGA OF PHARMACY

OOTY

Analysis Report

SOP document	
Validated	Design
Description :	
Analysis	C:\CAMAG\winCATS\Data\JanarLupeol.cna
Created/used by	JSS COLLEGE OF PHARMACY Friday, August 08, 2008 4:17:10 PM
Current user	JSS COLLEGE OF PHARMACY

Stationary phase

Executed by	JSS COLLEGE OF PHARMACY Friday, August 08, 2008 3:47:06 PM
Plate size (X x Y)	10.0 x 10.0 cm
Material	HPTLC plates silica gel 60 F 254
Manufacturer	E. MERCK KGaA
Batch	
GLP code	
Pre-washing	No
Modification	No

Definitions - Quantification

Executed by JSS COLLEGE OF PHARMACY Friday, August 08, 2008 3:57:47 PM

Calibration parameters

Calibration mode	Single level
Statistics mode	CV
Evaluation mode	Peak area

Samples

Sample ID: Lupeol

Sample ID: PE

Sample ID: Ea

Sample ID: Chl

Sample ID: Eth

Substance name	Rf	Window size	Deviation	Purity
Lupeol	0.56	1.7 mm	10.00 %	1.0000

Standards absolute

Standard level1

Substance	Amount/fraction
Lupeol	1.0000 µg

User : JSS COLLEGE OF PHARMACY
Friday, August 08, 2008 4:17:11 PM

Approved :. . . . . .
Report ID : 07D808080610110A

SN 1410W009, V1.4.3
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Detection - CAMAG TLC Scanner 3

Information	
Application position	10.0 mm
Solvent front position	75.0 mm
Instrument	CAMAG TLC Scanner 3 "Scanner3_061121" S/N 061121 (1.14.28)
Executed by	JSS COLLEGE OF PHARMACY Friday, August 08, 2008 4:16:42 PM
Number of tracks	5
Position of first track X	14.0 mm
Distance between tracks	13.0 mm
Scan start pos. Y	16.5 mm
Scan end pos. Y	83.4 mm
Slit dimensions	3.00 x 0.30 mm, Micro
Optimize optical system	Light
Scanning speed:	20 mm/s
Data resolution:	100 µm/step
Measurement Table	
Wavelength	254
Lamp	D2 & W
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	285 V
Detector properties	
Y-position for 0 adjust	16.5 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (33)

Integration

Properties	
Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	19.9 mm
Track end position	75.0 mm
Display scaling	Automatic

User : JSS COLLEGE OF PHARMACY
Friday, August 08, 2008 4:17:11 PM

Approved :. . . . . .
Report ID : 07D808080610110A

SN 1410W009, V1.4.3
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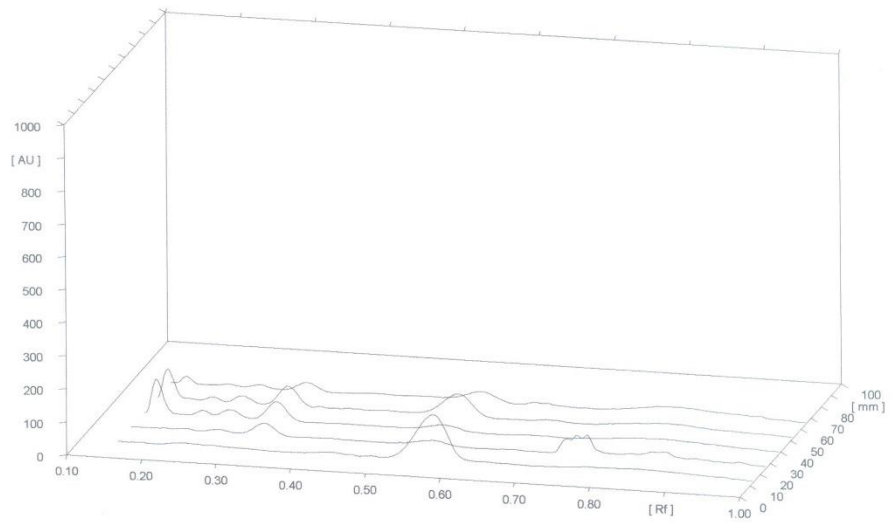
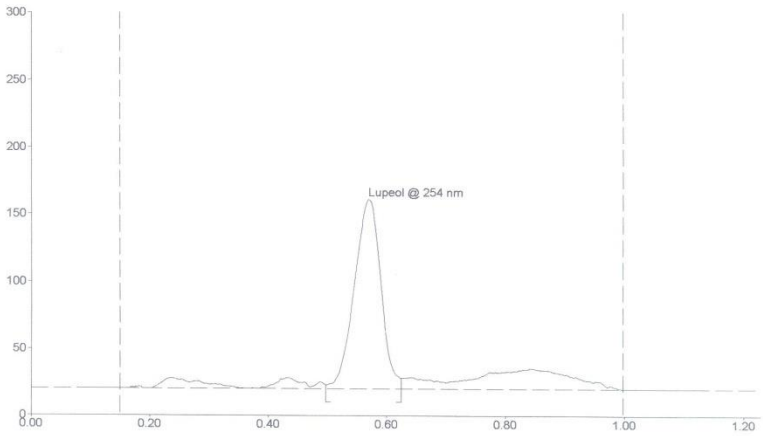
All tracks at Wavelength



Track 1, ID: Standard1



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.50	2.9	0.57	141.3	100.00	0.63	8.1	4792.7	100.00	Lupeol

User : JSS COLLEGE OF PHARMACY
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Report ID : 07D808080610110A

SN 1410W009, V1.4.3
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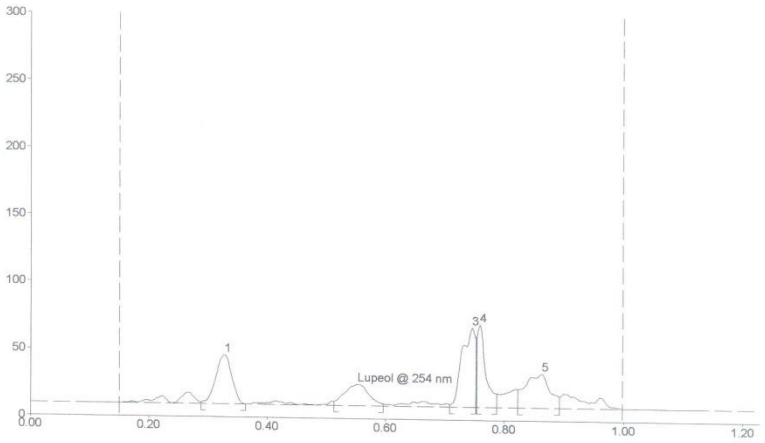
Track 2, ID: PE



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.29	1.4	0.33	36.3	18.40	0.36	0.1	748.5	19.93	unknown *
2	0.51	3.3	0.55	16.1	8.13	0.60	2.0	503.9	13.42	Lupeol
3	0.71	2.2	0.75	58.6	29.68	0.75	51.1	1011.8	26.95	unknown *
4	0.76	52.3	0.76	61.0	30.90	0.79	10.5	685.8	18.26	unknown *
5	0.82	13.9	0.86	25.5	12.89	0.89	8.8	804.8	21.43	unknown *

User : JSS COLLEGE OF PHARMACY
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Report ID : 07D808080610110A

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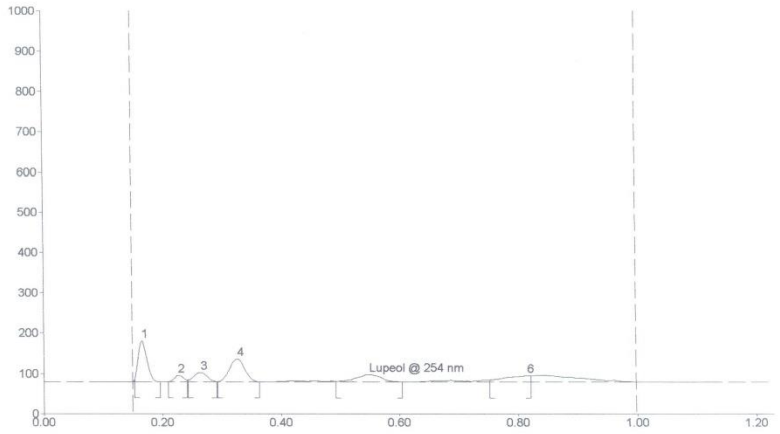
Track 3, ID: Ea



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.15	4.7	0.17	100.5	43.52	0.20	0.1	1135.4	28.33	unknown *
2	0.21	0.4	0.23	16.3	7.04	0.24	5.0	200.5	5.00	unknown *
3	0.24	5.2	0.27	23.3	10.11	0.29	0.9	412.4	10.29	unknown *
4	0.29	1.1	0.33	56.2	24.33	0.36	0.1	1116.0	27.85	unknown *
5	0.49	1.6	0.55	18.5	8.00	0.61	0.2	609.7	15.22	Lupeol
6	0.75	5.8	0.82	16.2	7.00	0.82	15.3	533.3	13.31	unknown *

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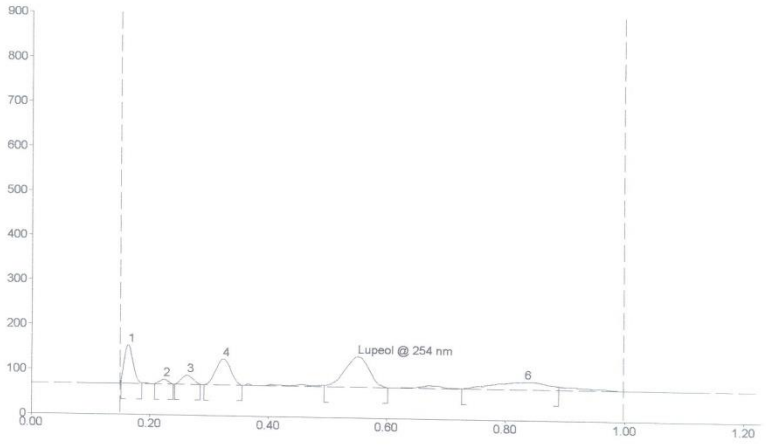
Track 4, ID: Chl



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.15	8.3	0.16	85.7	33.01	0.19	2.0	960.1	16.42	unknown *
2	0.21	0.4	0.22	10.9	4.21	0.24	0.1	120.7	2.07	unknown *
3	0.24	0.2	0.26	20.3	7.83	0.29	1.0	307.7	5.26	unknown *
4	0.29	0.1	0.32	57.7	22.21	0.36	0.1	1102.3	18.86	unknown *
5	0.50	3.6	0.55	67.6	26.01	0.60	0.6	2122.2	36.30	Lupeol
6	0.73	1.5	0.83	17.5	6.74	0.89	8.6	1233.1	21.09	unknown *

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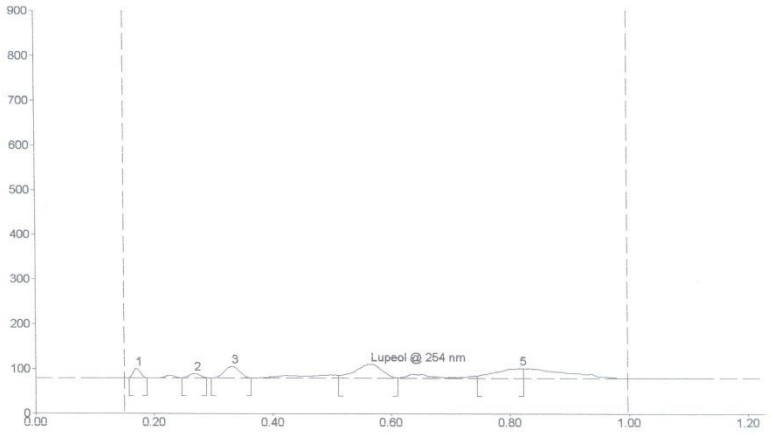
Track 5, ID: Eth



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.16	0.2	0.17	20.8	18.89	0.19	0.1	196.7	7.27	unknown *
2	0.25	0.7	0.27	10.5	9.53	0.29	1.1	144.3	5.33	unknown *
3	0.30	0.0	0.33	25.5	23.17	0.36	0.3	501.2	18.53	unknown *
4	0.51	6.3	0.57	31.2	28.34	0.61	1.6	1069.3	39.54	Lupeol
5	0.75	5.9	0.82	22.1	20.07	0.82	21.4	792.9	29.32	unknown *

Spectrum scan

Executed by	JSS COLLEGE OF PHARMACY Friday, August 08, 2008 4:00:25 PM
Mode	All detected peaks
Slit dimensions	3.00 x 0.30 mm, Micro
Optimize optical system	Resolution
Scanning speed	100 nm/s
Data resolution	1 nm/step
Reference spectrum, pos X	14.0 mm
Reference spectrum, pos Y	16.5 mm

Measurement Table

Lamp	D2 & W
Start wavelength	200 nm
End wavelength	400 nm
Measurement type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector Mode	Automatic

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Report ID : 07D808080610110A

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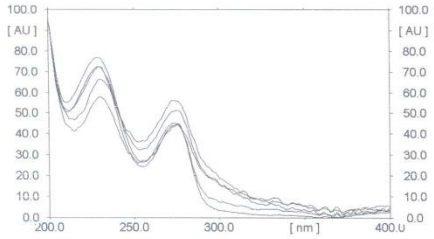
Detector properties

Y-position for 0 adjust	0.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (24)

Lupeol on all Tracks



T	Rf	Substance	Max. @
1	0.57 Rf	Lupeol	200 nm
2	0.55 Rf	Lupeol	200 nm
3	0.55 Rf	Lupeol	200 nm
4	0.55 Rf	Lupeol	200 nm
5	0.57 Rf	Lupeol	200 nm

Evaluation results

Evaluation Sequence

Track	Track type	Vial	Sample ID
1	Standard1	1	
2	Sample	2	PE
3	Sample	3	Ea
4	Sample	4	Chl
5	Sample	5	Eth

Table of substances

Substance	Position MD mm	Tracks 1	2	3	4	5
Lupeol	46.7	A	A	A	A	A

Results per track

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Report ID : 07D808080610110A

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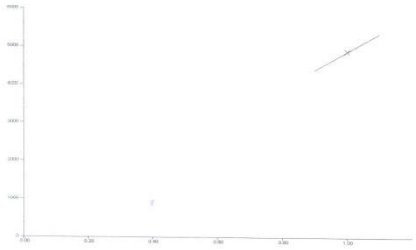
Substance: Lupeol @ 254 nm

Regression via area: Single level Y = 0 + 4793 * X r = 0.00000 sdv = 0.00

Track	Vial	Rf	Amount	Height	X(Calc)	Area	X(Calc)	SampleID/Remark
1	1	0.57	1000.00 ng			4792.69		
2	2	0.55				503.92	<900.00 ng	PE
3	3	0.55				609.71	<900.00 ng	Ea
4	4	0.55				2122.20	<900.00 ng	Chl
5	5	0.57				1069.26	<900.00 ng	Eth



winCATS summary report

Calibration results per Analysis

Sample from vial 2: PE
Result via area

Substance	Rf	X(average)	CV [%]	n	Remark
Lupeol	0.55	0.0 unknown	0.000	0	

Sample from vial 3: Ea
Result via area

Substance	Rf	X(average)	CV [%]	n	Remark
Lupeol	0.55	0.0 unknown	0.000	0	

Sample from vial 4: Chl
Result via area

Substance	Rf	X(average)	CV [%]	n	Remark
Lupeol	0.55	0.0 unknown	0.000	0	

Sample from vial 5: Eth
Result via area

Substance	Rf	X(average)	CV [%]	n	Remark
Lupeol	0.57	0.0 unknown	0.000	0	

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Report ID : 07D808080610110A

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Table No:20 Effect of Medicinal plants on Modified pylorus Ligated (Shay) Rat Model (MPL)

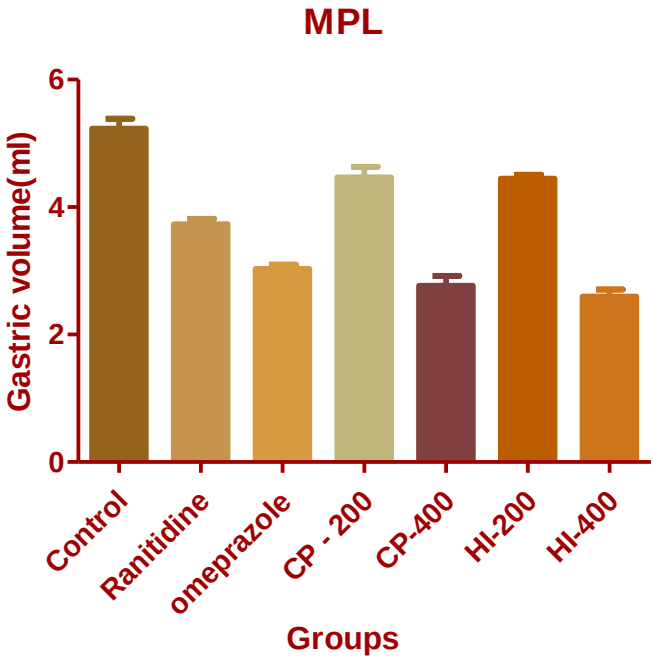
Group	Gastric Volume(ml)	pH	Ulcer index	Free acidity (Meq/l/100g)	Total acidity (Meq/l/100g)	Pepsin (µg/ml)	Total protein (µg/ml)
Solvent control	5.233 ±0.156	2.468 ±0.04	3.3 ±0.007	39.95 ±0.307	61.62 ±0.164	13.88 ±0.112	457.7 ±1.62
Ranitidine (27 mg/kg)	3.733 ±0.084***	4.1 ±0.2***	1.39 ±0.015***	31.27 ±0.305***	44.54 ±0.223***	7.220 ±0.085***	320.3 ±1.43***
Omeprazole (2 mg/kg)	3.03 ±0.067***	3.9 ±0.08***	1.143 ±0.015***	21.75 ±0.263***	31.94 ±0.204***	4.50 ±0.113***	255.0 ±0.976***
Cyathula prostrata (200 mg/kg)	4.467 ±0.167**	3.49 ±0.081***	3.153 ±0.035*	38.28 ±0.392**	64.01 ±0.9773*	13.03 ±0.289*	450.1 ±2.29*
Cyathula prostrata (400 mg/kg)	2.767 ±0.156*** a	3.990 ±0.18*** a	1.517 ±0.054*** a	18.52 ±0.210*** a	28.57 ±0.227*** a	7.117 ±0.116*** a	263.1 ±0.528*** a
Heliotropium indicum (200 mg/kg)	4.450 ±0.056**	3.3 ±0.082**	3.15 ±0.04*	38.33 ±0.422*	63.73 ±0.365*	12.97 ±0.260**	449.7 ±1.931**
Heliotropium indicum (400 mg/kg)	2.6 ±0.106*** b	4.083 ±0.090*** b	1.240 ±0.011*** b	22.85 ±0.2135*** b	33.77 ±0.55*** b	5.983 ±0.060*** b	244.4 ±0.264*** b

Values are expressed as mean±SEM; n=6
Oneway ANOVA followed by Turkey's multiple comparison test
* P <0.05, ** P<0.01; *** P<0.001 when compared with solvent control
a P<0.001 when compared CP 200 Vs CP 400
b P<0.001 when compared HI 200 Vs HI 400

Table No: 21 Effect of Medicinal plants on Biochemicals parameters of Modified Pylorus Ligated (Shay) Rat Model (MPL)

