PHARMACOLOGICAL EVALUATION OF INDIAN MEDICINAL PLANT *Clitoria ternatea* (L) IN EXPERIMENTAL ANIMAL MODELS

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In partial fulfillment of the requirement for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

By

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This is to certify that this dissertation entitled **Pharmacological Evaluation of Indian medicinal Plant *clitoria ternatea* (linn) in experimental animal models** by under for the award of **"MASTER OF PHARMACY"** in Pharmacology, comprises of the bonafide work done by **Reg No: 261225351** in the Arulmigu Kalasalingam College of Pharmacy, Krishnakoil. His work was supervised by Mr.N.R.Livingston raja, Associate Professor A.K. College of Pharmacy Anand Nagar, Krishnankoil.

I recommend this piece of work for acceptance as project for the partial fulfillment of the degree of **"MASTER OF PHARMACY"** of the Tamil Nadu Dr. M.G.R. Medical University, Chennai, for the academic year 2013-2014.

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EVALUATION SHEET

This dissertation work entitled "**Pharmacological Evaluation of Indian Medicinal Plant *Clitoria ternatea* (L) in Experimental Animal Models"** was evaluated for the parial fulfillment of the requirements for the degree of **"MASTER OF PHARMACY"** in the Tamil Nadu Dr. M.G.R. Medical University.

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The plant specimen brought to me by S.Gopi Krishnan second year M.Pharmcy dept of pharmacology (2013-2014) studying in Arulmigu Kalasalingam College of Pharmacy,Anand Nagar Krishnan koil, srivilliputhur for identification is **CLITORIA TERNATEA** belongs to the family **FABACEAE**

Date: 17.07.2013.

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Chapter 1

1.1. GENERAL INTRODUCTION

Natural products, obtained from plants, animals and minerals have been the basis of treatment of human diseases since time memorial. In India the use of different parts of several medicinal plants to cure specific aliments has been in vogue from ancient times[(1)]. The medicinal use of the plants is found in the Rigveda, the oldest repository of human knowledge, having been written between 4500 and 1600 BC. The properties of various drugs have been given in detail in *Ayurveda*. 'Susrutha Samhita' which was written not later than 1000 BC contains comprehensive chapter on therapeutics and 'Charaka Samhita' gives a remarkable description of the material medica of *Ayurveda*. Later, during Buddhist period considerable progress was made and medicinal plants were cultivated under the direction of highly qualified specialists. Contacts with Greece and Rome and later with Arabia and Persia, contributed to the enrichment of Indian material medica and a large number of medicinal plants, vegetable and other products came into use for the treatment of various diseases[(2)].

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of our country. The material medica of various indigenous systems of medicines practiced in India has become extensive and heterogeneous. Out of about 2000 items recorded in Indian medical literature ,less 200 are mineral and animal origin; the rest are derived from vegetable sources[(2)]. Our knowledge of medicinal plants has mostly been inherited traditionally. Spreading and preserving the knowledge on medicinal plants and their uses in important for the continued welfare of human beings. There is a growing tendency all over the world to shift from synthetic to natural based products including medicinal plants.

The practitioners of various Indian systems of medicines in different parts of India have utilized locally growing plants as far as possible and accepted those which were found useful after repeated trials for treatment of diseases. Thus medicinal properties have been attributed to a large variety of plants growing in different parts of the country. Many vegetable drugs are used in different regions; others are used as household remedies by the common people[(2).].

The demand for medicinal plants made by the modern Pharmaceutical industries has also increased manifold. In modern medicine too, plants occupy a very significant place as raw material for some important drugs, especially for the treatment of cancer although synthetic drugs and antibiotics have brought about a revolution in controlling different diseases[(1)].

Our pharmaceutical industry is fairly advanced and sophisticated. However, there is continuous search for more potent and cheaper medicines. With concerted research and development efforts, many medicinal plants could provide raw material in abundance to the indigenous pharmacies and local herbalists. It is now well understand. It is now well understand that strong linkages should be developed between medicinal plants growers, health experts and pharmaceutical industries for developing novel products and also for laying down a scientific basis on which system of medicine are working. It is now well believed that an integrated system of the indigenous of medicine based on natural products and synthetics may yield the most effective and cheap package for WHO's goal of "health for all by the year 2000".

The great importance of collecting good herbarium material for taxonomic identification of the collected species, and also cultivation, maintenance and assessment of germplasm for future use, since among the most vulnerable plant species in India, the most overexploited are the medicinal plants[(1)].

Most of the pharmacological actions reported are on crude extract or active fractions. In case of many plants, active principles have been identified and studied pharmacologically. In general, the traditional and Ayurvedic uses of plants are so wide & varied and that it was not possible to correlate with the uses of the plants. Very limited numbers of plants have been reported to have confirmed activities in their fractionated extracts.