Group	Total carbohydrates	Hexosamine (µg/ml)	Fucose (µg/ml)	Sialic acid (µg/ml)	Total hexoses (µg/ml)	Na^+ conc	K^+ conc	C:P-ratio
Solvent control	369.8 ±0.0692	147.5 ±0.408	51.83 ±0.1667	20.10 ±0.153	150.3 ±0.441	17.00 ±0.57	6.33 ±0.056	0.809 ±0.0032
Ranitidine (27 mg/kg)	1022 ±4.841***	407.5 ±2.141***	159.3 ±0.614***	37.28 ±0.32***	418 ±3.33***	7.30 ±0.052***	11.17 ±0.149***	3.191 ±0.02***
Omeprazole (2 mg/kg)	1120 ±6.410***	467.5 ±2.141***	173.4 ±00.490***	42.92 ±0.287***	435.8 ±4.73***	5.70 ±0.08***	11.88 ±0.135***	4.390 ±0.033***
Cyathula prostrata (200 mg/kg)	685.3 ±8.3***	159.2 ±1.537*	57.0 ±0.775**	23.58 ±0.200**	255.0 ±4.830***	15.2 ±0.1054**	8.217 ±0.05**	1.155 ±0.086**
Cyathula prostrate (400 mg/kg)	833.8 ±7.12*** a	380.0 ±2.887*** a	144.5 ±0.619*** a	35.25 ±0.478*** a	284.2 ±5.388*** a	6.975 ±0 .415*** a	12.0 ±0.32*** a	3.2 ±0.024*** a
Heliotropium indicum (200 mg/kg)	708.4 ±2.507***	159.2 ±3.516*	56.33 ±1.67**	23.78 ±0.302**	269.2 ±3.52***	15.08 ±0.2386**	8.223 ±0.105**	1.201 ±0.1336**
Heliotropium indicum (400 mg/kg)	898.7 ± 4.722*** b	420.8 ± 3.005*** b	135.0 ±0.571*** b	42.38 ±1.5*** b	310.8 ±3.005*** b	6.233 ±0.1783*** b	13.75 ±0.704*** b	3.669 ±0.021*** b

Values are expressed as mean±SEM; n=6
Oneway ANOVA followed by Turkey's multiple comparison test
* P <0.05, ** P<0.01; *** P<0.001 when compared with solvent control
a P<0.001 when compared CP 200 Vs CP 400
b P<0.001 when compared HI 200 Vs HI 400



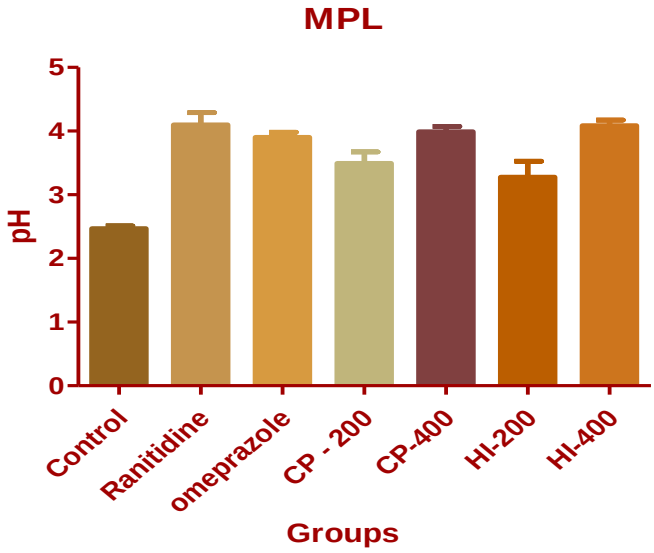
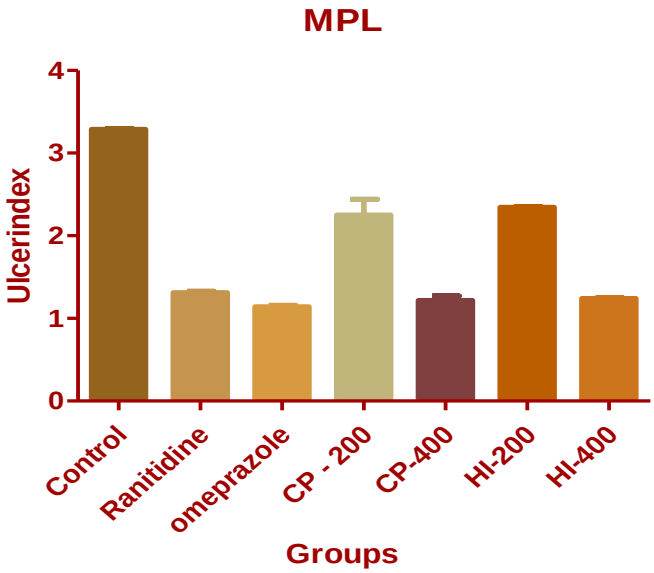


Fig No: 5 & 6 Effect of Medicinal Plants on Biochemical parameters of Modified pylorus ligated (shay) rat model. (MPL)



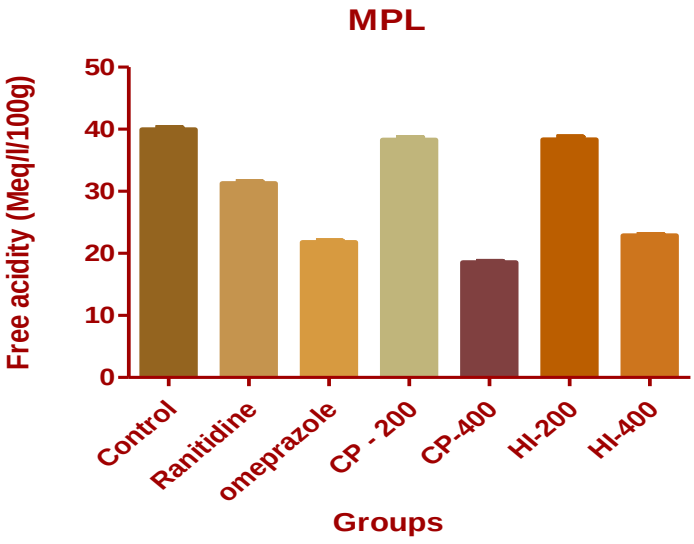
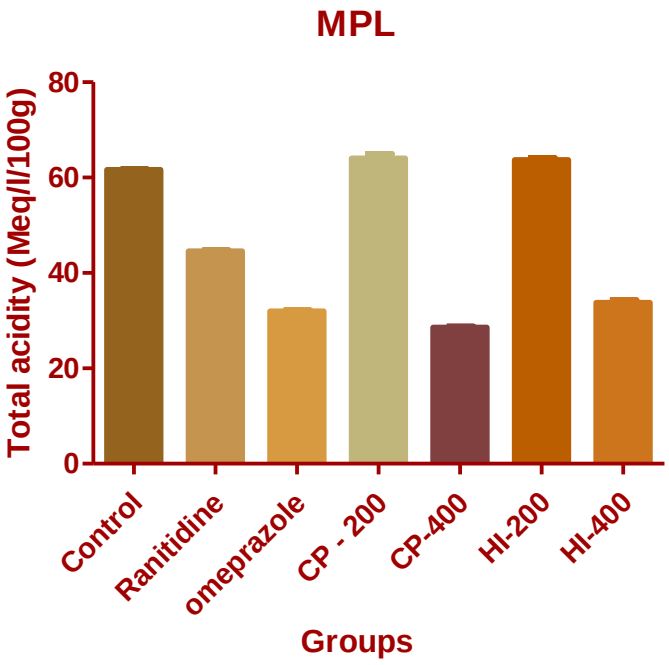


Fig No: 7 & 8 Effect of Medicinal Plants on Biochemical parameters of Modified pylorus ligated (shay) rat model. (MPL)



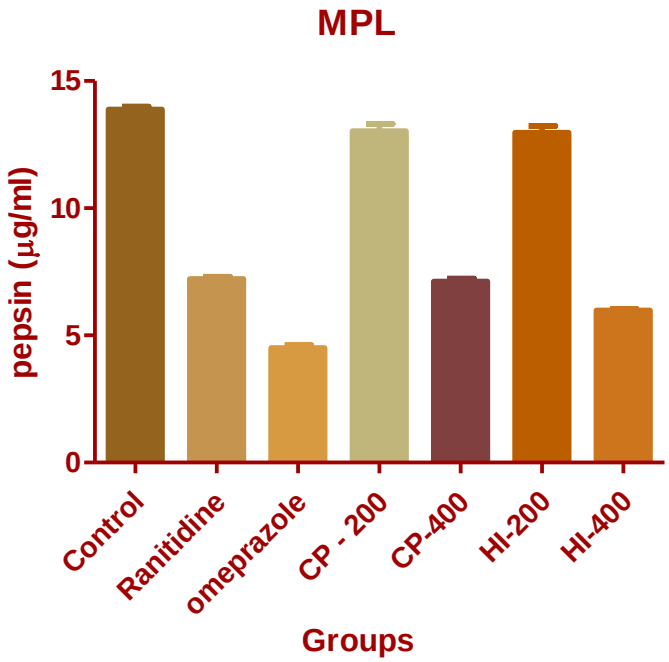
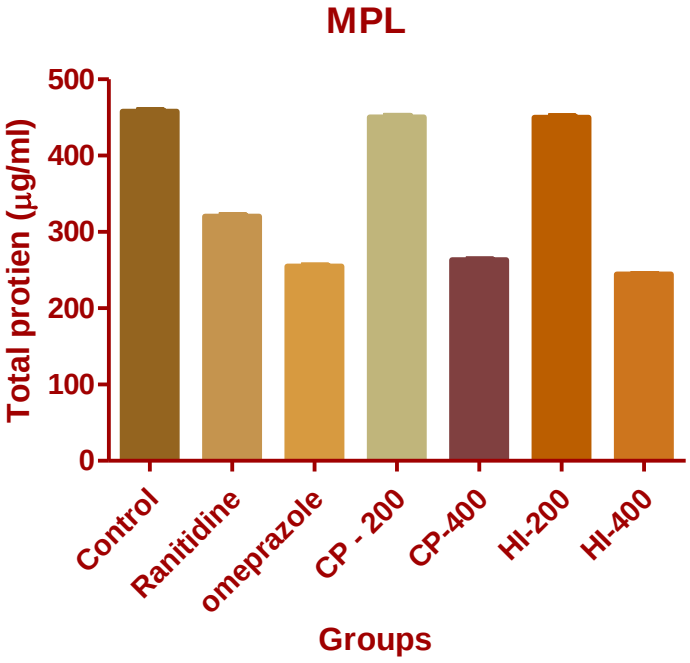


Fig No: 9 & 10 Effect of Medicinal Plants on Biochemical parameters of Modified pylorus ligated (shay) rat model. (MPL)



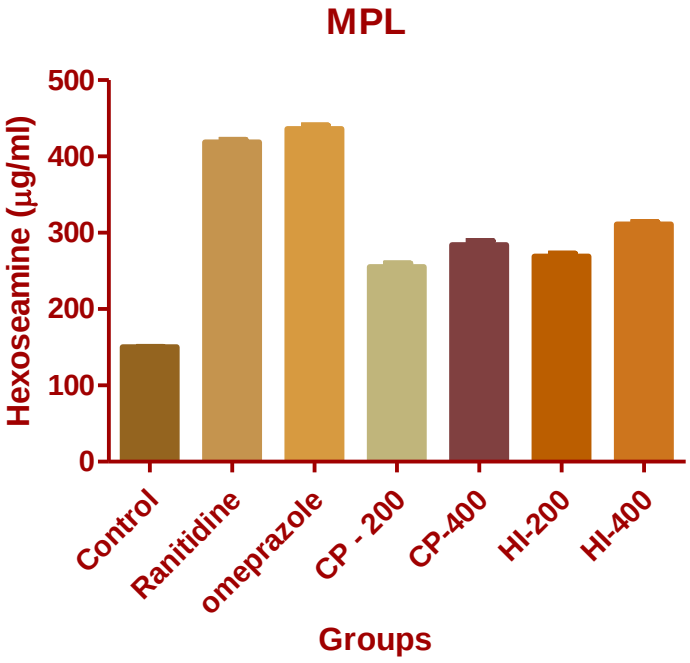
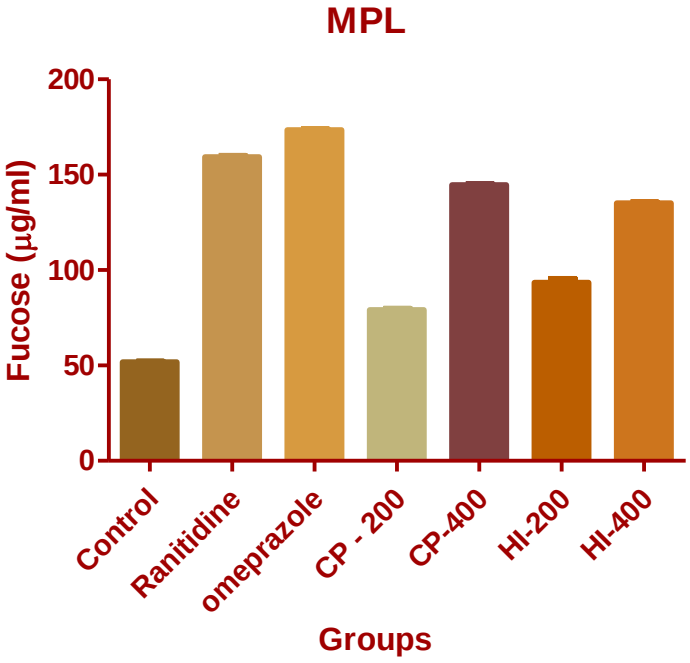


Fig No: 11 & 12 Effect of Medicinal Plants on Biochemical parameters of Modified pylorus ligated (shay) rat model. (MPL)



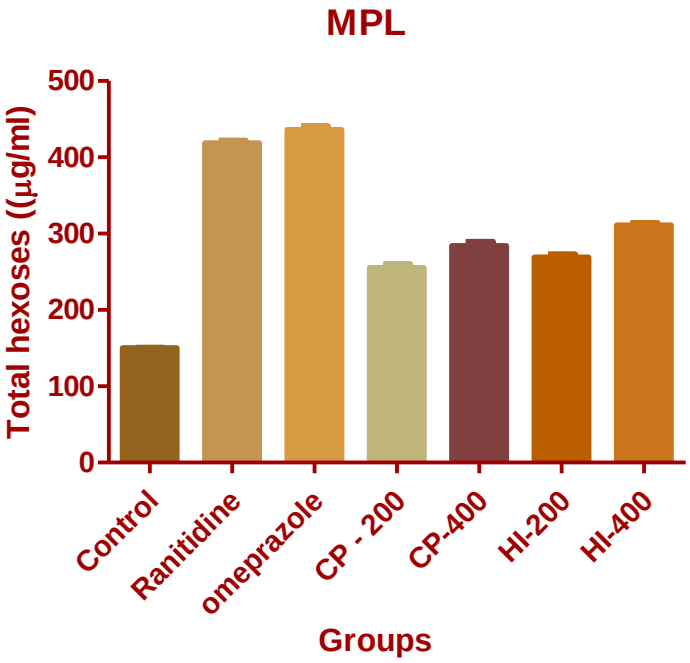
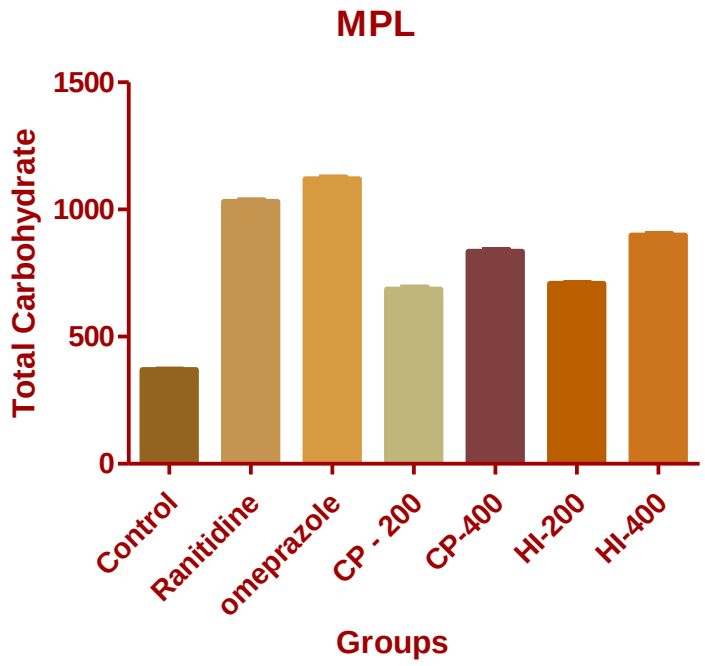


Fig No: 13 & 14 Effect of Medicinal Plants on Biochemical parameters of Modified pylorus ligated (shay) rat model. (MPL)



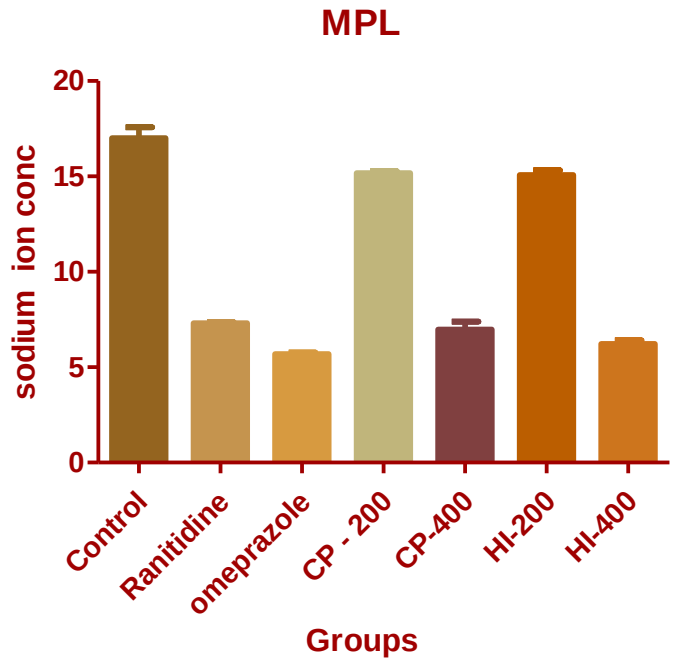
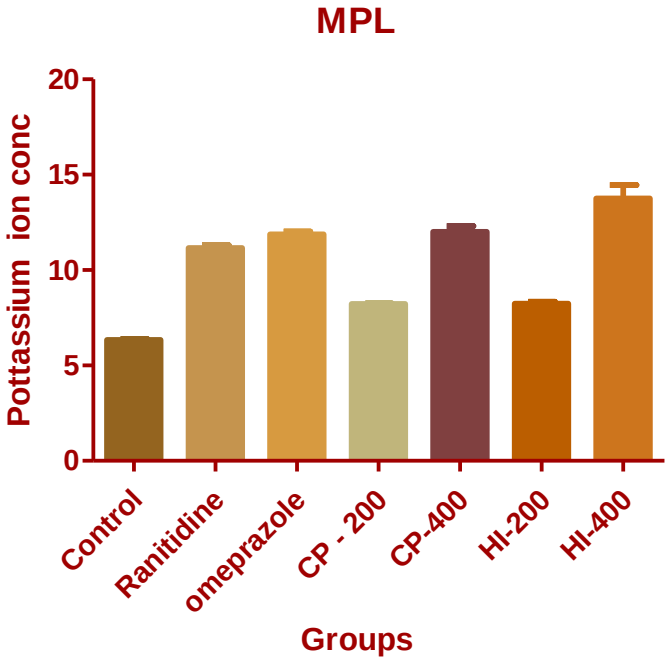


Fig No: 15 & 16 Effect of Medicinal Plants on Biochemical parameters of Modified pylorus ligated (shay) rat model. (MPL)



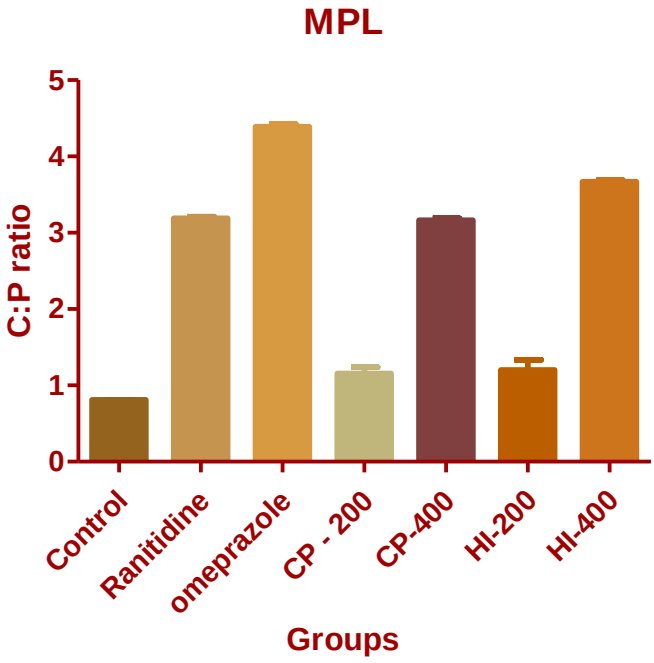


Fig No: 17 & 18 Effect of Medicinal Plants on Biochemical parameters of Modified pylorus ligated (shay) rat model. (MPL)

MPL- Modified pylorus ligation (Shay) rat model

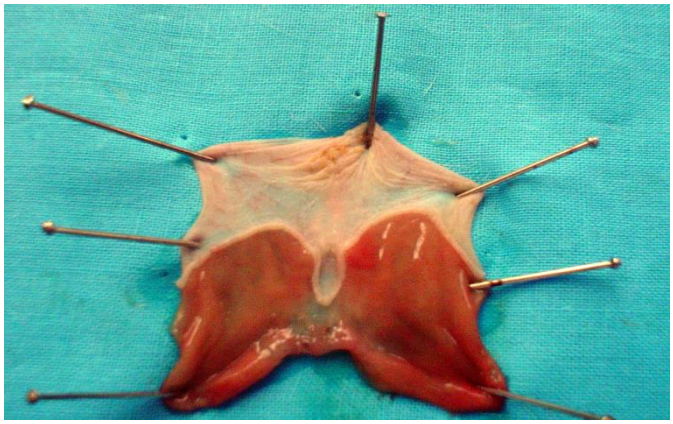


Plate No:**1**

Control 0.3% CMC

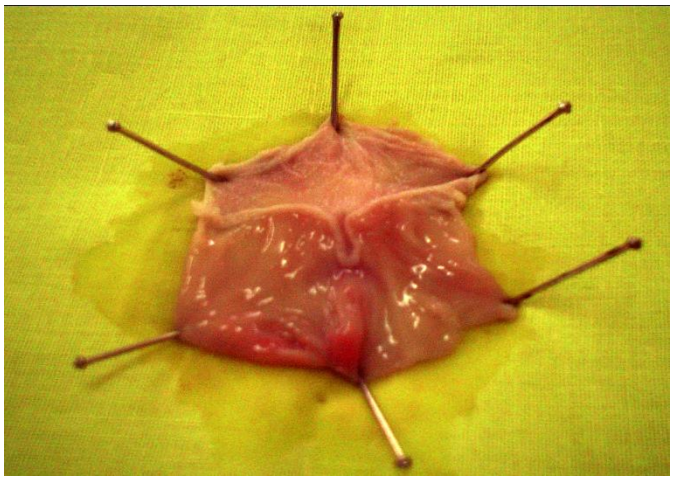
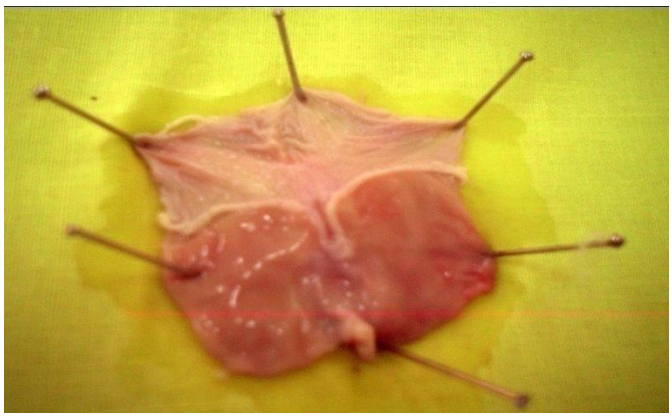


Plate No:2

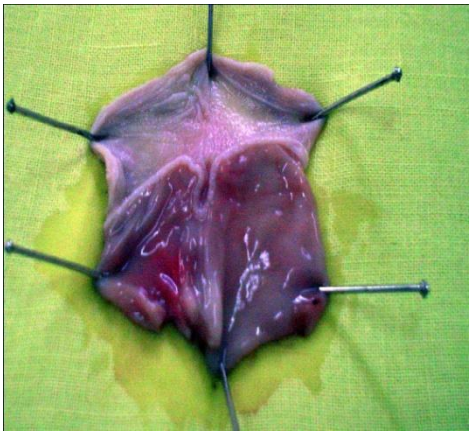
Ranitidine 27mg/kg

Plate No:3



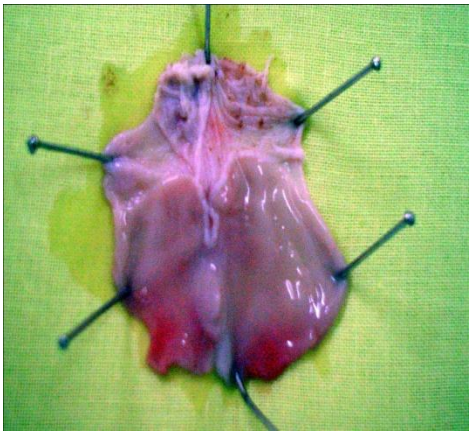
Omeprazole 2mg/kg

Plate No:4



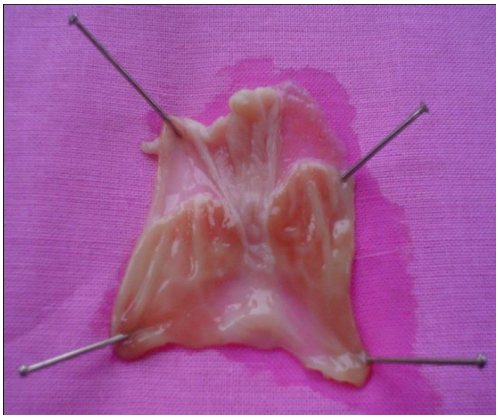
CP-200 mg/kg

Plate No:5



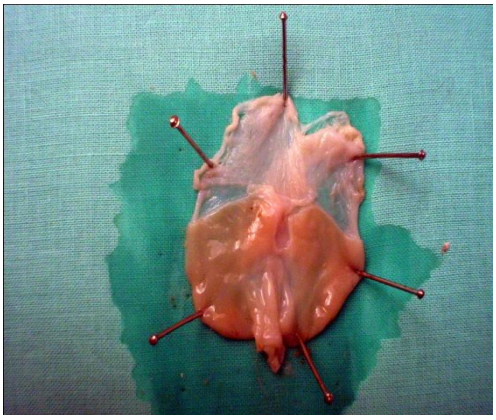
CP-400 mg/kg

Plate No:6



HI-200 mg/kg

Plate No:7



HI-400 mg/kg

Pharmacological evaluation of the hydroethanolic extracts of

i) C*yathula prostrata*

ii) *Heliotropium indicum*

Acute toxicity studies:

Based on the acute toxicity studies and the literature survey, 200 mg/kg and 400 mg/kg were selected for the further pharmacological studies, to observe the effect of this extracts on various experimentally induced ulcers. The anti-secretory effect of two medicinal plants namely C*yathula prostrata* and *Heliotropium indicum* hydroethanolic extracts was studied at two different dose level 200 mg/kg and 400 mg/kg b.w.o using this model.

Modified pylorus ligated (shay) rat model

The data are shown in table no 20 & 21.and figure no 5 to 18 The test extracts results showed that there was a significant decrease in gastric volume, [CP 4.5,2.8 and HI 4.5,2.6ml] ulcer index, [CP 3.2,1.5 and HI 3.2, 1.2] free acidity, [HI 38.3,18.5 and 38.3,22.8] total acidity, [CP 64.1, 28.6 and HI 63.73, 33.17] Na^+ ion concentration [CP 15.2,7 and [HI 15.1, 6.2] pepsin [CP 13.03,7.12 and HI 13,6] ($P< 0.05$, $P <0.01$, $P<0.001$) and significant increase in pH, (CP 3.5,4 and HI 3.3, 4.12 and carbohydrates (total hexose, hexosamine, fucose, sialic acid) and K^+ ion concentration (CP 8.2, 12 and HI 8.2, 13.75 which reflected a significant increase in the carbohydrates/protein (CP)

ratio, 1.2, 3.2 and HI 1.2, 3.7 ($p< 0.01$, $P<0.001$) when compared with the control group (figure no:18).

The test extract of C*yathula prostrata* hydroethanolic extract at both dose level 200 mg/ 400 mg/kg b.w.o showed a significant reduction in ulcer index, gastric volume (figure no: 5,7) when compared with solvent control, but it was equipotent to positive control standard drugs in decreasing the gastric volume at 400 mg/kg b.w.of CP and HI.. The pepsin activity and total protein were decreased at both test dose levels 200 mg/ 400 mg/kg b.w.o. It was observed that the hydroethanolic extract of C*yathula prostrata* was at 400mg/kg dose level equipotent with the positive control omeprazole 2 mg/kg .

The test drug *Heliotropium indicum* hydroethanolic extract at both dose level 200 mg /400 mg/kg b.w.o showed a significant reduction in ulcer index, gastric volume when compare with solvent control ($P< 0.01$, $P<0.001$) table no:20 & figure no:7,5.

The pepsin activity and total protein were significantly reduced at both dose level treated animals when compared with control group animals. The result showed that there was a significant decrease in gastric volume ulcer index, free acidity, total acidity, Na^+ ion concentration and pepsin ($P< 0.01$) and significant increase in p^H, carbohydrates (total hexose, hexosamine, fucose, sialic acid) and K^+ ion concentration which reflected a significant increase in the carbohydrates/protein (CP) ratio, $p< 0.01$, when compared with the control group.

C*yathula prostrata* and *Heliotropium indicum* hydroethanolic extract showed dose-dependent ulcer protective effect against pyloric ligation induced gastric ulcer and also in other models

CP & HI in both pylorus ligated aspirin plus pylorus ligated , induced ulcers did not have any significant effect on acid-pepsin secretion, but were found to increase mucin secretion quantified in terms of C:P ratio of the gastric juice. [131]

Increase in mucin was due to significant increase in individual mucopolysaccharides like sialic acid and total hexoses leading to significant increase in total carbohydrates. Both test extracts (CP & HI) were significantly increased glycoprotein content of mucosal cells as seen from the increase in the TC: P ratio of gastric mucosa (Goel et al., 1994). Consequently, it is learnt that both the test extracts induce turnover of glycoprotein in the mucosal cells, thus increasing the quantity of cellular mucous.

Ulcer index was taken into consideration as the prime parameter for the evaluation of anti-ulcer activity because the ulcer formation is linearly proportional to the factors involved in the ulcer induction. Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as reduction in gastric volume, decrease in free and total acidity. It is significant to note when the p^H reached 3, the ulcer score appeared less that is born out by the decrease in free acid which might have contributed to the anti-ulcer property of the tested extracts.[111]

The mucosal defense mechanism may be due to the epithelial cells of the gastric mucosa, which are impermeable to hydrogen ions thereby forming a physical barrier. Carbohydrates/ protein ratio also supports the above observation. The anti-ulcer effect is also supported by the decrease and an increase in the resistance factors like p^H, hexose, hexosamine, fucose and sialic acid.

The anti-ulcer activity of the drugs may be due to the presence of saponins, terpenoids, flavonols and amino acids. The anti-ulcer agent may protect the mucosa from acid effects by selectively increasing prostaglandin PGF2α, protection against experimental ulcers may be due to the effect of prostaglandins in the parietal cells, as prostaglandins enhance the mucosal resistance, perhaps by increasing the secretion of mucous and bicarbonates, strengthening the mucosal barrier, decreasing the gastric motility, increasing the release of endogenous mediators scavenging the free radicals, decreasing the release of endogenous amines and stimulation of cellular growth and repair.

In our present study supports that the above finding and the presence of chemical sontitiutents such as triterpenoids, saponins, phenolic compounds , flavanol in cyathula prostrate and lupeol, [phenolic compound and flavanol in Heliotropium indiucm. Promote ulcer healing.[166]

Table No: 22 Effect of Medicinal plants on Aspirin plus modified pylorus ligated (Shay) rat model (APL)

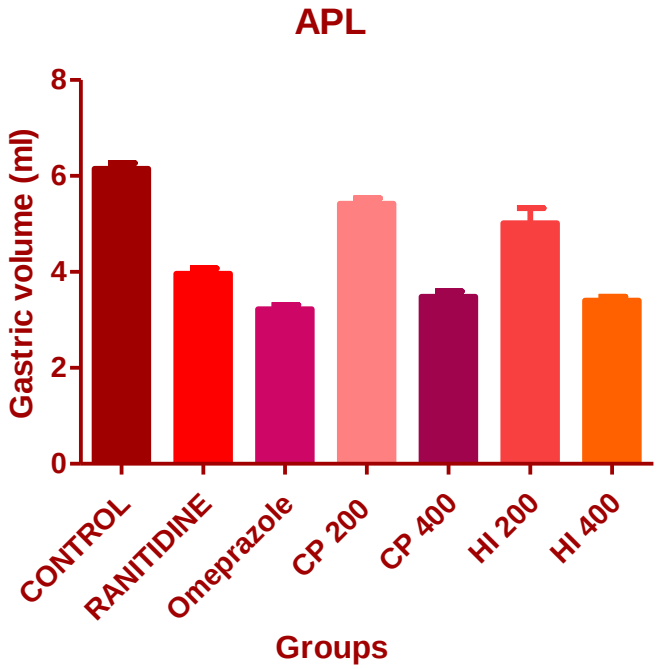
Group	Gastric Volume (ml)	pH	Ulcer index	Free acidity (Meq/l/100g)	Total acidity (Meq/l/100g)	Pepsin (µg/ml)	Total protein (µg/ml)
Solvent control	6.150 ±0.120	2.093 ±0.045	2.9 ±0.06	63.08 ±0.387	90.16 ±0.298	11.23 ±0.175	545.9 ±3.88
Ranitidine (27 mg/kg)	3.96 ±0.108***	3.933 ±0.2***	1.528 ±0.09***	40.52 ±0.382***	59.75 ±0.395***	8.337 ±0.183***	373.4 ±1.75***
Omeprazole (2 mg/kg)	3.225 ±0.083***	4.32 ±0.065***	1.493 ±0.022***	36.57 ±0.55***	55.22 ±0.35***	5.255 ±0.143***	375.0 ±01.496***
Cyathula prostrata (200 mg/kg)	5.53 ±0.455**	2.693 ±0.0148***	2.53 ±0.031*	59.16 ±0.09*	87.22 ±0.93**	10.34 ±0.123*	542.5 ±1.58*
Cyathula prostrata (400 mg/kg)	3.483 ±0.117*** a	3.970 ±0.134*** a	1.417 ±0.105*** a	37.71 ±1.12**a	40.21 ±0.339*** a	6.3 ±0.203*** a	321.1 ±02.270*** a
Heliotropium indicum (200 mg/kg)	5.520 ±0.011**	2.717 ±0.151**	2.525 ±0.032**	59.17 ±0.93*	87.34 ±0.065*	10.32 ±0.185**	530.6 ±2.308**
Heliotropium indicum (400 mg/kg)	3.44 ±0.088*** b	4.12 ±0.075 *** b	1.27 ±0.08*** b	34.05 ±01.045*** b	39.69 ±0.55*** b	5.450 ±0.025*** b	293.2 ±1.94*** b

Values are expressed as mean±SEM; n=6
Oneway ANOVA followed by Turkey's multiple comparison test
* P <0.05, ** P<0.01; *** P<0.001 when compared with solvent control
a P<0.001 when compared CP 200 Vs CP 400
b P<0.001 when compared HI 200 Vs HI 400

Table No:23 Effect of Medicinal plants onBiochemicals parameters of Aspirin plus modified pylorus ligated (Shay) rat model (APL)

Group	Total hexoses (µg/ml)	Hexosamine (µg/ml)	Fucose (µg/ml)	Sialic acid (µg/ml)	Total carbohydrates	Na^+ conc	K^+ conc	C:P-ratio
Solvent control	248.3 ±0.061	252.1 ±1.273	53.67 ±1.247	21.58 ±0.453	577 ±5.605	17.20 ±1.07	7.0 ±0.288	1.057 ±0.032
Ranitidine (27 mg/kg)	465.0 ±3.65***	398.3 ±3.032***	190.5 ±4.515***	40.62 ±0.32***	1158 ±5.714***	7.93 ±0.052***	18.25 ±0.149***	2.898 ±0.072***
Omeprazole (2 mg/kg)	451.7 ±6.91***	461.2 ±2.141***	192.5 ±2.391***	45.17 ±0.756***	1088 ±16.22***	6.42 ±0.18***	18.75 ±0.57***	3.100 ±0.01***
Cyathula prostrata (200 mg/kg)	271.2 ±2.674*	272.7 ±1.022*	65.67 ±1.23*	24.68 ±0.694*	689.2 ±2.630**	14.5.2 ±0.554**	9.125 ±0.26*	1.235 ±0.012**
Cyathula prostrate (400 mg/kg)	463.2 ±1.74*** a	405.5 3.212*** a	144.7 ± 2.231 *** a	30.17 ±0.833*** a	1002 ±5.178*** a	7.483 ±0.215*** a	19.0 ±0.402*** a	2.232 ±0.021*** a
Heliotropium indicum (200 mg/kg)	272.3 ±1.5*	273.5 ±1.15*	66.5 ±0.886*	24.28 ±0.498*	693.2 ±2.62**	14.0 ±0.23**	9.083 ±0.105**	1.256 ±0.011**
Heliotropium indicum (400 mg/kg)	427.0 ± 4.722*** b	405.2 ±3.15*** b	145.7 ±23.5*** b	39.93 ±0.55*** b	1047.8 ± 4.865*** b	6.67 ±0.211*** b	19.5 ±0.342*** b	3.570 ±0.05*** b

Values are expressed as mean±SEM; n=6
Oneway ANOVA followed by Turkey's multiple comparison test
* P <0.05, ** P<0.01; *** P<0.001 when compared with solvent control
a P<0.001 when compared CP 200 Vs CP 400
b P<0.001 when compared HI 200 Vs HI 400