1.2Plant used to treat diseases:

Diseases always co existed with livings, detecting their remedies also always continuing, going through the commencement of drug therapy for disease, drugs comes to the force in sudden, in the ancient time human knowledge found the absence of some forms the base for the development of some disease, they were tried to use the particular disease and they got success in that work. These motivate the plant researches to use different plants, plant parts for different disease. In olden days they used plants as such and then made into different formulations for their convenience such as powder, juice, decoction, extracts etc.,

Our tradition system of medicines siddha categorized nearly 5000 plants species and their usage. Later on the allopathic system of medicine comes to force and dominate the siddha and due to the fast relieving nature it reaches the world as quickly and diminished the usage of plant medicine as maximum.

But allopathic system cannot provide ultimate solution to some diseases, and also their side effect in particularly the long term therapy, limits their usage still the plant medicine is recommended and used in such cases. This suggests the plant medicine to researches as and scientific world as alternate to allopathic system of medicine. The world health organization also recognize and motivate the plant researches and centre, hence the plant medicine now considered to be alternative system of medicine.

Even usage of plants are known since plants species consists of mixture of compound, isolating the single compound and identifying the component is responsible for that particular activity is a major question in front of plant researches and also it is very difficult to say only these are all the compounds available from particular plant.Nowadays due to the development of science and technology such as chromatographic technique and spectroscopical technique it is possible to isolate almost all the components of plant and characterize them. Isolation and characterization are very important to improve effectiveness, minimizing the dose and on set of action.

Now this study is considered as a separate discipline called “Phytochemistry” defined as a branch of science somewhere in between natural products organic chemistry and plant biochemistry concerned with organic substance accumulated by plants and deals with the chemical structure of the substance their biosynthesis, turnover and metabolism their natural distribution and their biological function.

Since detecting the compound responsible for the particular activity, isolating characterizing the compound and monitoring the activity is of prime importance the basic requirement needed for the medicinal world, it is the duty of the chemist to do these work.

Chapter 2

REVIEW OF LITERATURE

2.1. General

Since the beginning of human civilization, medicinal plants have beenused by mankind for its therapeutic value. Nature has been a source ofmedicinal agents for thousands of years and an impressive number ofmodern drugs have been isolated from natural sources. Many of theseisolations were based on the uses of the agents in traditional medicine. Theplant-based, traditional medicine systems continues to play an essential rolein health care, with about 80% of the world's inhabitants relying mainly ontraditional medicines for their primary health care.India has several traditional medical systems, such as Ayurveda and Unani,which has survived through more than 3000 years, mainly using plant-baseddrugs. The materia medica of these systems contains a rich heritage ofindigenous herbal practices that have helped to sustain the health of mostrural people of India. The ancient texts like Rig Veda (4500-1600 BC) andAtharva Veda mention the use of several plants as medicine.In addition to their natural role, plant secondary metabolites also represent a vast resource of complex molecules that are valued and exploited by man for their pharmacological and other properties(Table 2.1)

Table 2.1: Some examples of plants as source of drugs[(3)]

Plants	Part used	Uses
Atropa belladonna	Whole plant	Sedative
*Adhatoda zeylanica*Medicus.	Leaf	Asthma, Cold
*Aloe vera*Linn.	Leaf	Wound healing
Betel piperL.	Leaf	Pimples
Cardiospermum canescens Wall.	Leaf	Dysentery
Cassia fistula Linn.	Leaf	Laxative
Erythrina indica Lam.	Leaf	Menstrual problem
Eucalyptus globules Labill.	Leaf	Body ache, Re-freshener
Euphorbia anticaram L.	Latex	Edema
Ficus bengalensis L.	Latex	Wound healing
Lawsonia inermis L.	Leaf	Heeling crack
Ocimum santum L.	Leaf	Dry cough
Phyllanthus amarus Schum.	Leaf	Jaundice
Quercus infectoria	Seed husk	Wound ,Anti-inflammatory

In India, the ayurvedic system has described a large number of such medicines based on plants or plant product and the determination of their morphological and pharmacological or pharmacognostical characters can provide a better understanding of

their active principles and mode of action. However a large number of tropical plants have not been studied in detail for their chemical constituents, pharmacological properties of the extracts, and their pharmacognostical characterization including DNA sequencing etc. In the present review focused various aspects in selected medicinal plant*Clitoria ternatea* (L).

2.2SELECTION OF PLANT FORSTUDIES

Clitoria ternatea (L)commonly known as Shangupushpam is widely used in traditional medicinein India. Extracts of roots, bark, and leaves ofthis plant are widely used in the treatment ofgastroenteritis, vomiting, laxative, and diuretic, ulcers, toothache, coughs, asthma, and a number of other skin diseases.

2.2.1Plant preferred for present study:

Sl. No.	Botanical Name of the Plant	Family	Part selected
01.	*Clitoria ternatea* (L).	Fabaceae	Leaves

2.2.2MORPHOLOGY of plant

***Clitoria ternatea* (WHITE VARIETY)** [4]

Botanical Name **:** *Clitoria ternatea*

Kingdom : Plantae

Subkingdom : Viridaeplantae

Division : Magnoliophyta

Genus : *Clitoria*

Species : *ternatea*

Family : Fabaceae

Clitoria ternatea

Vernacular Names:

Tamil	:	Shangupushpam
Hindi	:	Kokkattan
Malayalam	:	Aparajita
Telugu	:	Dintena
English	:	Butterly pea

2.2.3DESCRIPTIONOFPLANT[(5)]

Clitoria ternatea commonly known as Butterfly pea belonging to the family fabaceae and sub-Family Papilionaceae is a perennial leguminous twinned. *Clitoria* linn. Comprises 60 species distributed mostly within the tropical belt with a few species found in temperate areas. The mostly frequently reported species is *Clitoria ternatea*. The plant is mainly used as a forage as it is highly palatable for live stock and it is well adapted to various climates. The plant orginates from tropical Asia and later was distributed widely in South and Central America.

Clitoria ternatea has twining fine stems, 0.5-3 m long. The leaves are pinnate, with 5-7 elliptic to lanceolate leaflets, 3-5 cm long and shortly pubescent underneath. Flowers are solitary, deep blue to blue mauve; very short pedicellate and 4-5 cm long fig [(4)]. Pods are flat, linear, beaked, 6-12 cm long, 0.7-1.2 mm wide and slightly pubescent with upto 10 seeds. The seeds are olive, brown or black in colour, often mottled, 4.5-7mm long and 3-4mm wide.[(6)]

Clitoria ternatea resembles a conch shell ; therefore it is commonly called "Shankhpushpi" in the Sanskrit language where it is reported to be good "Medhya "(brain tonic) drug and therefore used in the treatment of "Masasika roga" (mental illness).

Roots, seeds and leaves are the reported plant part used from ancient times. The major phyto constituents found in *Clitoria ternatea* are the pentacyclic triperpenoids such as taraxerol and taraxerone. Phytochemical screening of the roots shows the presence of ternatins, alkaloids, flavonoids, saponins, tannins, carbohydrates, protein, resins, starch, taraxerol and taraxerone. The seeds contains anti fungal proteins and has been shown to be homologous to plant defense and also contains amino acid sequence similar to insulin, delphinidin-3,3,5- triglucoside, essential amino acids, pentosan, water soluble mucilage, adenosine, tannic acid etc. phytochemical analysis has revealed that the stem contains phytosterols , phenol compound, flavonoids and carbohydrates.[(7)]

Clitoria ternatea fixes nitrogen and therefore also used to improve soil quality. The useful parts are leaf, root, bark, seeds and flower. The plant used in colic gonorrhea and skin disease. Root is used as laxative and demulcent. Powdered seeds are used in the treatment of ascites, enlargement of abdominal viscera, weakness of sight and skin disease.the seeds containing nucleoprotein with its amino acid sequence similar to insulin but for the absence of histidine, threonine, proline, and crystine. Seeds have cinnamic acid, flavonol glycosides leaves contain glycosides of kaempferol. In south india the seeds and roots constitute the drug shankhapushpi used as nerve tonic.[(8)]

2.3 PEPTIC ULCER:

Peptic ulcer disease is one of the most common chronic infections in human population.Peptic ulcer disease has a tremendous effect on morbidity and mortality until the last decades of the 20th century. Development of new effective and potent acid

suppressants and the discovery of helicobacter pylori are two important steps that cause a reduction in the prevalence of peptic ulcer.[9] Peptic ulcer disease(PUD) affects 10 % of the total population. H.Pylori infection and the use of non steroidal inflammatory drugs(NSAID) are the principle factors associated with the peptic ulcer disease. H.Pylori infection plays a major role in peptic ulcer disease and non ulcer dyspepsia.[10]

A gastric ulcer, also called stomach ulcer is a break in the normal gastric mucosa integrity that extends through the muscularis mucosa into sub mucosa (or) deeper . The normal stomach mucosas maintain a balance between protective and aggressive factors. Some of the main aggressive factors are gastric acid, abnormal motility, pepsin, bile salts, use of alcohol and non steroidal anti inflammatory drugs (NSAID), as well as infection with micro organism. On other hand mucus secretion, bi carbonate production, gastro protective prostaglandin synthesis. Although in most cases the etiology of ulcer is unknown yet, it is generally that aggressive factor (endogenous, exogeneous/ or infectious agents) overcome mucosal defense mechanism[11].

2.3.1GASTRO PROTECTIVE FACTORS:

The stomach is lined by a complex epithelium that forms a selective barrier between the external environment (lumen) and the body, which is folded into several branching, tubular gastic glands that reach deep into the muscularis mucosa. In general, gastric defence mechanism consist of

1. Gastric mucosal barrier
2. Epithelial barrier.

2.3.2GASTRIC MUCOSAL BARRIER:

The barrier constitutes the first line of mucosal defence and is formed by mucus gel, bicarbonate and surfactant phospholipids which cover the mucosal surface. The regular exposure of the stomach to endogenously produced acid and degrading enzymes require the presence of an efficient gastric mucosal “barrier”.[12] The gastric mucus consists of a viscous, elastic, adherent and transparent gel secreted by apical expulsion from surface epithelial cells. It consists of 95% water and 5% mucin, glycoprotein that cover the entire gastro intestinal mucosa.