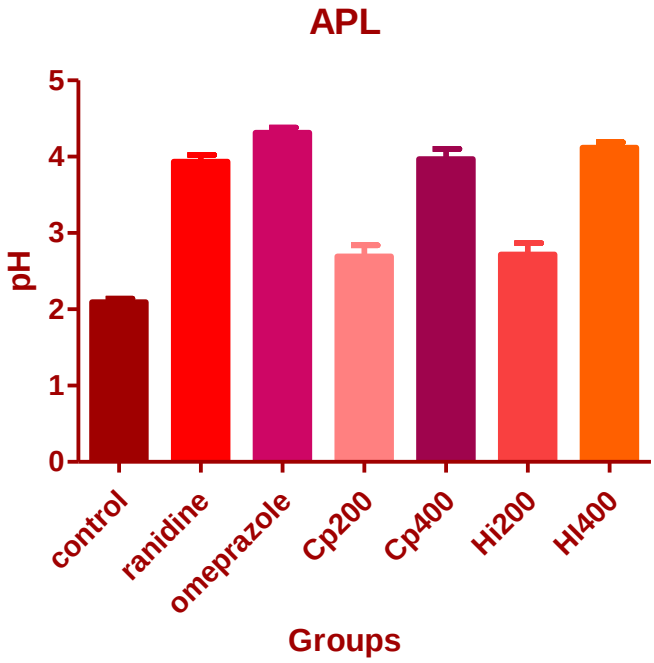
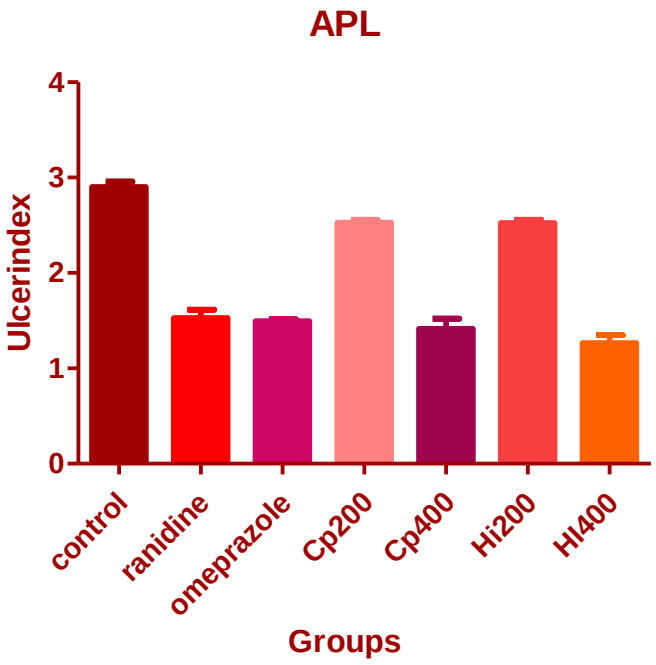


Fig No: 19 & 20 Effect of Medicinal Plants on Biochemical parameters of Aspirin plus pylorus ligated (shay) rat model. (APL)

Phytochemcial and pharmacological Studies on
Cyathula prostrata (Linn) Blume (CP) and Heliotropium indicum Linn (HI)



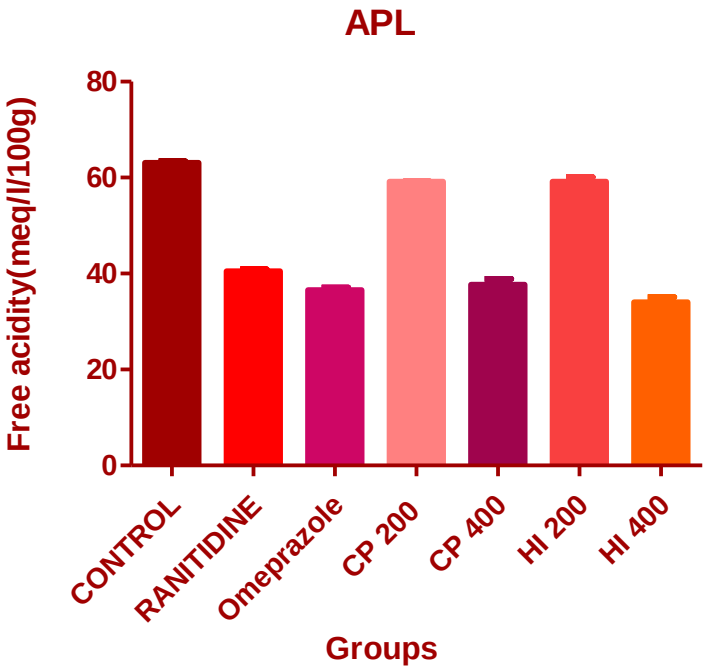
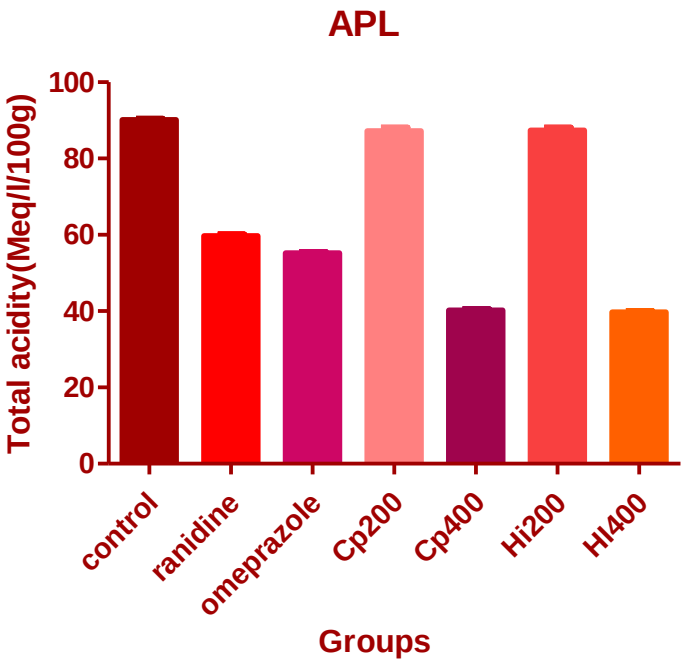


Fig No: 21 & 22 Effect of Medicinal Plants on Biochemical parameters of Aspirin plus pylorus ligated (shay) rat model. (APL)



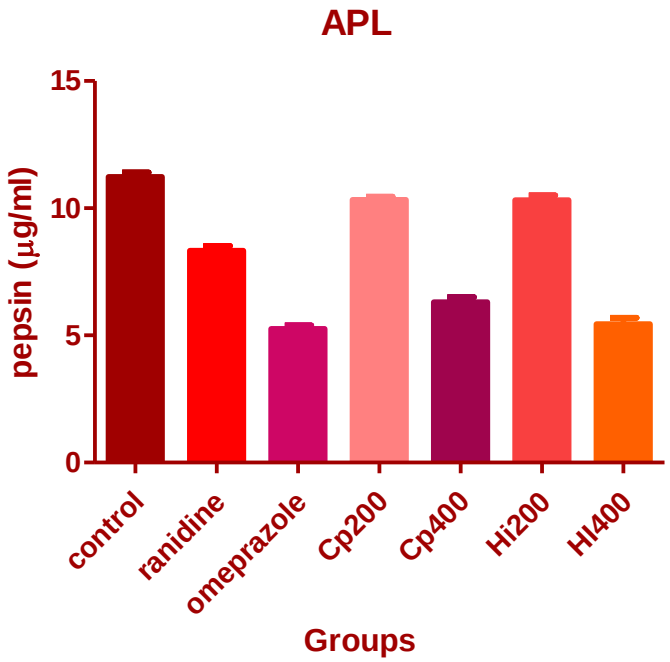
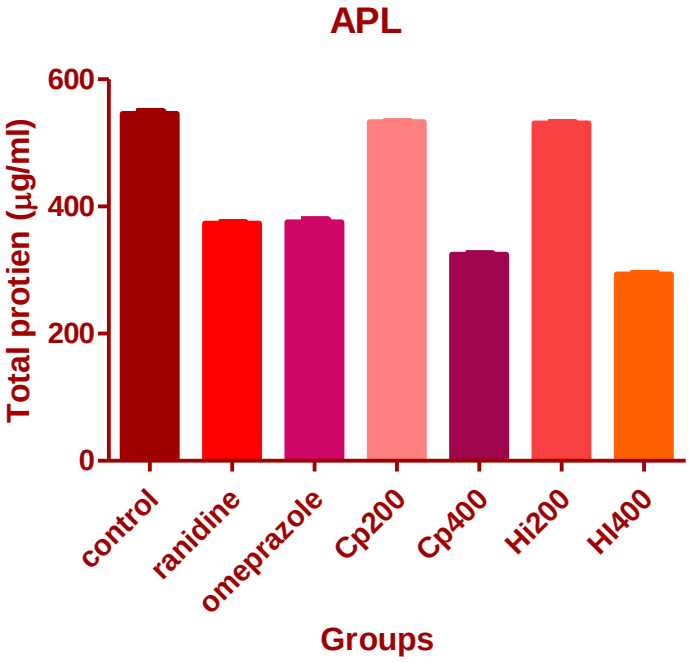


Fig No: 23 & 24 Effect of Medicinal Plants on Biochemical parameters of Aspirin plus pylorus ligated (shay) rat model. (A



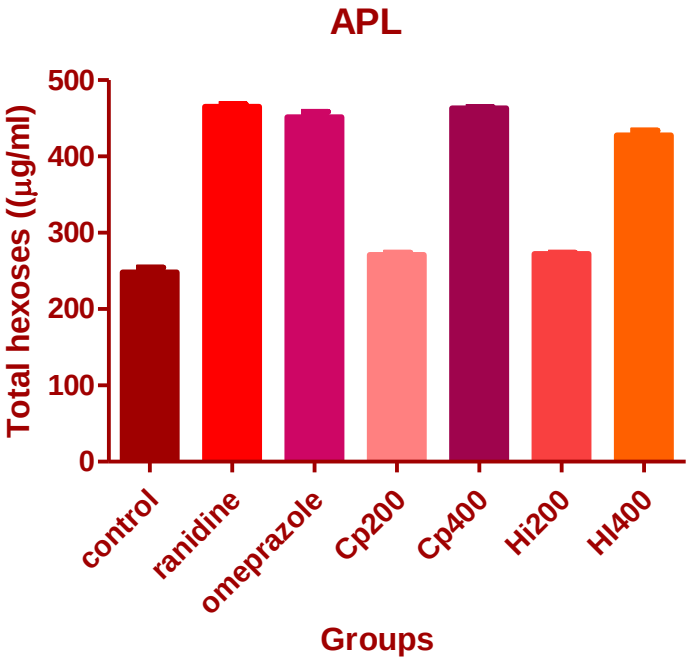
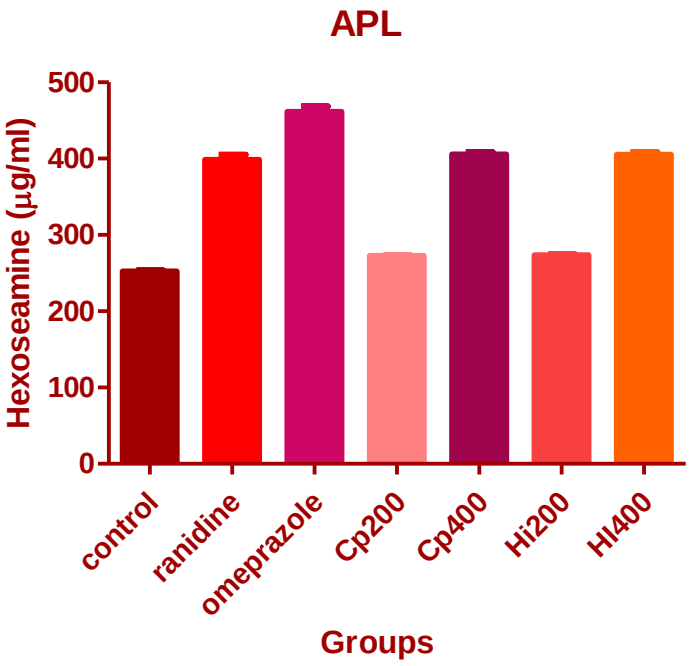


Fig No: 25 & 26 Effect of Medicinal Plants on Biochemical parameters of Aspirin plus pylorus ligated (shay) rat model. (APL)



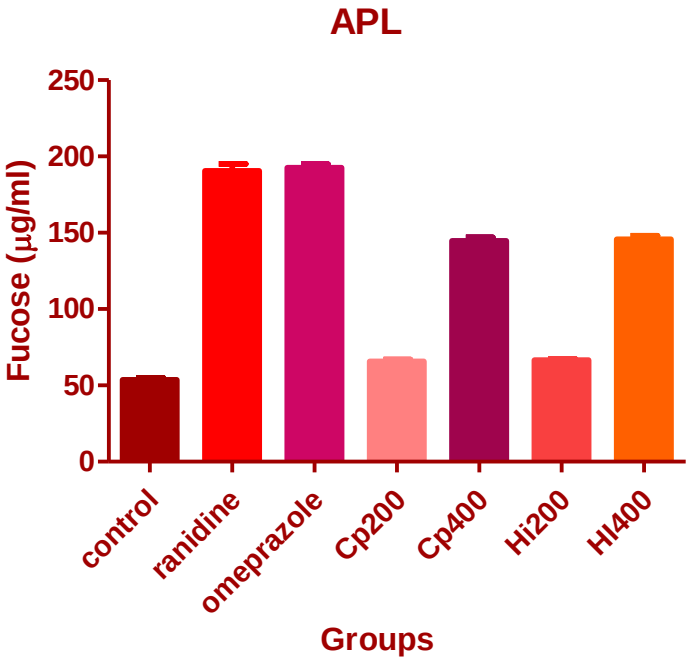
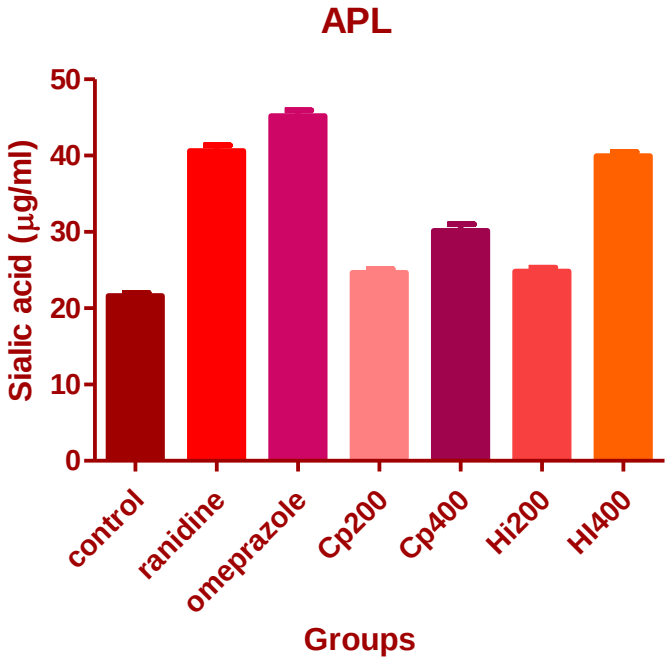


Fig No:27 & 28 Effect of Medicinal Plants on Biochemical parameters of Aspirin plus pylorus ligated (shay) rat model. (APL)



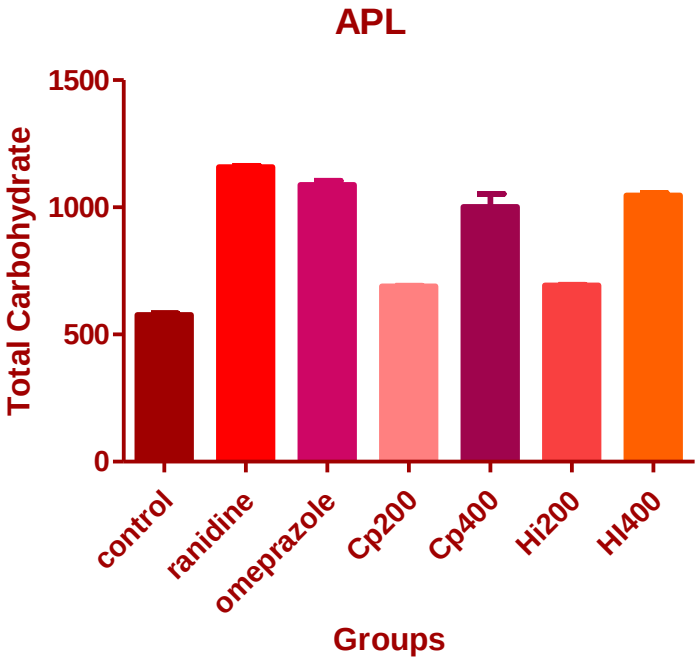
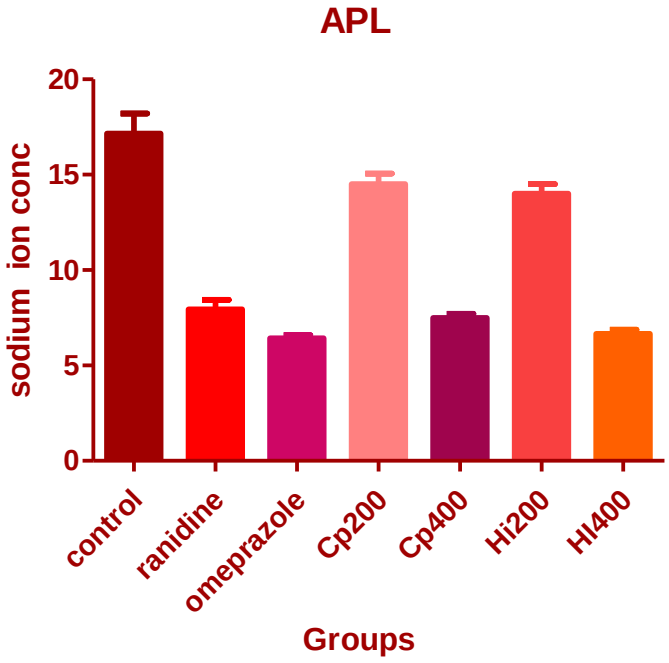


Fig No: 29 & 30 Effect of Medicinal Plants on Biochemical parameters of Aspirin plus pylorus ligated (shay) rat model. (APL)



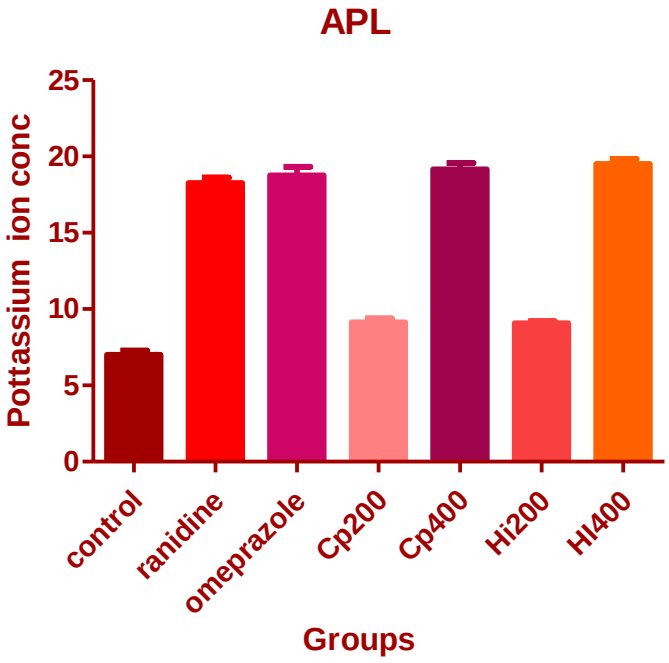


Fig No: 31 & 32 Effect of Medicinal Plants on Biochemical parameters of Aspirin plus pylorus ligated (shay) rat model. (APL)