2.3.3EPITHELIAL BARRIER

Epithelial layer secrete mucus and bi carbonate and generate prostaglandins (PGs), heat shock protein, trefoil family peptides (TFFS) and cathelicidins. Maintenance of epithelial integrity requires a precise balance between cell proliferation and cell death.

Prostaglandins are also synthesis by gastric mucosal epithelial cells from arachidonate metabolism through the action of cyclo oxygenase (COX). The ability of the endogenous PGs to attenuate or even completely prevent mucosal damage caused by corrosive substance such as absolute ethanol, concentrated bile has been termed as "cytoprotection". In addition , other mediators such as nitric oxide(NO) calcitonin gene related peptide (c CRP) as well as some hormones including gastrin and cholecystokinin (CCk) , ghrelin, leptin and gastrin relasing peptide (GRP) have been also found to protect gastric mucosa against the damage induced by corrosive substances.[13]

2.3.4GASTRIC AGGRESSIVE FACTOR:

Most important of which are acid secretion, bacteria and their products, NSAID, alcohol, reactive oxygen species, as well as different chemical compounds. The effect on the gastric barrier represent important mechanism of the pathogenic of gastric ulcer, chronic gastritis and other gastric disease, which are frequently generated through an imbalance between mucosal aggressive and defense factors.[14]

Helicobacter pylori is a common pathogen and public health problem associated with the pathogenesis of gastritis and the peptic ulcer, 90% in developing population. This micro organism is the second common pathogen for human being. It is a non-sporulating, gram negative and micro aerophilic bacilli, spiral shaped, having one to six polar sheathed flagella, emerging from one of its rounded ends and a smooth surface.[15] This pathogen multiplies with great efficiency in the hostile environment with in the stomach. Both survive poorly in the gastric lumen. Mainly found in the mucous layer.

This pathogen synthesis urease, which is virulent. When virulent strains are present organism, adhere to the gastric epithelium, which affect the membrane, and induce host cells to release toxic protein, cytotoxins, platelet activating factors and lipo polysaccharides that all further damage the gastric mucosa.[16]

2.3.5NSAID induced ulcer

Non steroidal anti inflammatory drugs is an important factor for injury. Studies shows that NSAID are among the most commonly used drugs in the world. The major problem with the use these drugs in that indicates, that NSAID induce gastric mucosal injury. The major mechanism via which NSAID causes ulcers and gastro intestinal complication is inhibition of cyclo oxygenase (COX). Which is a key enzyme for the bio synthesis of PGs.? There is COX 1 and COX 2 is present in most tissues produce PGs that play an essential protective role in the stomach by stimulating the synthesis and secretion of mucus and bicarbonate and promote epithelial proliferation.[17] Whereas COX2 has no expression in most tissues, but is rapidly induced in response to inflammatory stimuli. Therefore, isoform is primary target for anti inflammatory drugs. However the prostaglandins derived from the COX 2 can be generated at the ulcer healing through triggering the cell proliferation, promotion of angiogenesis and restoration of mucosal integrity.

2.3.6GASTRIC ACID SECRETION:

Parietal cells secrete hydrochloric acid at a concentration of approximately 160 mmol/L or pH 0.8, acid facilitates the digestion of proteins and absorption of calcium, iron and vitamin B 12, as well as it is the first line mucosal defense to avoid micro organism colonization thus preventing the bacterial over growth and consequent enteric infection (such as H.Pylori)[18]. However, when levels of acid (and pepsin) overwhelm mucosal defense mechanism, serious acid related clinical condition occur, including gastro esophageal reflux disease, peptic ulcer disease and stress related erosion/ ulcer disease.[19]

2.3.7ALCOHOL induced ulcer:

It is one of the commonly abused drugs, related to a wide range of physical, mental, and social harms and responsible for 3.8% of death and 4.6% of disability adjusted life years lost world wide. Among the various organ system that mediate alcohol effect on the human body and its health, the gastro intestinal tract plays a major role. The alcohol absorption into the blood stream occurs throughout the GIT and its direct contact with the mucosa can induce numerous metabolic and functional changes. These alterations may lead to marked mucosal damage, which can result in a broad spectrum of acute and chronic disease, such as gastro intestinal bleeding and ulcers.[20]

2.3.8OXIDATIVE STRESSinduced ulcer:

The relative oxygen species (ROS) such as superoxide anions, hydrogen peroxide and hydroxyl radicals are involved in the etiology and physiopathology of several human diseases.Including neurogenerative disorder, viral infection, inflammation, auto immune pathologies as well as in digestive disturbances.[21] Such as GI inflammation and gastric ulcer. During gastric oxidative stress the imbalance of aggressive and defensive factors in the stomach plays a vital role in gastric hemorrhage and ulcer formation.

Over production of ROS has been concerned as one of the major pathogenic factors that directly results in oxidative damage, including lipid per oxidation, protein oxidation and DNA damage, which can lead to cell death.[22]

2.3.9PREVENTION

Patients with a recent complicated peptic ulcer are at very risk and it is best in such cases to avoid NSAID treatment entirely; however anti inflammatory treatment must be undertaken, COX-2 inhibitors plus misoprostol , PPI , H2 blockers therapy should be employed.

2.4Constipation

Constipation is prevalent all over world. Many factors including aging of the population, misconceptions about the normal (and desirable) frequency of bowel movements, fear of the consequences of constipation, and the availability of laxatives over the counter have resulted in their widespread use. On the other hand, concern about potential side effects may result in underuse by patients who would profit from laxatives for regulation of bowel habits.

2.4.1Laxatives:

Bulk laxatives such as bran, methylcellulose and ispaghula husk, stretch and stimulate the gastrointestinal tract.Osmotic laxatives (such as lactulose, magnesium sulphate (Epsom salts), macrogols, magnesium hydroxide mixture, phosphate enemas and sodium citrate enema) draw water into the gastrointestinal tract, thereby increasing the bulk of residue in the colon.Faecal softeners, such as liquid paraffin (not recommended), docusate sodium, mineral oils and arachis oil enema.

Stimulant laxatives or purgatives are generally reserved for 'rescue therapy'. They irritate the gastrointestinal tract and include: senna, figs, rhubarb, castor oil (not recommended), bisacodyl, glycerol, dantron (carcinogenic in rodents, therefore use limited to terminal illness), docusate sodium and sodium picosulfate.

2.4.2 Indications:

On initiation of opioid therapy when administration of opioids is expected to last more than five to seven days. Laxative therapy should not be delayed, as opioids predispose to gastrointestinal spasm and obstruction. In palliative care, stimulant laxatives are usually combined with faecal softeners or lactulose.

If straining would exacerbate another condition, for example angina, anal fissure and hemorrhoids. Faecal softeners or bran or another bulk laxative are first choice.

2.4.3Bowel investigations.

Gastrointestinal disease, for example irritable bowel syndrome, diverticular disease and colostomy (bran or another bulk laxative is first choice).

Colonic constipation:

1. Serious pathology has been excluded, including gastrointestinal obstruction, cancers of the gastrointestinal tract, hypothyroidism, and potassium deficiency.

2. Drugs causing constipation have been reviewed or eliminated, as far as possible, for example, iron tablets, sedatives, non-prescription 'cold cures', opioids (including codeine in non-prescription cough medicines and analgesics), salbutamol, beta blockers, calcium channel blockers, some NSAIDs (not aspirin), some anti-emetics, most antipsychotics, some anti-depressants, aluminium-containing antacids, amphetamines(including ecstasy), cocaine, long-term laxatives, drugs causing dehydration, including diuretics and alcohol.

3. Physiological measures have failed, for example: drinking one or two glasses of water with each meal, encouraging exercise, ensuring privacy, and encouraging toileting immediately after meals, particularly breakfast, including more than 20g of dietary fibre/day in the diet. For example, each fruit and vegetable portion contains 2–4g ofdietary fibre. Beans and other legumes contain up to 8g fibre/serving. Bran cereal gives about 10g fibre/helping. Recommend five portions (15 ounces/375g) of fruit or vegetables daily.

Management of faecal incontinence, due to dementia, decreased storage capacity or overflow, may involve controlled defecation twice weekly.Failure to pass faeces within three days of childbirth (single dose). Lactulose is prescribed in advanced liver disease to minimize the associated central nervous system disturbances (known as hepatic encephalopathy). Doses are usually higher than those prescribed for constipation.

2.5Review of various studies on *Clitoria ternatea*

S.P. Anand et al.,2011 carried out the study of anti bacterial activity on leaves of *Clitoria ternatea* linn. Using various organic solvent (pet ether, ethyl acetate, methanol) extracts against *Bacillus cereus, staphylococcus Aureus, Klebseiella Pneumonia,Salmonella Typhi* by agar disc and diffusion method and reported that methanolic extract shows anti bacterial activity. The study validates the methanolic extract of this species in ethnomedicine, favouring the isolation of anti bacterial agents from the leaf extracts of *Clitoria ternaea.*(23)

P. Daisy et al.,2009 reported that the hypoglcycemic effects of *Clitoria ternatea* linn in leaves and flowers on alloxan induced diabetes in rats. The results validate the significant reduction in serum glucose level, glucose -6 phosphatase and increase in serum insulin level.The aqueous extracts of *Clitoria ternatea* leaves and flowers significantly ($P<0.05$) reduced Serum glucose, glycosylated hemoglobin and the activities of gluconeogenic enzyme, glucose-6-phosphatase, but increased serum insulin, liver and skeletal muscle glycogen and the activity of the glycolytic enzyme, glucokinase. For all the biochemical tests performed, the leaf extract-treated ratshowed essentially the same profile as those treated with the flower extract.(24)

Manish Gunjan et al., 2010 reported the pharmacognostic and anti diabetic study of *Clitoria ternatea* . There is a significant decrease in blood glucose level in the 7^{th} and 14^{th} days of diabetes induction showing anti diabetic activity. The effect was comparable to that of standard antidiabetic drug Glibenclamide.(25)