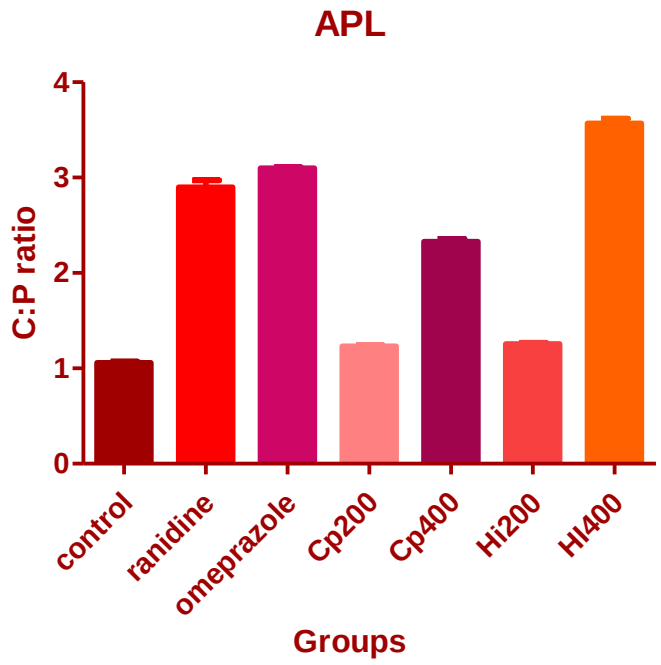
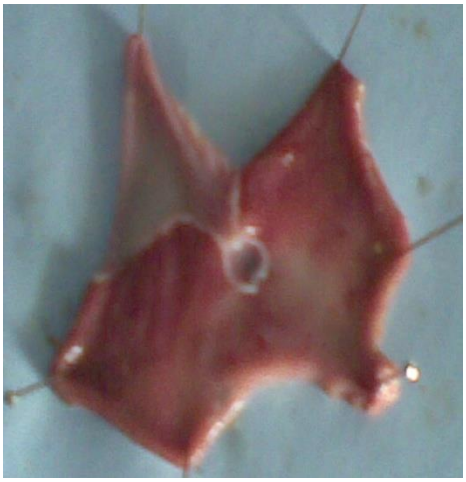


Fig No: 33 Effect of Medicinal Plants on Biochemical parameters of Aspirin plus pylorus ligated (shay) rat model. (APL)

Aspirin plus modified pylorus ligation (Shay) rat model

Plate No:8

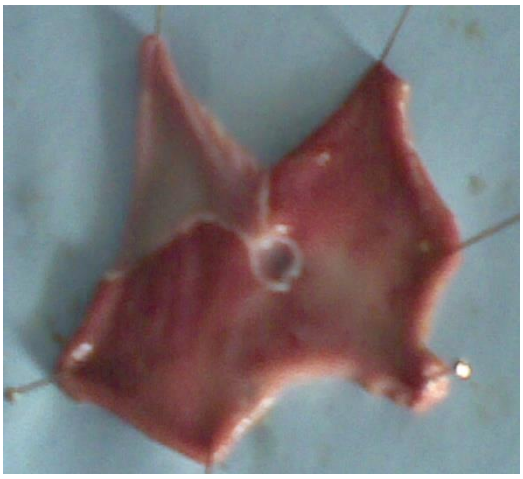


Control 0.3% CMC

Plate No:9

Ranitidine 27mg/kg

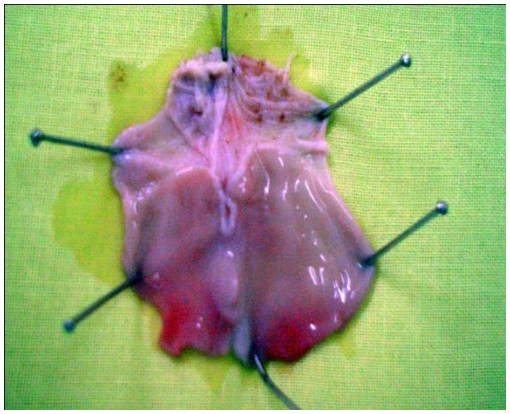
Plate No:10



Omeprazole 2mg/kg

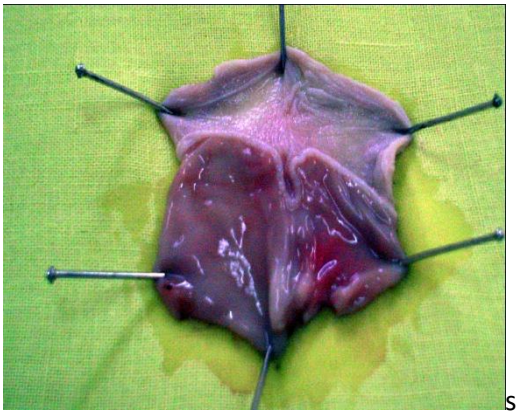
Aspirin plus modified pylorus ligation (Shay) rat model

Plate No:11



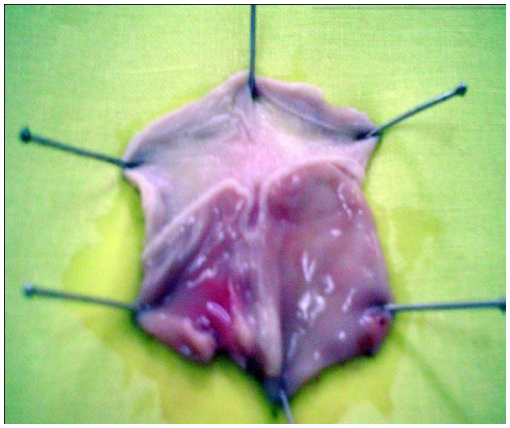
CP-200 mg/kg

Plate No:12



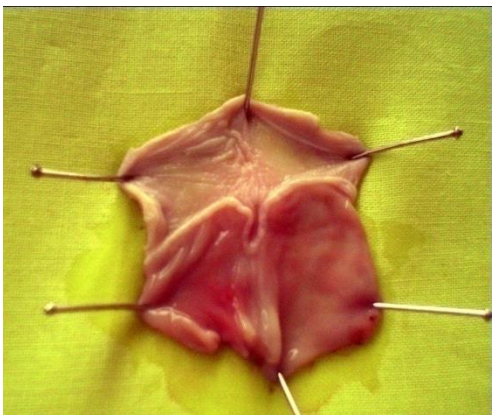
CP-400 mg/kg

Plate No:13



HI-200 mg/kg

Plate No:14



HI-400 mg/kg

Aspirin plus pylorus ligated (shay)s rat model

The tabulated parameter of ulcer inhibition was described in materials and methods. The value of mean and standard error (n=6) in each experiments in all group P**<0.01 and P* <0.05 values were compared to control group and evaluated by using one ANOVA followed by Turkey's multiple comparison test

The result have expanded on table no 22 ,23 .and fig no 19,33 the extract treated groups showed the gastric volume, [CP 5.5,3.5 and HI 5.5,3.4] free acidity, [59.2, 37.7 and HI 59.1, 34, total acidity, [CP 87.2, 40.2 and HI 87.3,39.6] ulcer index [CP 2.5,1.4 and HI 2.5, 1.3] and pepsin levels [CP 10.4,6.3 and HI 10.3, 5.45} were significantly reduced (*P<0.05, &P**<0.01, P***0.001)when compared to control group

The table no 22,23 and fig no 20,30,33 showed, extract treated groups parameter of gastric pH, total carbohydrates and C:P ratio were significantly increased (P**<0.01 and P* <0.05) (P**<0.01, (P***<0.001) when compared with control group.

The table number 23 .and fig no 31,32 indicated the estimation of Na^+ and K^+ ion present in gastric acid. The extract treated groups gastric acid indicating $Na^{+\,level}$ decreases and K^+ ion level was gradually increased P<0.01 and P <0.05, P<0.01 and P<0.001 when compared with control group.

Indigenous drug system can be source of variety of new drugs which can provide to relieve the ulcer ailments. Our present study is claimed to develop the novel plant based anti ulcer drug.

Interestingly the treatment of aspirin one hour before the ligation changed the acidity of the herbs (extracts) in comparison with the pyloric ligation alone.

Pretreatment of aspirin along with pylorus ligation aggrevated the ulcer index and acid secretion in the control groups. In other words, aspirin produces its pharmacological action by inhibiting cox1 and cox2 enzymes which are responsible for PG synthesis . It is well known that reduction in PG levels mostly leads to more ulcer formation. [45] Further the free acidic group in the aspirin may also contribute to the increased ulcer index.

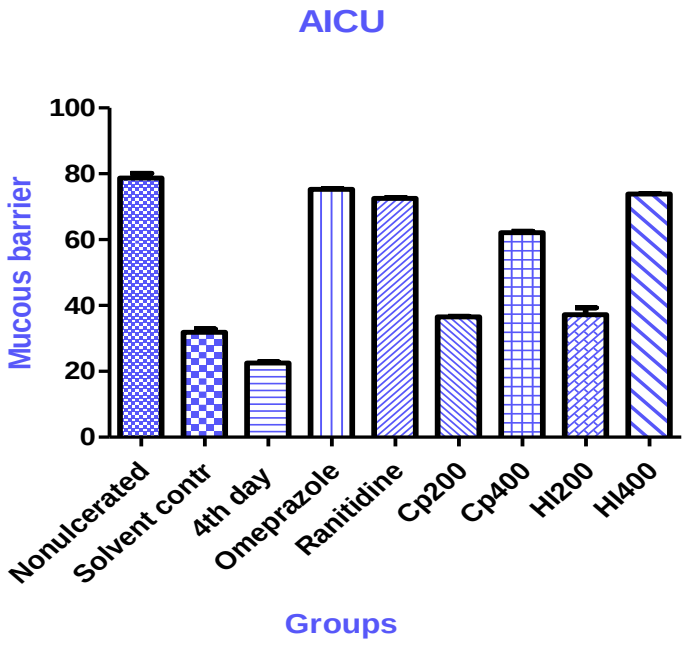
Ulcer caused by chemical inducers like ethanol and aspirin could be due to their direct effect of release of noxious substances including free radicals. Disruption of prostanoid synthesis is another contributing factor for aspirin induced ulcer .[105]

Table No:24 Effect of Medicinal plants on Acetic acid induced Gastric mucosal damage model

S.N.	Group	Ulcer index	Mucose barrier (mg/Alcian blue/gm)	Non protein sulfhydryl (NP-SH) glandular tissue/gm	Anti oxidant Estimation		
					Catalase (iu/mg)	Superoxide dismutase (iu/mg)	Lipid peroxidase (iu/mg)
1.	Non-Ulcerated	-------	78.26±1.216	0.871±0.007	28.89 ±0.539	3.62±0.282	0.29±0.02
2.	Solvent control Ulcerated	2.91±0.06	31.88 ±0.942	0.271±0.010	15.17±0.313	1.91±0.08	1.22±0.069
3.	Ulcerated sacrificed on fourth day	3.2±0.07	22.58±0.282	0.1565±0.002	10.79±0.460	0.83±0.064	1.52±0.051
4.	Ranitidine 27 mg/kg	1.13 ±0.39 *** ***	72.54 ±0.275 *** ***	0..819 ±0.01 *** ***	25.58 ±0.383 *** ***	3.01 ±0.207 *** **	0.40 ±0.010*** ***
5.	Omeprazole 2 mg/kg	1.33 ±0.088 *** ***	75.31±0.248 *** ***	0.862±0.007*** ***	27.86 ±.477 *** ***	3.52 ±0.164*** ***	0.365±0.01 95 *** ***

6.	Cyathula prostrata (200 mg/kg)	2.38±0.03 *** *	36.58±0.117 *** *	0.332±0.009*** *	16.61±0.25*** ***	2.898 ±0.024*** **	0.9833 ±0.053*** *
7.	Cyathula prostrata (400 mg/kg)	1.38±0.098*** ***	62.15±0.366 *** ***	0.436±0.017*** ***	28.57±0.382*** ***	4.198 ±0.033*** ***	0.87 ±0.034*** ***
8.	Helio tropium indicum 200 mg/kg	2.23±0.04*** ***	37.23±2.055 *** **	0.3212±0.018*** ***	17.08±0.186*** *	2.8451 ±0.232*** *	0.9717 ±0.075 *** **
9.	Helio tropium indicum 400 mg/kg	1.43±0.04*** ***	73.83±0.180*** ***	0.837s±0.009*** ***	27.83±0.383*** ***	4.417± 120*** ***	0.375 ±0.01*** ***

Values are expressed as mean±SEM; n=6
Oneway ANOVA followed by Turkey's multiple comparison test
* P <0.05, ** P<0.01; *** P<0.001 when compared with solvent control (ulcerated) and ulcerated-sacrificed on 4th day
^{a} P<0.001 when compared CP 200 Vs CP 400
^{b} P<0.001 when compared HI 200 Vs HI 400



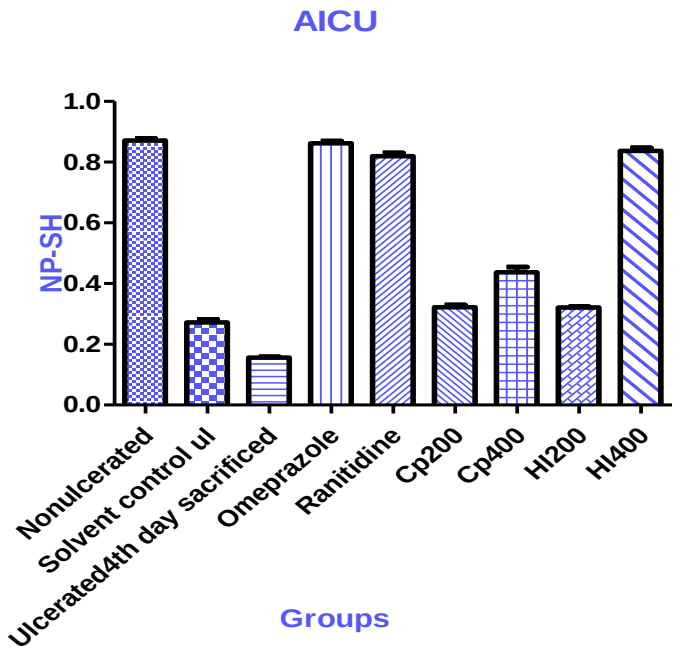
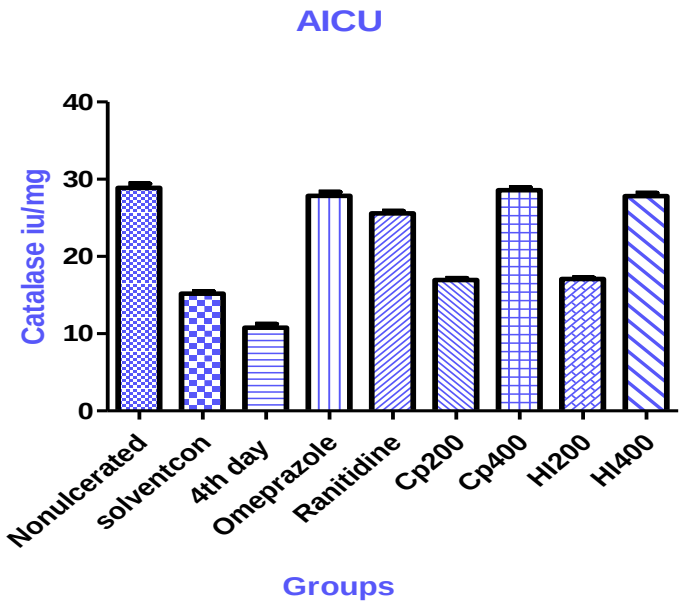


Fig No: 34 & 35 Effect of Medicinal Plants on Acetic acid Induced Gastric Mucosal Damage Model



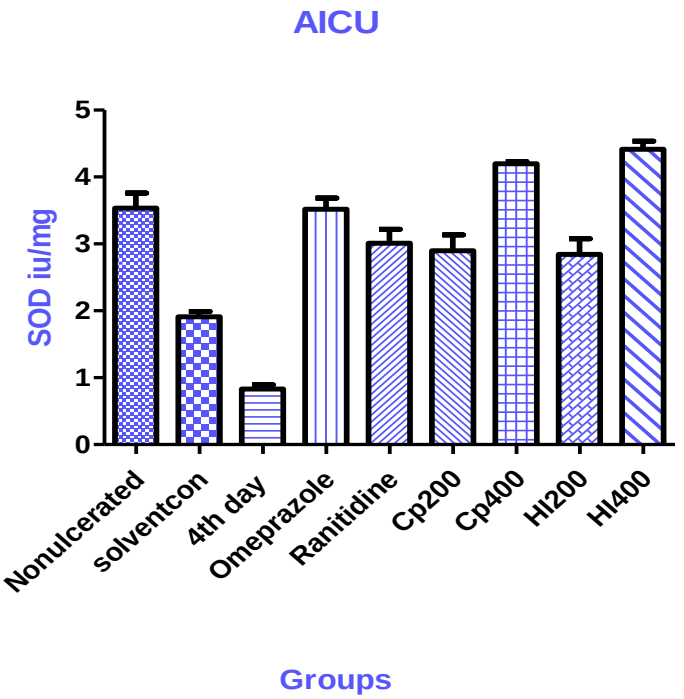


Fig No: 36 & 37 Effect of Medicinal Plants on Acetic acid Induced Gastric Mucosal Damage Model

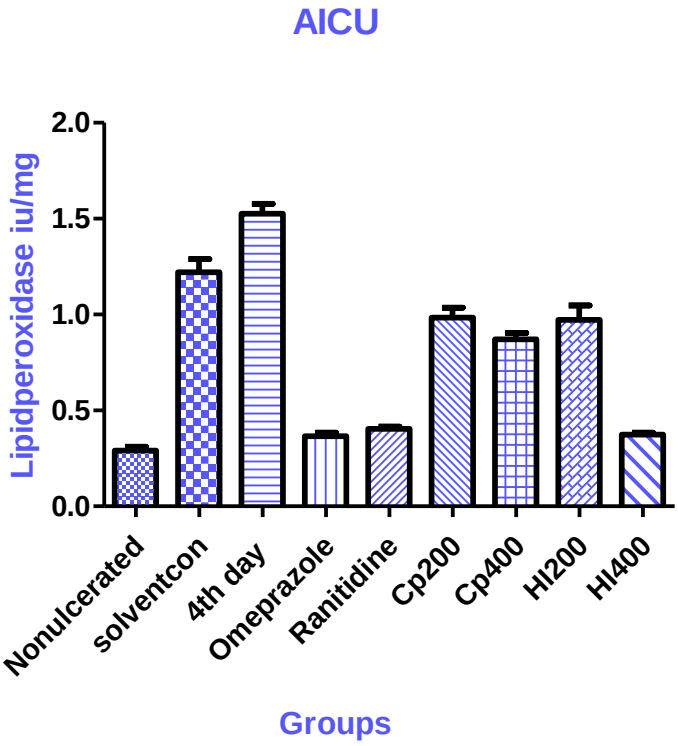


Fig No: 38 & 39 Effect of Medicinal Plants on Acetic acid Induced Gastric Mucosal Damage Model

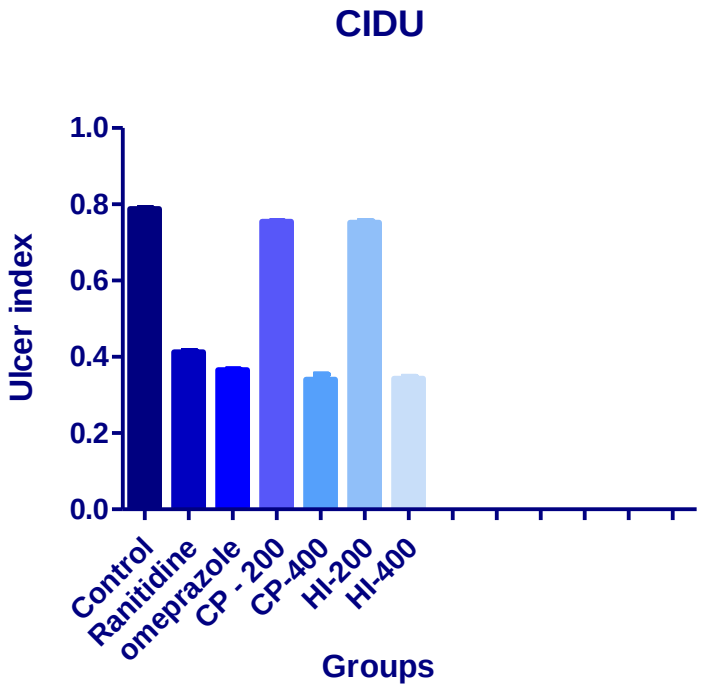


Fig No: 39 Effect of Medicinal Plants on Cyestamine induced duodenal ulcer model. (CIDU)