Shammy sarwar et al.,2014 reported *Clitoria ternatea* is well adapted to heavy cracking clay soils due to superphosphate on the soils.CNS depressant activity showed that the extract decreased the dose dependent motor activity and exploratory behavior of mice in hole cross and open field test. The number of field crossed in open field test and hole crossed in hole cross test decreased as time approached.(26)

Shyamkumar et al., 2012 carried out the study of anti inflammatory, analgesic and phytochemical studies of *Clitoria ternatea* linn flower extract. The results show that due to the presence of taraxerol, pentacyclic tri terpenoids responsible for the pharmacological activity of the extract. The study reveals that the test drug shows significant protection against carrageen induced paw edema.(27)

Neelmani chauhan et al., 2012 reported the pharmacognostical, phytochemical and pharmacological evaluation of *Clitoria ternatea* for anti asthmatic activity. The study shows that *Clitoria ternatea* extract posses many pharmacological activity such as anxiolytic activity, anthelmintic activity, CNS depressant activity, immunomodulatory activity, anti microbial activity, CNS depresstant activity, anti stress activity, effects on general. Behavior, immune modulatory effect, larvicidal effect, proteolytic activity, diuretic activity.And also reported that crude extract from seeds of *Clitoria ternatea* showed maximum zone of inhibition (22±0.5 mm) against *E. coli* at 0.75 mg concentration and minimum with *M. flavus* of (14±1 mm) and the callus extract showed maximum zones of inhibition (16±2mm) against *S. typhi* while the lowest with *E. coli* and *S. aureus* (12±1 mm and 12±0.9mm) respectively. Alcoholic and Aqueous extracts from in vitro raised calli were tested for antibacterial activity by agar well diffusion method against Gram-negative bacteria. Antibacterial activity was shown against *Salmonella* spp. and *Shigella dysenteriae*; organisms causing enteric fever. In addition, the methanol crude extracts showed anti-bacterial activity against *K. pneumonia* and *P. aeruginosa*.[28]

A.Jayachitra et al., 2012 were analysed enzymic anti-oxidant activity in both flowered leaf of *Clitoria ternatea*, by using in vitro model study as alternative to live animals (goat liver slices).. The enzymic antioxidants analyzed in the leaves were superoxide dismutase (SOD), catalase, peroxidase, polyphenol oxidase, glutathione reductase and glutathione transferase**.** The leaves of *Clitoria ternatea* were found to be good sources of all the enzymic antioxidants analyzed (SOD, CAT, POD, GST and PPO).The results showed that the activities of all the enzymic antioxidants analyzed were found to be more in the white flowered leaves than inthe blue flowered leaves.[29]

Varsha jadhav et al., 2013 investigate the anti-oxidant activites of different fractions from different extracts (leaves, stem and root) were evaluated by using antioxidant assay like DPPH, FRAP, Metal Chelating Ability, Reducing Power assay. Methanolic extract of*C. ternatea* root showed highest value 87.75±0.05% and 74.26±0.04% in DPPH and Ferrous ion chelating activity whereas its stem extract 0.588±0.2 and leaves extract 2.132±0.037 mg of AAE per 100 g in FRAP and Reducing power assay respectively. Almost all the fractions of white variety showed highest activity as compare to blue variety. This study showed that both variety of *C. ternatea* have antioxidant properties which provide a basis for the traditional use of plant and could be harnessed as drug formulation.[30]

Neda et al., 2013 reported the anti proliferative property of *Clitoria ternatea* extracts were examined by colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide) assay Preliminary results showed that the water extract of CT had significant effects ($p < 0.05$) against MCF-7 with an IC_{50} value of 175.35 µg/ml. Furthermore, the aqueous and methanolic extracts were investigated by Gas Chromatogram-Mass spectrometry (GC-MS). The GC-MS chromatogram analysis of the water extracted had shown five peaks that represented components in the water extract namely mome inositol (38.7%) and pentanal (14.3%). Fifteen chemical constituents were identified in the methanol extract and the major chemical constituents were mome inositol (33.6%), cyclohexen, 1-methyl-4-(1-methylethylideme)- (7.1%), acetic acid, cyano- (6.5%) and hirsutene (5.7%). Heavy metals tested were at very low levels. The analysis conducted on the flowers provides a strong basis for emphasizing the medicinal and nutritional value of CT.[31]

Selvamaleeswaran ponnuswamy et al., 2011 estimated that primary metabolites such as protein, lipid, starch, phenol and carbohydrate in different plant parts containing different proportion in *Clitoria ternatea.*The highest amount of soluble sugar was observed 40.92 mg/100g in stem of C. ternatea, protein 13.96 mg/100g in seed of *Clitoria ternatea*, carbohydrates 36.24 mg/100g in stem of *Clitoria ternatea*, Total Ash 9.95 mg/100g leaf of *Clitoria ternatea* and lipid 12.3 mg/100g seed of *Clitoria ternatea*.[32]