AICU- Acetic acid induced gastric mucosal damage

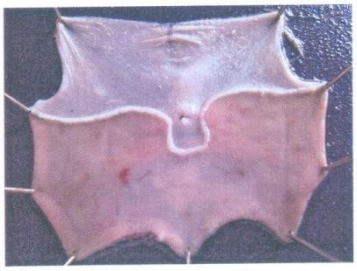
Plate No:15

Normal control

Plate No:16

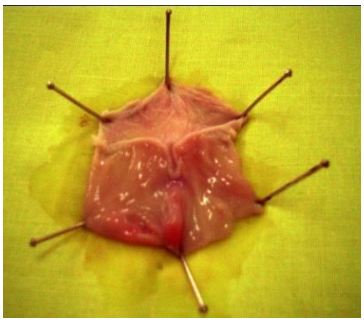
Ulcerated control

Plate No:17



4th day ulcerated control

Plate No:18



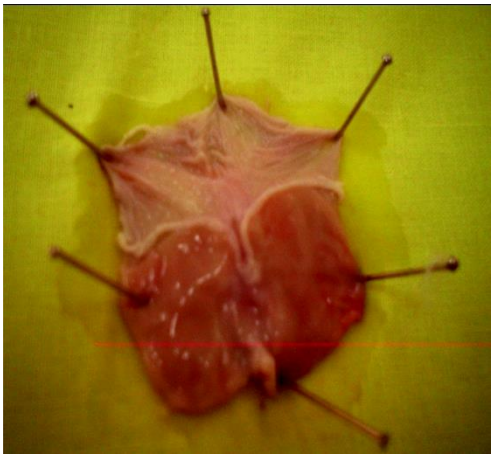
Ranitidine 27mg/kg

Plate No:19

omeprazole 2mg/kg

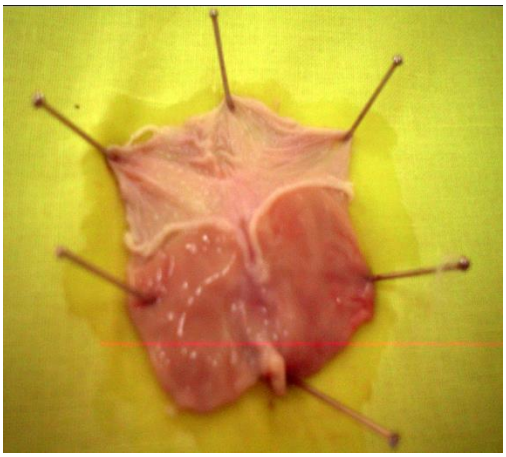
AICU

Plate No:20



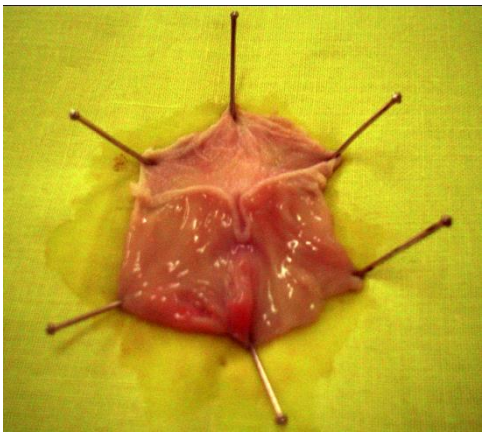
CP-200 mg/kg

Plate No:21



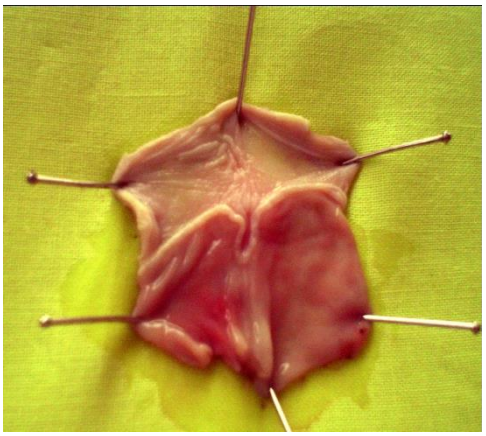
Cp-400 mg/kg

Plate No:22



HI-200 mg/kg

Plate No:23



HI-400 mg/kg

Acetic acid induced chronic ulcer model

Mucous barrier

The above tabulated parameters showed the healing of ulcers was measured by (n=6) in each experiments in each group (P** <0.01 and P* <0.05) values were compared to solvent .control and 4th day ulcerated group evaluated by using one way ANOVA followed by Turkey"s multiple comparison test..

Animals in the solvent group showed a significantly decreased (P<0.01) in the mucous barrier when compared with those of the non ulcerated control group. When another group of ulcerated animal were sacrificed on the 4th day the mucous membrane size was reduced (22.58 ±0.2817/µg) alcian blue/g of glandular tissue P<0.01 when compared with vehicle control.

Administration of *Cyathula prostrata* (cp) and *Heliotropium indicum* extracts(pH) produced significant (P<0.01) recovery of the mucous membrane size in a dose dependent manner. The size of the mucous membrane increased up to 36.58± 0.117 µg and 62.15± 0.366 µg alcian blue /g of glandular tissue when the ulcerated animals treated with *Cyathula prostrata* at 200 and 400 mg/kg b.w respectively. The size increased up to 37.23 ±0.05 µg and 73.83 ± 0.18 µg when the ulcerated animal treated with *Heliotropium indicum* at 200 and 400mg/kg b.w respectively. The recovery of mucous membrane in the ulcerated animal when treated with omeprazole (2mg/kg) and ranitidine (27mg/kg). The size of the mucous membrane in the animals treated with the standard drugs was 75.26±0.25 µg Omeprazole 2mg/kg and 72.54±0.275 µg Ranitidine 27mg/kg, alcian blue/g of glandular tissue respectively (Table no: 24).

Non-protein sulfhydryl group

The level of the non-protein sulfhydryl group in the non-ulcerated control group found to be 0.870±0.007, in solvent control ulcerated (0.271±0.01) and fourth day (0.156±0.002) were significantly (P<0.01) decreased. Ulcerated animals when treated with *Cyathula prostrata &Heliotropium indicum* at dose levels of 200 and 400mg/kg body weight, showed a dose dependent and significant (P<0.01) increase in the non-protein sulfhydryl groups. The levels increased up to 0.332±0.009 and 0.436±0.017. Treatment with *Heliotropium indicum* at the same dose levels of 200 and 400 mg/kg b.w, increased the levels in a dose dependent manner up to 0.321±0.018 and 0.836±0.009 respectively. The increase observed with *Heliotropium indicum* was significant (P<0.01) at 200mg/kg b.w dose level and was more significant (P< 0.001)) at 400 mg/kg dose level. The increase was comparable with that of the standard drugs with omeprazole (2mg/kg; 0.862±0.009 and (P<0.05) ranitidine (27mg/kg; 0.819±0.010 and P<0.01) Table no: 24

Catalase

The level of the catalase in the non-ulcerated group was found to be 28.89±0.539 iu/mg proteins, in solvent group the levels were decreased significantly (P<0.01) (15.17±0.313 iu/mg protein) and on fourth day (10.79±0.460). Ulcerated animals when treated with *Cyathula prostrate* at dose levels of 200 and 400 mg/kg b.w showed a dose dependent and significant (P<0.01) increase in the catalase levels. The levels increased up to 16.95±0.350 1u/mg protein and 28.57±0.038 1u/mg protein on *Cyathula prostrata* treated animals at a dose level of 200 and 400 mg/kg. b.w respectively , on treatment with *Heliotropium indicum* at the same dose levels of 200 and 400 mg/kg b.w increased the levels significantly (P<0.01) in a dose dependent manner up to 17.08±0.186 and 27.83±0.383 1u/mg proteins respectively. The increase was comparable with that of the

standard drugs with Omeprazole(27.86±0.477 1u/mg protein) and Ranitidine (25.58±0.30 iu/mg protein) table no: 24.

Super oxide dismutase

Super oxide dismutase(SOD) levels were measured in all the control and treatment groups. In the normal non-ulcerated control group animals the SOD level was found to be 3.62±0.282 iu/mg protein. This level had decreased up to 1.91±0.0079 1u/mg protein on the first day and 0.83±0.068 1u/mg protein on the fourth day sacrificed ulcerated group of animals.

Animals treated with *Cyathula prostrata* showed SOD levels of 2.9±0.24 iu/mg protein. and *Heliotropium indicum* showed a signicant increase at 200mg/kg dose level, but not significant at 400mg/kg dose level 2.845±0.23, 4.42±0.12 iu respectively. The standard drugs omeprazole and ranitidine increased the level of SOD up to 3.52±0.164 Omeprazole 2mg/kg and 3.012±0.207 1u/mg protein ($p<0.001$) respectively. (Table no: 24)

Lipid peroxidase:

The Lipid peroxidase activity was measured in all the control and treatment group animals. In the non-ulcerated control group the activity was found to be 0.29±0.019 µ mol/mg. the Lipid peroxidase activity in the solvent control group were increased and found to be 1.22±0.07 on the first day and 1.52±0.051 µ mol/mg on the fourth day. The decrease in the activity observed when the ulcerated animals were treated with *Cyathula prostate* and *Heliotropium indicum*, at a dose level of 200mg/kg was 0.983±0.29 µ mol/mg and at 400mg/kg dose was 0.87±0.034 m mol/mg. both the dose levels were found to produce significant ($P<0.001$) decrease in the lipid peroxidation activity. *Heliotropium indicum* produced significant ($P<0.001$) decreased at 200mg/kg dose level (0.972±0.075 µ mol/mg) and the effect was moret significant ($P_{,}<0.001$) at 400 mg/kg dose (0.375±0.01 µ mol/mg). The decrease level observed in the animals treated with the

standard drugs omeprazole and ranitidine was 0.36±0.01 µ mol/mg and 0.04±0.010 µ mol/mg respectively. (Table no: 24)

Discussion

The defense mechanism of the gastrointestinal mucosa agent aggressive factors, such as hydrochloric acid, bile acid and non-steroidal anti-inflammatory drugs mainly consists of functional, humoral and motility act as functional factors while prostaglandins and nitric oxide act as humoral factors and capasaicin sensitive sensory neurons act as neuronal factors. All the above factors are known to contribute to mucosal protection. (yashiro et.al.(2001)).

Oxygen derived free radicals play a key role in the mechanism of acetic acid induced mucosal lesion. Many reports have demonstrated that most injury of gastric mucosa can be reduced with pretreatment with scavengers of reactive oxygen species. Oxygen handling cells have antoxidant enzymes such as CAT, SOD, GST, GPX and GSH which are the first line of cellular defense against oxidative injury, decomposidity O2 and H2O2 before they interact to form more reactive(OH-) radicals. SOD and CAT enzymes are highly specific in their catalytic mode of action and it decreases the gastric mucosal damaging effect of aspirin.(Jainu. Mallika et.al.2004).

Oxygen derived free radical may play important role in delaying the healing of acetic acid induced chronic gastric ulcers in rats (kanoka hamaishi et.al 2006). Acetic acid is reported to produce ulcers by gastric construction leading to increase in acidic gastric juice. Perhaps increase in defensive mucosal factors may have beneficial role in protecting ulcers induced by acetic acid (sairam.et.al.2003).

The catalase in metabolic oxidizing enzyme and which is involved in the conversion of, from hydrogen peroxide to oxygen and water(goodmann and gilman p480).

The super oxide dismutase is involved in gene mutation process and catabolism of potentially toxic superoxide radicals in stomach.

The lipid peroxidase enzyme involved in the prostaglandin Geosynthesis and specific prostoglandia p(GE2) activators the mucus barrier in stomach.

The gastric mucosa continuing the high concentration of reduced ghitathime, the major component of endogenous NP-SH pool might involve in scavenging oxygen derived free radicals. The mucus also continuously coats over the gastric mucosa and mucus might implicate in scavenging oxygen derived free radicals. (chen sheng et.al.2005)

These are evidences for the participation of reactive oxygen species in etiology and pathophysiology of human disease, such as neurodegenerative , inflammation, viral infection, auto immune gastrointestional inflammation and gastric ulcer(Repetto et.al.2002)

The importance of mucous secretion as a response to gastric mucosal has long been recognized investigated the protective role of mucous on gastric mucosa. Suggested the defense mechanism of gastric mucosa is superior to others. More the production of mucosa, the less was the degree of ulceration. Mucous also protects the mucosa and sub-mucosa from inflammatory reaction.(Anoop.et.al,2003).

From the data of chronic acetic acid induced ulcer model it is concluded that the hydroethanolic extract of cyathula prostrate and Heliotropiom indicum effectively increased the enzymatic antioxidants such as CAT, SOD and on the other hand it decreased LPO level. Also it increased the different defense factors of ulcer such as NP-SH, mucous production and decreased the ulcer index.

The present study is attempted on plant based anti ulcer drug and this work, believe that it will be useful for further anti ulcer research workers, such as isolation of compound from extract, IR, UV, MS, NMR spectroscope analysis to conform the identity as well as the molecular structure and to patent the drug, related to anti ulcer property.

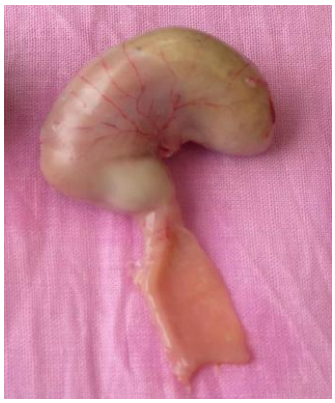
Table No:25 Effect of Medicinal plants on Cysteamine induced duodenal ulcer model (CIDU)

Group Drug/Dose	Ulcer Incidence		Ulcer index
	Number	%	
Solvent control 0.3% w/v conc	6/6	100	0.7880 ± 0.003
Ranitidine(27 mg/kg)	2/6	33.3	0.4120 ± 0.0037***
Omeprazole (2 mg/kg)	0/0	0	0.3652 ± 0.0018***
Cyathula prostrata (200 mg/kg)	3/6	50	0.7547 ± 0.001**
Cyathula prostrata (400 mg/kg)	2/6	33.33	0.3400 ± 0.014*** a
Heliotropium indicum 200 mg/kg	3/6	50	0.7518 ± 0.002**
Heliotropium indicum 400 mg/kg	2/6	33.33	0.3430 ± 0.0051*** b

Values are expressed as mean ± SEM; n=6
Oneway ANOVA followed by Turkey's multiple comparison test
** P<0.01; *** P<0.001 when compared with solvent control
a P<0.001 when compared CP 200 Vs CP 400
b P<0.001 when compared HI 200 Vs HI 400

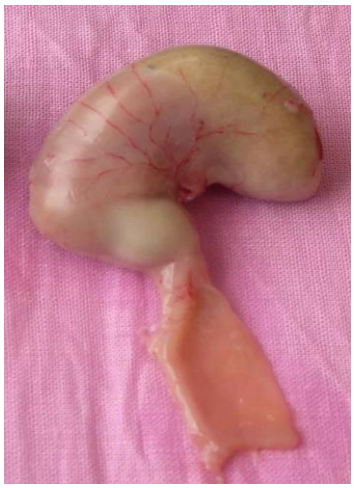
CYSTEAMINE INDUCED DUODENAL ULCER MODEL

Plate No:24



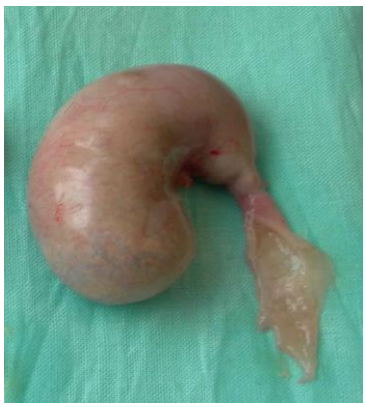
Control 0.3% CMC

Plate No:25



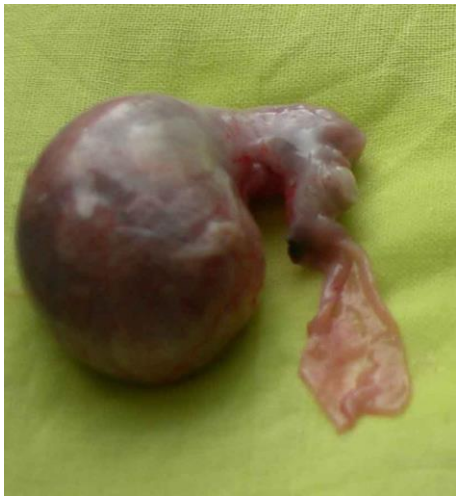
Ranitidine 27 mg/kg.

Plate No:26



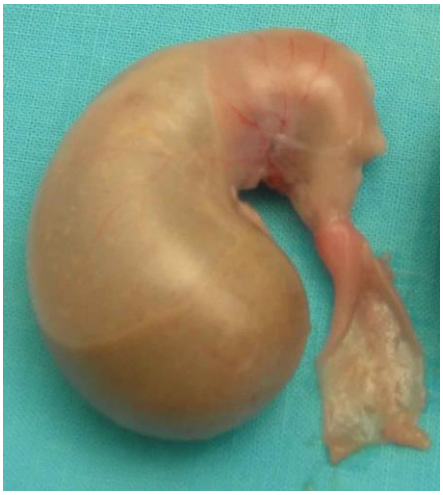
Omeprazole 2 mg /kg

Plate No:27



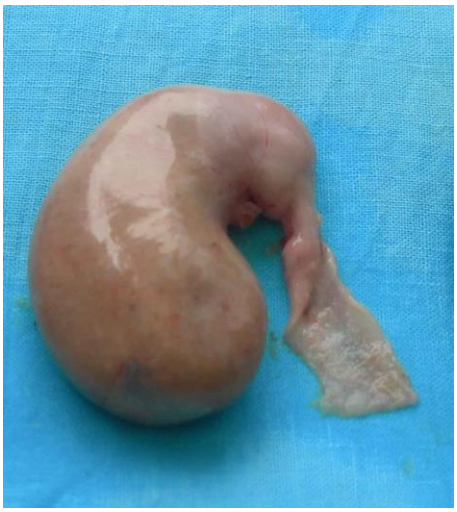
C P- 200 mg / kg

Plate No:28



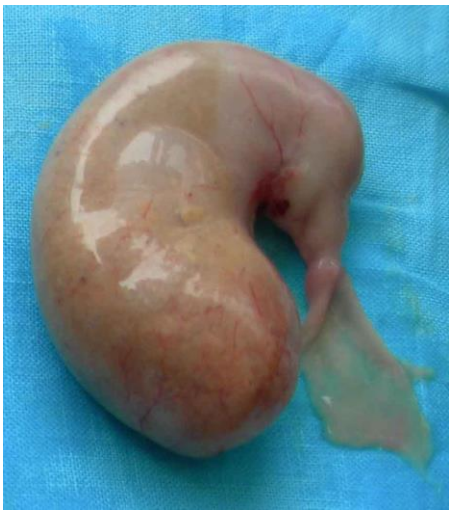
CP- 400 mg / kg

Plate No:29



HI- 200 mg / kg

Plate No:30



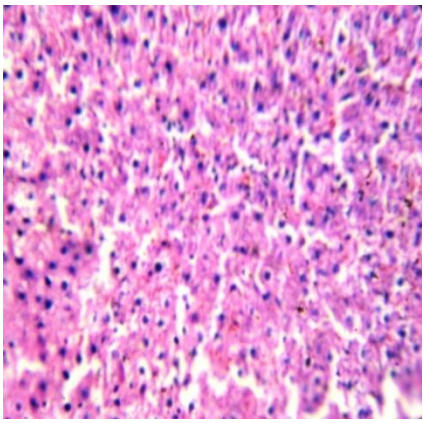
HI- 400 mg / kg

Cysteamine induced duodenal ulcer

The duodenal antiulcer activity of the plant extracts namely *Cyathula prostrate* and *Heliotropium indicum* at two different dose level as 200mg/kg and 400mg/kg b.w were studied using cysteamine induced duodenal ulcer model in rats.