Kuppan Nithianantham et al., 2011 evaluates the hepatoprotective and anti oxidant activity of *Clitoria ternatea* in methanolic extract. The antioxidant property of methanolic extract (ME) of *Clitoria ternatea* leaf was investigated by employing an established *in vitro* antioxidant assay. The hepatoprotective effect against paracetamol-induced liver toxicity in mice of Methanolic extract of *Clitoria ternatea* leaf was also studied. Activity was measured by monitoring the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and billirubin along with histopathological analysis.[33]

Kohei Kazuma et al., 2003 reported that 3 flavonol glycosides such as kaempferol, quercetin, myricetin were isolated from the petals of *Clitoria ternatea* . The structure were identified using UV, MS, NMR spectroscopy. kaempferol and quercetin 3-(2G- rhamnosylrutinoside), kaempferol, quercetin, and myricetin 3-neohesperidosides, 3-rutinosides, and 3-glucosides in the same tissue. In addition, the presence of myricetin 3-O-(200-O-a-rhamnosyl-600-O-malonyl)-b-glucoside was inferred from LC/MS/MS data for crude petal extracts. The flavonol compounds identified inthe petals of C. ternatea differed from those reported in previous studies.[34]

Saxena abhishex et al.,2013 evaluate the hypoglycemic effect of methanol ratio extract of clitoria ternatea leaves, shows significant (P<0.001) reduction in blood glucose level in alloxan induced method.The methanolic extract of the drug showed marked effect for decreasing the blood glucose level and rectifying the problem like fatigue and irritation associated with the disease. Two concentration of the extract were used for the investigation i.e. 400 mg/kg and 200 mg/kg against the standard glibenclamide 10 mg/kg dose showed 23.12 % decrease in blood glucose level, 200mg /kg showed 21.92% decrease and standard drug showed 28.52% decrease during the study of two week when compare with the standard drug. 400mg/kg dose of methanolic extract was near about as effective as standard drug (glibenclamide).[35]

Shyam kumar et al., 2011 studied the in vitro cyto toxic activity of *Clitoria ternatea* flower extracts(ethanol & pet ether) using trypan blue dye exclusion method. Both extract exhibit significant cell cyto-toxic activity. There was a dose dependent increase in cytotoxic activity for all the concentrations tested. petroleum ether extract the concentration 10 µg/ml showed a reduction of 8 % and 100% reduction observed at 500µg/ml. In case of ethanolic extract at 10 µg/ml concentration 1.33 % reduction was observed and at 500µg/ml 80 % reduction in cell count were observed.[36]

Murugalakshmi et al., 2013 reported anti pyretic and purgative activities of ethanol and acetone extract using albino rats. The present study highlights the significant anti pyretic and purgative than the standard drug. The results obtained showed that the extracts were found to exhibit antipyretic and purgative activities. Present study highlights the significant antipyretic activity of the ethanol and acetone extracts of *Clitoria ternatea* leaves. The antipyretic activity of *Clitoria ternatea* leaves extract was found to be higher than the standard drug paracetamol. The purgative activity reveals that the induction of purgation was significant for acetone extract. Ethanol extract of *Clitoriaternatea* was found to exhibit higher degree of purgation than the standard sodium picosulphate. The reduction in rectal temperature was found to be 0.55^{0}C than the standard paracetamol (0.35^{0}C).[37]

Iracema lima Alnouz et al.,1994 reported the proteolytic activities in seeds of *clitoria ternatea* during germination.The data indicate the presence of different groups ofproteolytic enzymes in cotyledons and axis of *Clitoriaternatea* L.[38]

Babu uma et al., 2009 reported the phytochemical analysis and anti microbial activity of clitoria ternatea linn against extended spectrum beta lactamase producing enteric and urinary pathogens. Anti microbial activity was carried out by disc diffusion and minimum inhibitory concentration by two fold serial dilution method using various extract(aqueous, methanol, chloroform) against uropathogenic E.coli, Enteropathogenic E.coli, Enterotoxigenic E coli, Typhimurium, Pseudomonas aureginosa.(39)

Kavitha R. et al., 2013 carried out Phytochemical analysis of ethanolic extract of clitoria ternatea. Kiranmai S.Rai et al 2001 studied the learning and memory in rats of clitoria ternatea root extracts. The result revealed that the root extract has memory enhancing property in neonatal rats, treated with 100 mg/kg body weight for 30 days.(40)

Amol P.Patil et al 2011 reported the evaluation of in vitro anti oxidant activity of seeds of blue and white flowered variety of *clitoria ternatea* linn, methanolic extract of *clitoria ternatea* shows more significant anti oxidant activity.Methanol extracts seeds of Blue and White flowered varieties of *Clitoria ternatea* (CT) were studied for DPPH free radical scavenging assay, reducing power assay and hydroxyl radical scavenging assay. Pet Ether, Chloroform and Methanol extracts seeds of White flowered variety of CT were significantly inhibited the DPPH free radical at the concentrations ranging from 50-600 µg/ml, showed highest inhibition at 600 µg/ml i.e. 52.07%, 56.20% and 76.46% respectively. Pet Ether, Chloroform and Methanol extracts seeds of Blue flowered variety of CT were showed highest inhibition of DPPH free radical i.e. 46.44%, 54.03 % and 70.68% at 600µg/ml respectively. Methanol extracts of CT also showed significant reductive ability as well as hydroxyl radical scavenging activity. Methanol extract of seeds of white flowered variety of CT showed more significant antioxidant activity as compared to blue flowered variety of CT.(41)