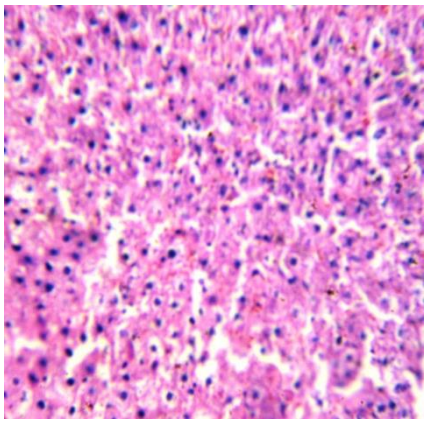
The results data are shown in table no and fig no .On light of these results revealed that there was a significant decrease in the ulcer index on tested herbal extracts at 200mg/kg (P**< 0.01 and 400mg/kg showed the significance (P***< 0.001) when compared with solvent control. The extracts of *Cyathula prostrate* exhibited a equipotent effect of Omeprazole at a dose level of 400mg/kg b.w.o, and *Heliotropium inicum* also showed the similar effect (equipotent) to Omeprazole at a dose level of 400mg/kg b.w.o. but, at a dose of 200mg/kg b.w.o level of both extracts showed less effect when compared with standard positive control drugs as ranitidine and Omeprazole

Plate No: 31



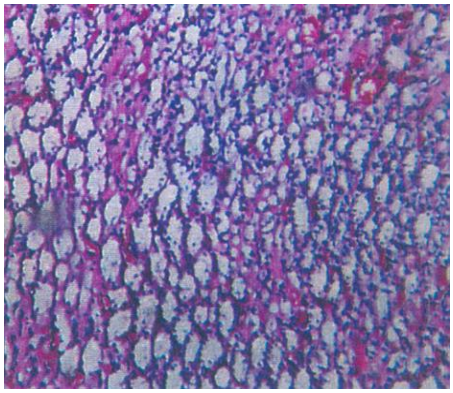
CP-200 mg/kg

Plate No:32



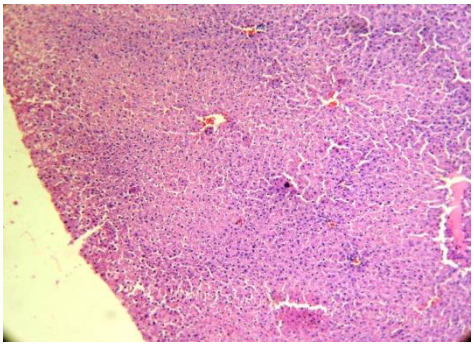
CP-400 mg/kg

Plate No:33



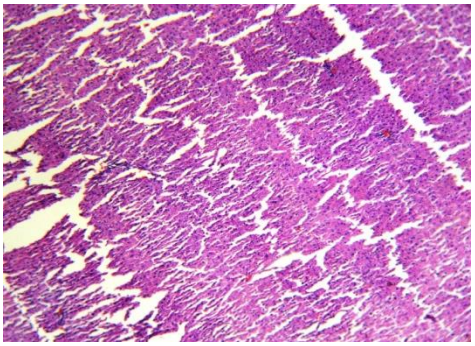
Normal Kidney

Plate No:34



HI-200 mg/kg

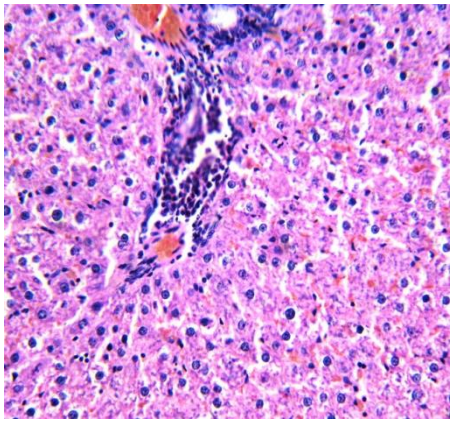
Plate No:35



HI-400 mg/kg

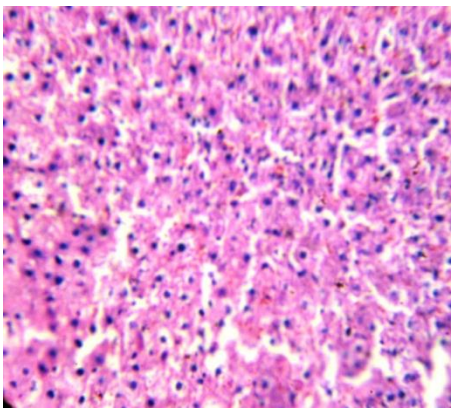
Effect of Cyathulaprostrata (CP) and Heliotropium indicum (HI) extracts on Rat Liver

Plate No: 36



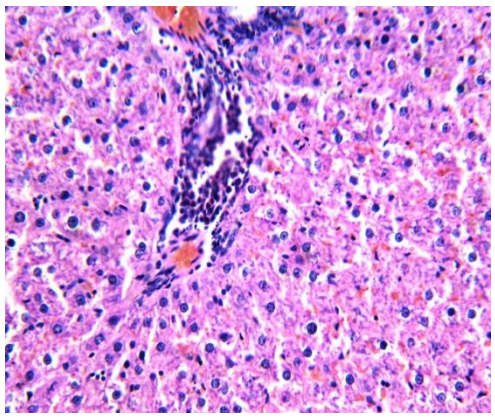
CP-200 mg/kg

Plate No:37



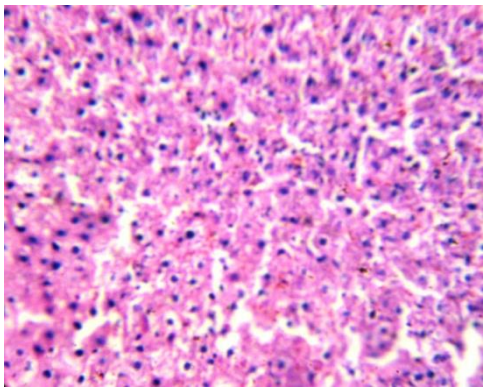
CP- 400 mg/kg

Plate No:38



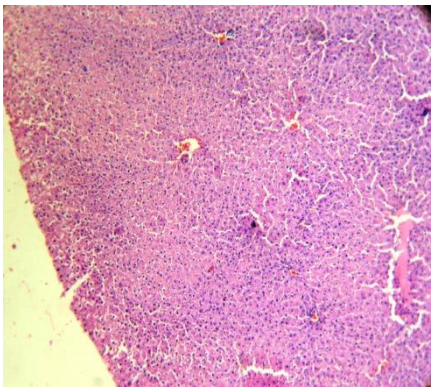
Normal liver

Plate No:39



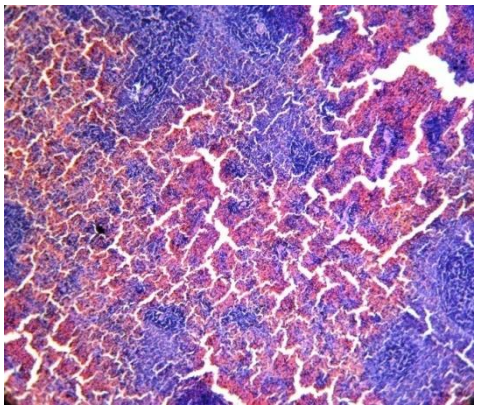
HI-200 mg/kg

Plate No:40



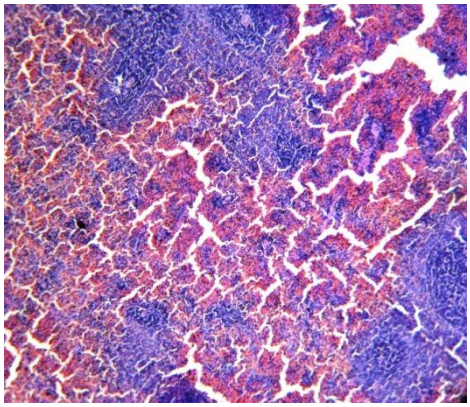
HI- 400 mg/kg

Plate No: 46



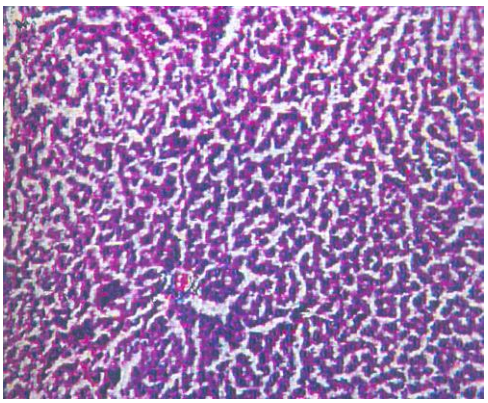
CP-200 mg/kg

Plate No: 47



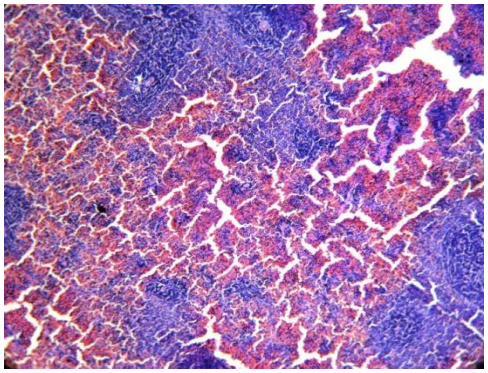
CP-400 mg/kg

Plate No:48



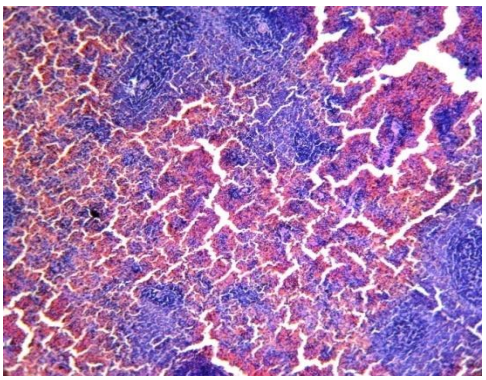
Normal Spleen

Plate No:49



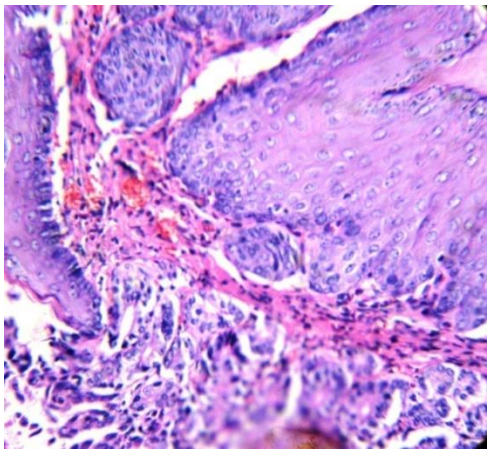
HI-200 mg/kg

Plate No:50



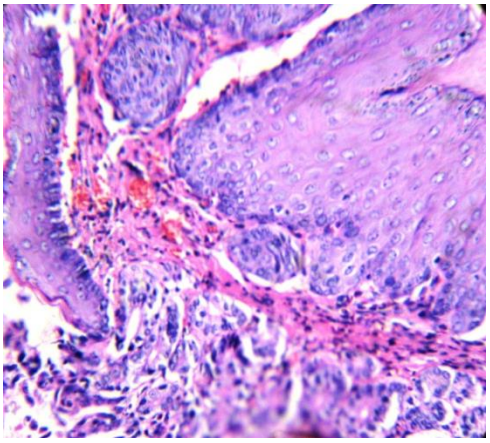
HI-400 mg/kg

Plate No:41



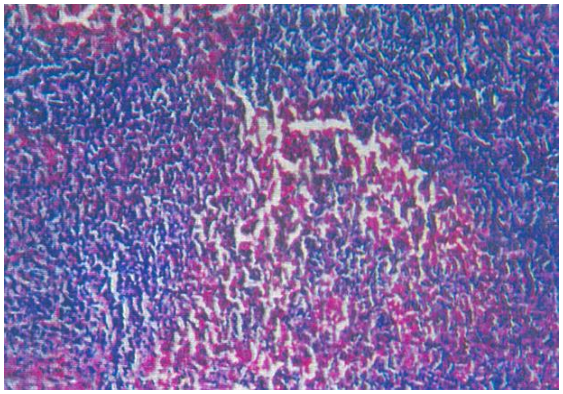
CP-200 mg/kg

Plate No:42



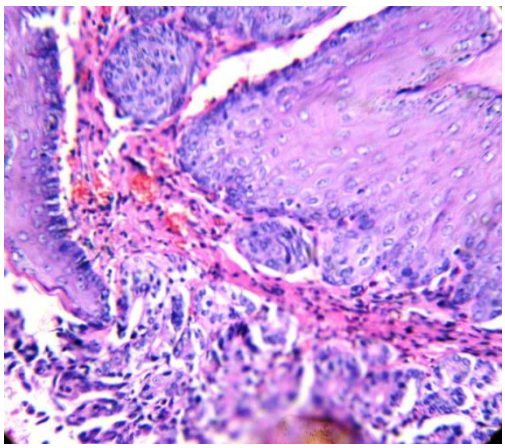
CP-400 mg/kg

Plate No:43



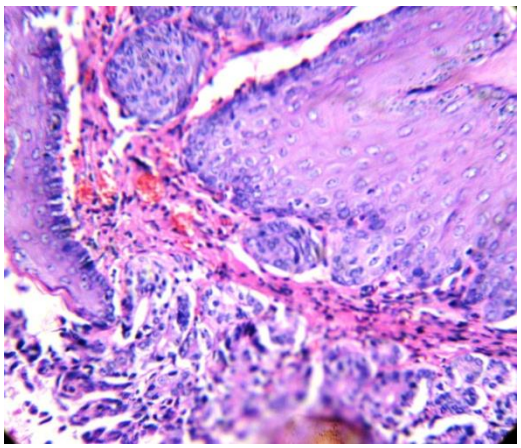
Normal Stomach

Plate No:44



HI-200 mg/kg

Plate No:45



HI-400 mg/kg

Table No: 26 Effect of Medicinal plant extract on haematological parameters after 28 days treatment in rats.

S.No	Drug & Dose	Hb%	RBC X 10^5 cells/cumm	WBC X 10^5 cells/cumm	Differential Leucocyte count			
					Lymphocytes %	Neutrophils %	Monocytes %	Esinophils %
1.	Solvent control	13.85 ±0.2074	5650 ±0.487	8140 ±209.3	36.50 ±±4.59	61.50 ±1.875	1.000 ±0.26	0.8333 ±0.307
2.	CP-200	10.78 ±2.0778	4.755 ±0.729	7400 ±11.5	40.83 ±11.70	57.00 ±5.34	0.333 ±0.21	2.00 ±1.065
3.	CP-400	12.65 ±1.130	3.758 ±0.906	8542 ±107.8	41.50 ±13.60	56.50 ±4.98	0.333 ±0.33	1.667 ±0.760
4.	HI-200	11.71 ±2.171	4.642 ±0.918	6442 ±483.5	44.33 ±11.66	53.00 ±4.940	2.167 ±0.703	0.666 ±0.666
5.	HI-400	10.50 ±1.275	3.783 ±0.672	7750 ±778.7	48.83 ±14.01	48.33 ±6.07	0.833 ±0.307	1.167 ±0.477

Sub acute toxicity studies

The sub acute toxicity evaluation shows no mortality rate at both dose level for the two tested extracts namely cyathula prostrate and Heliotropium indicum with reference to the pre and post treatment body weight in all the extracts treated animals concerned. There is no significance change in body weight as compared with the control group. The fact may be co-related to the side effects of the drugs on long term administration .

The Hematologivsl parameters shows the decrease in Hemoglobin, RBC, WBC at both dose levels significantly indicated the chemical constituents of the both plant extracts possess the capability to interfere with the formation an d destruction of blood cell

Table No:27 Quantitative data for the effect of plant extract on SGOT, SGPT and ALP enzyme levels after 28 days administration in rats.

Group	Treatment & days	SGOT 1 U/L	SGPT 1 U/L	ACP 1 U/L
1.	Solvent control	21.08 ±0.3430	19.10 ±0.3742	23.13 ±0.398
2.	CP-200	21.08 ±0.3430	29.93 ±1.332	45.42 ±2.338
3.	CP-400	21.68 ±1.015	23.43 ±1.012	41.75 ±1.570
4.	HI-200	19.67 ±0.6706	±17.95 ±0.352	34.03 ±0.907
5.	HI-400	25.58 ±1.204	22.80 ±0.82	40.57 ±1.758

In our hematological study, the RBC and the total WBC counts are found to be decreased which may be attributed to the physiological side effects of the drugs on chronic administration for 28days.

In regard to the biochemical estimation of select enzyme, SGOT, SGPT and ALP levels are concerned, all the enzymes are found to show marked indicating adverse reaction in the body due to chronic drug administration. During this study, higher the dose, greater

the enzyme elevation was observed for both the tested extracts indicating at the higher dose levels the degree of side effect is considerably increased.

Histopathological changes indicate that at all dose levels, the two test extracts produce marked changes in the anatomical profile of Kidney, Liver, Stomach and Spleen reflecting adverse side effects of the extracts in chronic administration for a period of 28 days.

The sub acute and hispathological results also show that higher dose levels for longer duration definitely produce tissue changes. The preliminary report can be further confirmed by providing a comprehensive toxicological studies in higher animals at different dose levels. The selection of smaller dose in the therapeutic range for long term administration in normal and in ulcer condition, maintaining similar metal ion concentration in body fluids and monitoring of metal ions in vital organs need to be carried out through future studies to throw more light in this direction.

Ethanopharmacology and drug discovery using natural products remain important issues in the current target for rich and lead poor Scenario (Patwardhan,et al., 2004). Many modern drugs have their origin in ethnopharmacology. Globally, there is a positive trend in favour of traditional and integrative health science in both research and practice. There are common approaches to drug discovery including the use of chemical biology, serendipity, chemical synthesis, combinatorial chemistry and genomics. However, the innovative approach involves ethanopharmacology, reverse pharmacology, holistic system biology and personalized medicine. There are clear trends to show that the mainstream in pharmaceutical research is moving away from single molecule or single target approach to combinations and multiple target approaches.[125]

The ethanopharmacology knowledge and experimental base allows drug research from 'Clinics to Laboratories' is a true reverse pharmacology approach. In this process 'Safety' remains the most important stating point and the efficacy becomes a matter of validation.

> A golden triangle consisting of traditional knowledge, modern medicine modern science with system orientation will converge to form an innovative discovery engine for newer, safer, affordable and effective therapies.[126]

An analysis of the origin of the drugs developed between 1981and 2002 showed that natural products of natural product derived drugs comprised 28 % of all new chemical entities launched into market. In addition 24 % of these new chemical entities were synthetic or natural mimic compounds, based on the study of pharmacophores related to natural product.[127] The combined % (52 % of all new chemical entities) suggest that natural products are important source for new drugs and are also good lead compounds suitable for further modification during drug development.

Macroscopical features:

The pharmacognostical features such as macroscopical characters, preliminary phytochemcial studies of *Cyathula prostrata* and *Heliotropium indicum* were carried out and presented. The results revealed that the plant *cyathula prostrata* appeared as leaves-simple, opposite, exstipulate, short petiole, flowers-reddish violet in terminal spikes, fruits-ovoid, Seed-single, oblong inverse, green color, no specific odour and taste.

Heliotropium indicum plant appeared as leaves-simple, alternate, unequal, hispid, pubescent, flowers-nut lets, combined in pairs, seed- each lobe 4 ribbed and no specific odour and taste is bitter (Table no:6).

The organoleptic characters may authentify the plants some extent but, that required additional information such as the parameters like- physio-chemical constants were done to helpful in determining the quality and purity of crude drugs in the powder form according to the standard

procedures. From the results, it was observed on the plants, *Cyathula prostrate and Heliotropium indicum* that the total ash value, acid insoluble ash, water soluble ash , sulphated ash and loss on drying at 110ºC were 2.1%w/w, 0.95%w/w,1.0%w/w,3.2%w/w,8% w/w and 2.95%w/w, 1.3%w/w,1.6%w/w, 4.4%w/w –11 %-respectively.8(Table no:7) These values may help to identify the authentic crude drugs from the closely related species

Phytochemical studies

The results of extraction and phytochemical analysis of *Cyathula prostrata* and *Heliotropium indicum* are given in Table no:8 Among the five successive extracts, the yield of alcohol and aqueous extracts of both the plants were found to be high (Table no: 9)

The preliminary phytochemical studies of both plants, revealed that the plant extract of *Cyathula prostrate* i.e petroleum ether extract showed the presence of steroids and terpenoids and the successive extraction with chloroform, Ethylacetate , and ethanol & aqueous showed the presence of flavanoids, phenolic compds, and tannins

And the plant *Heliotropium indicum*, petroleum ether extract yielded dark yellow solid mass showed no steroids and terpenoids but, on the successive extracts gave positive tests for the presence of alkaloids, flavanoids , phenolic compds, and tannins (Table no:15)

The fluorescence characters of the powdered *Cyathula perostrata and Heliotropium indicum* when treated with various chemical reagents (Table no: 11, 12 and 13) and those of its extracts

have been studied. The cortex (CP) showed reddish pink color with sbcl2 indicating the presence of steroids and triterpenoids. And the paraenchymatous tissues showed the brown colour with dragendroff's reagent indicating (HI) the presence of alkaloids. This study may be helpful to provide supplement information to distinguish the authentic crude drug (s) from the closely related species.

The table No: 14 & 15 showed the characteristic behavioural change with different reagents which may give the basic phyto constitiuents presence in each of the extract that indicate the possible activity claimed with the respect to the folk medicine practice. The table No 9 represents the phytoconstiteunts found in *cyathula prostrata* with various reagents , such as terpenoids, as saponins, tannins, phenolic content and flavanol and in case of *Heliotropium indicum* the phytoconstituents are alkaloids, tannins, phenolic content, flavanol with various chemical reagents.

Quantitative phytochemical analysis

The phyto chemical analysis as quantification of total phenol, flavanol estimated by spectroscopic method and estimation of lupeol by HPTLC method.(Table No: 16,17 and 18). The total phenolic content determined in *cyathula prostrata* comprises the chloroform extract 40.0mg/g, ethyl acetate extract 38.2mg/g, ethanolic extract 50.22mg/g and the flavanol content in chloroform extract 20mg/g, ethyl acetate extract 184.2 mg/g ethanolic extract 26.0mg/g .The total phenolic content determined in *Heliotropium indicum* comprises the chloroform extract 32mg/g, ethyl acetate extract 350.32mg/g, and ethanolic extract 216.6mg/g. The special content

as lupeol determined by HPTLC, found that the pet.ether extract 0.213%, chloroform extract 0.28%, ethyl acetate0.478% and ethanolic extract 0.089% respectively.

Lupeol (3B-lup-20(29)-en-3-ol) is a pentacyclic tripernene, which exists widely in plants as aglycones of tripernoids saponins. Molecular wight of 426.73, m.p 215-217^{o}c . It crystallizes in the form of needles from alcohol or acetone.

Lupeol belonging to the group of lupine triterpenoids largely possess similar pharmacological properties. The survey revealed wide distribution in plants, variety of used of lupeol as pure compound and its existence in folk medicine and its biological activity. The traditional uses of plants containing lupeol in folk medicine are many. New pharmacological properties of lupeol have also been discovered after the isolation of it from medicinal plants using the folklore knowledge.The multiple uses of lupeol include anti-inflammatory, kidney stone development inhibition, antitumor, antibacterial, antifungal, antimalarial, hypotensive and several others. Lupeol has the free radical scavenging property.

Pharmacological evaluation of hydroethnolic extracts of *Cyathula prostrata* and *Heliotropium indicum*

Acute Toxicity studies:

The both plant extracts tested at a dose level of 2000mg/kg didn't show any characteristic behavioural effects till the end of 72 h and no mortality was observed, hence, one tenth and fifth of the maximum tolerated dose were selected for further pharmacological studies, i.e 200mg/kg

and 400mg/kg were selected for the both the plant extracts. All the plant extracts were suspended on 0.3%w/w of carboxy methyl cellulose sodium and administered 1ml/kg of rat orally.

Gastric ulcer is a common disorder where discontinuity in the gastric mucosa is observed. The conventional treatment of ulcer comprises of regular feeds and adequate rest, antacid, various drugs and avoidance of ulcerogenic agents such as coffee, alcohol and tobacco. The drugs used for the treatment of ulcer includes proton pump inhibitor, receptor blockers, drugs affecting mucosal barrier and act on central nervous system .[128] Even though a range of drugs are available for the treatment of ulcer, many of these do not fulfill all the requirements and side effects such as arrythmiasis, impotence, hemopoietic changes are noted.[129a,b]

Anti secretary evaluation

Modified Pylorus ligation (Shay) rat model

Pylorus ligation induced ulcer is one of the most widely used methods for studying the effect of drugs on gastric secretion. Agents that decrease gastric acid secretion and increase mucus secretion are effective in preventing the ulcers induced by this method. The ligation of pyloric end of the stomach causes accumulation of gastric acid in stomach, leading to development of ulcers in the stomach.

The causation of ulcer in the gastric mucosa due to pyloric ligation may be twofold: stress induced increase in gastric hydrochloric acid secretion and stasis of the acid.[130] Development of ulcers in pylorus ligated model may be due to increased metabolism of

carbohydrates, synthesis of nucleic acids and also exhaustions of carbohydrate and other compensatory mechanisms. Further the status of mucous secretion is important to determine the status of the mucosal barrier.

In present study, it was observed that whole plant extract of CP and HI showed their anti-ulcer activity by inhibiting the offensive factors such as free acidity and total acidity in a dose dependent manner showing that higher dose (400 mg/kg) possessed a very significant ($p < 0.001$) activity, reflecting, increase in the pH and decrease in the gastric volume. Also, the effect of the extracts in decreasing the pepsin activity at higher dose was very well reflected in the decrease in total protein content.

The anti- ulcer effect is also supported by decrease in the aggressive factors like pepsin and proteins and an increase in the defensive factors like p^H, hexosamine, hoxose, fucose and sialic acid. This was supported by the increase in the C: P ratio at both dose levels tested.

The C: P ratio is a direct index of the dissolved mucosubstances in the gastric juice. The increased total carbohydrate content for an effective mucosal barrier.[131] The increase in carbohydrate protein ratio is a direct reflection of mucin activity .[132a,b] This suggests the increase in glycoprotein content of the gastric mucosa. The decrease in protein content of the gastric juice suggests the decrease of leakage of plasma into the gastric juice.[133] Similar to liquorice, the mechanism of action may be due to the coating property that has protective effect on the gastric mucosa.[134]

The increase in the K^+ ion in-turn reflects the increase in H^+ ion concentration and HCO_3^- ion concentration. The increase in HCO_3^- ion concentration plays an important role in protecting the gastric and duodenal mucosa against hydrochloric acid.[135] In present study, it was estimated particularly K^+ ion concentration which was found to be increased at higher dose level for both the plant extracts and Na^+ was found to be decreased significantly and equipotent to positive control. All the parameters studied were finally in supportive to the anti-ulcer activity and it was very well reflected in the ulcer index. Ulcer index was taken as the prime parameter for the evaluation of anti-ulcer activity because the ulcer formation is linearly proportional to the factors involved in the ulcer induction. At 400 mg/kg dose levels of both plant species produced a significant decrease in the ulcer index which was very well comparable and equipotent to the positive controls tested.

Aspirin plus modified pylorus ligation (Shay) rat model

Aspirin, phenylbutazone and some non-steroidal anti-inflammatory drugs are also known to cause duodenal and gastric ulceration.[136] Prostaglandin E_2 and I_2 are predominantly synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate. Hydrophobic surfactant –like phospholipid secretion in the gastric epithelial cells is also stimulated by the prostaglandin.[137]

Pretreatment of aspirin along with pylorus ligation aggravated the ulcer index and acid secretion in control groups. In other words aspirin produces its pharmacological action by inhibiting COX_1 and COX_2 enzymes which are responsible for PG synthesis.[138] It is well known that reduction in PG levels mostly leads to more ulcer formations .[45] Further the free acidic group in the aspirin may also contribute to the increased ulcer index. Ulcer caused by chemical inducer like ethanol and aspirin could be due to their direct effect of release of noxious substances including free radicals. Disruption of prostanoid synthesis is another contributing factor to the aspirin in inducing the ulcers.[138]

Ulcer index was a parameter used for evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as gastric volume, free and total acidity. In present study, it was observed that aspirin plus pylorus increased the acid secretion, which in turn caused increase in gastric volume, low pH, increased free and total acidity by inhibiting the offensive factors such as free acidity and total acidity resulting into increase the ulcer index and these changes are in agreement with the earlier reports .[139]Whole plant extract of CP and HI showed their anti-ulcer activity by inhibiting the offensive factors such as free acidity and total acidity in a dose dependent manner showing that higher dose (400 mg/kg) possessed a very significant ($p<0.001$) activity, reflecting increase in the pH and decrease in the gastric volume. Also, the effect of the extracts in decreasing the pepsin activity at higher dose was very well reflected in the decrease in total protein content. The anti-ulcer effect is also supported by decrease in the aggressive factors like pepsin and proteins and an increase in the resistant factors like pH, hexosamine, hexose, fucose and sialic acid.

Aspirin plus pylorus ligation decreased the C: P ratio when compared to pylorus ligation method alone. The anti-ulcer effect was supported by the increase in the C: P ratio at both dose levels of the plants tested. The C: P ratio is direct index of the dissolved muco substances in the gastric juice. The increased carbohydrate index for an effective mucosal barrier.[131] The increase in carbohydrate protein is a direct reflection of mucin activity.[132a,b] This suggests the increase in glycoprotein content of the gastric mucosa. The decrease in protein content of the gastric juice suggests the decrease of leakage of plasma into the gastric juice.[133] Similar to liquorice, the mechanism of action may be due to the coating property that has protective effect on the gastric mucosa.[134]

The increase in the K^+ ion in-turn reflects the increase in H^+ ion concentration and HCO_3^- ion concentration. The increase in HCO_3^- ion concentration plays an important role in protecting the gastric and duodenal mucosa against hydrochloric acid (Suzuki, 1996). In present study, we have estimated particularly K^+ ion concentration which was found to be increased at higher dose level for both the plant extracts and Na^+ ion was found to be decreased significantly at both the dose levels for the plant species tested and equipotent to positive control.

Volume of gastric secretion is an important factor in the production of ulcer due to exposure of unprotected lumen of the stomach by the accumulating acid.[144] Treatment with higher doses of whole plant extract of CP and HI showed potential anti-ulcer activity by reducing the gastric secretion also. Herbs which are capable of controlling the COX_1 and COX_2 pathways may be beneficial in controlling the drug induced ulcers. Therefore it can be assumed that the Herbs *Cyathula prostrata* and *Heliotropium indicum* may possess their anti-secretory and

cytoprotective activity by controlling the COX enzymes. The cytoprotective action probably stimulated the prostaglandin synthesis, which in turn protects the gastric mucosa.

Acetic acid induced chronic ulcer model

Acetic acid induced chronic gastric ulcer model is quite useful for studying the effect of drugs on the healing of peptic ulcers. Application of glacial acetic acid on the serosal surface of the stomach produces deep penetrating gastric ulcer that resembles human peptic ulcer disease. Since the healing process of this ulcer closely resembles that of human peptic ulcers, this model was selected for testing the effect on chronic ulceritis .[145] Both CP and HI were effective in augmenting the gastric ulcer healing model.

The topical administration of acetic acid produced penetrating lesions in the pyloric portion of the gastric mucosa at the site of application and the observation are on par with reports.[145b] Pre-treatment with higher doses (400 mg/kg) of CP and HI resulted in a significant reduction of ulcer index and the activity was equipotent to the positive controls tested.

Mucin is viscous glycoprotein with physicochemical properties producing relatively resistant acid barrier.[146] The mucus gel layer is a complex secretion containing inorganic materials, secretion IgA, lactoferin, high molecular weight glycoprotein (which is composed of mainly galactose, galactosamine, glucosamine, fructose, carbohydrate) and proteins. It makes up the major part of the mucus, an important pre-epithelial factor that acts as a first line of defense

against ulcerogens.[147] Decrease in mucosal secretion is thus considered important in gastric ulceration.[148] Treatment with higher dose of CP and HI increased the mucosal content as well as non protein sulfhydryl content and the effect was equipotent to the positive controls tested, which shows the mucoprotective property of the extracts.

Oxygen derived free radicals play a key role in mechanism of acetic acid induced mucosal lesion. Many reports have demonstrated that most injury of gastric mucosa can be reduced with pretreatment with scavengers of reactive oxygen species (ROS). Oxygen handling cells have antioxidant enzymes such as CAT, SOD, GPX and GSH which are the first line of cellular defence against oxidative injury, decomposability of O_2 and H_2O_2 before they interact to form more reactive OH radicals. SOD and CAT enzymes are highly specific in their mode of action and it decreases the gastric mucosal damaging effect of acetic acid.[149]

Oxygen derived free radicals play an important role in delaying the healing of acetic acid induced chronic gastric ulcers in rats.[150] Treatment with higher doses of both CP and HI significantly increased the levels of SOD and CAT as well as decreased the LPO. Possession of antioxidant activity may also be a possible mechanism of action of *Cyathula prostrata* and *Heliotropium indicum* to exhibit the anti-ulcer property.

Cyestamine induced duodenal ulcer

Cysteamine induced duodenal ulcer in the rat is widely used as a model of peptic ulcer disease. Duodenal ulcers in rats given by cysteamine and in humans have similar

pathomorphologic history and are on the anterior and / or posterior wall ("kissing ulcers"), frequently penetrating the pancreas.[150] Cysteamine inhibits the alkaline mucus secretion from Brunner's glands in the proximal duodenum and stimulates gastric acid secretion rate. It induces delayed gastric emptying and increases serum gastrin concentration .[151] This chemically induced ulcer resembles duodenal ulcer in man to its location, histopathology and some aspects of pathophysiology.[152] In fact hyper secretion of acid, distributed gastroduodenal motility, hypogastrinemia and decreased mucosal resistance has all been implicated in the pathogenesis of cysteamine induced ulceration.[153]

Several studies shown that the passage of gastric acid content is mandatory for the development of the duodenal ulcer .[154,155] The duodenal ulcerogenic potency of these chemicals seems to be associated with two carbon (-C-C-) groups containing reactive radicals.[156] Furthermore, it has been reported that cysteamine induced ulcers can be prevented by anticholinergic agents, antacid and vagotomy .[157a,b]

In present study, cysteamine produced several anterior and /or posterior wall (kissing ulcers) duodenal ulcers in the control group animals. Pretreatment with CP and HI reduced the incidence of ulcer as well as ulcer index. Treatment with higher dose of CP and HI produced a significant reduction of ulcer index and the ulcer incidence was also reduced up to 50 %. They may possess the anti-ulcer activity by inducing the mucosal synthesis and by reducing the gastric emptying time.

The earlier findings with *Cyathula prostrata* showed anti-inflammatory, analgesic, anti-pyretic, anti-viral and anti-rheumatic activity.[158] which is associated with presence of saponins in it. The chemical components of *Heliotropium indicum* include pyrolizidine, alkaloids, sapoinins, tannins, indicine-N-oxide and heliotrine .[159] Its alkaloid component is already proved for its anti-inflammatory, wound healing ,[160a] antimicrobial, febrifuge, gastro protective and menstruation activator properties .[160b] It is also found to possess anti-cancer activity against.[162]

Tannins being an astringent might precipitated microproteins on the sites of ulcer thereby forming an impervious protective pellicle over the lining to prevent absorption of toxic substances and resist the attack of proteolytic enzymes to produced mucoprotective effec.[163] Several alkaloids have already been proved for their anti-ulcer activity.[164] Saponins have the foaming property, which is one of the reasons for its gastro protective property.[165] Presence of saponins in *Cyathula prostrata* and tannins, alkaloids and saponins in *Heliotropium indicum* may be responsible for their mucoprotective and anti-ulcer effect.

SUMMARY AND CONCLUSION

Cyathula prostata L. Blume is much branched slender prostrate or decumbent herbaceous perennial plant and *Heliotropium indicum* is succulent foetidy perennial herb with densely hirsute ascending branches. The two plants were collected from Kottakal area, Kerala state and aunthenticated. The two plants were undertaken for pharmacognostical standards such as Macroscopical, Organoleptic characters, and Physicochemical parameters like Total ash, acid insoluble ash, water soluble ash and sulphated ash values were also determined.

The phytochemical studies of the plant extracts showed the presence of terepenoids, flavonols, phenols, and tannins in both the plants. So, the total phenolic content and flvonoidal content was *Cyathula prostata* and *Heliotropium indicum* were estimated respectively. Whereas the estimation of lupeol in *Heliotropium indicum* was carried out by using HPTLC.

The hydroethanolic extracts of both plants were found to be effective on experimental and drug induced ulcer models such as Modified pylorus ligated (Shay) rat model, Aspirin plus Modified pylorus ligated (Shay) rat model, Acetic induced chronic ulcer model and Cysteamine induced duodenal ulcer model. The effect of whole plant extracts of *Cyathula prostata* and *Heliotropium indicum* on offensive and defensive factors of gastric content was studied on Modified pylorus ligated (Shay) rat model and drug induced ulcer model.

The study revealed that 200 mg/kg of hydroethanolic extracts of CP and HI regressed ulcer condition significantly. Whereas 400 mg/kg of both plant extracts effectively reduced the ulcers when compared to 200 mg/kg, showing the dose dependent activity.

The herbal extracts might control the ulcer formation by decreasing the secretion, drug induced damage and also by psychological stress. The herbal extracts were found to be more effective in controlling the ulcer in both the acute and chronic

models. Further, cysteamine induced duodenal ulcer study indicated that the extracts possess anti ulcer activity against duodenal ulcers in dose dependent manner.

Hence it can be stated that herbal extracts may be beneficial in preventing the stress (including psychological or environmental stress), drug induced ulcers and also act as anti secretary which may be attributed to the presence of phytoconstitiuents in plants. Reduction of gastric emptying time, Synthesis of mucosa, Inhibition of hyper-secretion of gastric acid are the possible mechanisms through which the herbal extracts are possessing anti-ulcer activity.

Even though the present study revealed the activity of herbal extracts and the mechanism of action, the future studies are needed to be carried out to isolate and screen the phytoprinciples responsible for the anti-ulcer and mucoprotective activity and to correlate them with the mechanism of action. For further understanding of mechanism, the other models of ulcer induction such as ethanol induced gastric ulcers can also be carried out. Works in this direction are in progress, in the institution.

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