Manoj salhan et al., 2010 reported anthelmintic activity of aqueous and ethanolic leaf extract of *clitoria ternatea.*The study involved the determination of time of paralysis (P) and time of death (D) of the worms. At the concentration of 100 mg/ml both the ethanolic and the aqueous extracts showed very significant activities as compared to the standard drug levamisole (0.55 mg/ml). In case of aqueous extract the time of paralysis and death time was observed as 18 ± 1.57 and 53.33 ± 0.33 and in case of ethanolic extracts 12.33 ± 0.80 and 32.33 ± 0.71 respectively.(42)

Pat.M.Lee et al., 2011 reported that thermal degradation behavior of anthocyanin extract of *clitoria ternatea* (blue flowered) showed a dramatic decrease in stability after a certain stabilizing period.(43)

Arumugam et al., 2012 studied the in vitro propagation and anti bacterial activity of *clitoria ternatea* linn. The anti bacterial activity were evaluated using disc diffusion method against gram positive bacteria such as bacillus cereus, bacillus subtilis & staphylococcus aureus. Result showed that in vitro derived callus and plants of *clitoria ternatea* exhibits anti bacterial activity against pathogenic bacteria.(44)

Selvamaleeswaran ponnusamy et al., 2010 studied the effect of leaves of *clitoria ternatea* linn against the fish pathogens. The fish pathogens include such as pseudomonas aeruginosa, E.coli, K.pneumonia, B.subtilis, A.formicans etc. were tested against agar well diffusion method. Different effect of *clitoria ternatea* showed inhibitory effect against various fish pathogens.(45)

Rishov mukhopadhyay et al., 2012 reported the in vitro free radical scavenging of*clitoria ternatea* leaf extracts by 1,1- diphenyl-2 picryl-hydrazyl (DPPH) radical scavenging assay. *Clitoria ternatea* exhibit potent in vitro free radical scavenging activity. Methanolic extract posses more activity than chloroform and pet ether extract.(46)

Arya suebkhampet et al.,2012 studied the effect of using aqueous crude extract from butterfly pea flowers as a dye on animal blood smear(chicken, pigeon, dog, and horse)staining.Preliminary results revealed that faint acidophilic staining was found in the nuclei of nucleated cells in the blood smears of all species. The cytoplasm of red blood cells stained grayish pink with differences of shading. Additionally, dull acidophilic staining was detected in the granules of the chicken heterophils and also the eosinophils of all species. The results indicated that using a crude extract from butterfly pea flowers for blood smear staining was able to differentiate the blood cells.(47)

Chapter 3

Aim and Objectives

Gastric ulcer is very common global problem today. It is now generally agreed that gastric lesions develop when the delicate balance between some gastro protective and aggressive factors are lost. Major aggressive factors are acid, pepsin helicobacter pylori and bile salts. Defensive factors involve mucous bicarbonate secretion and prostaglandins. Hyper secretion of gastric acid is a pathological condition, which occurs due to uncontrolled secretion of hydrochloric acid from the parietal cells of the gastric mucosa through the proton pumping H+K+ATPase. Even the normal rate of acid secretion may cause ulceration in the breached mucosa when some gastro protective factors are lost.

The modern approach to control gastric ulceration is to inhibit gastric acid secretion, to promote gastro protection, block apoptosis and stimulate epithelial cell proliferation for effective healing. Antisecretory drugs such as proton pump inhibitors (omeprazole, lansoprazole, etc.) and histamine H2-receptor blocker (ranitidine, famotidine, etc.) are extensively used to control increased acid secretion. But these drugs may produce unwanted effects if prolonged usage of these drugs.Herbal drugs reduce the offensive factors and are proved to be safe clinically effective, having better patient tolerance, relatively less expensive and lesser side effects. The need of present study reveals about the anti ulcer and laxative activity on animal models. Based on the on top of hypothesis the prospective medicinal plant specifically selected for investigation *Clitoria ternatea*(L) extracts for various experiments on animal models.

The objectives of the present study are:

1. Collection and authentication of plant and the plant parts
2. Extraction of plant materials with 50% methanol
3. Carrying out preliminary phytochemical screening
4. Carrying out TLC analysis for both plant extracts.
5. To evaluate the drug and excipient interactions by FT-IR spectral studies
6. To evaluate the following Pharmacological activities ofleafextractsof the selected plantusing the various standard experimental models.
    i. Anti Ulcer studies.
    ii. Laxative activity.

Chapter 4

Materials and Methods

4.1Collection and authentication of plant and the plant parts

The plant materials used in this study were leaves of *Clitoria ternatea* (L). (Family - Fabaceae)is collected from the Krishnankoil, Srivilliputur (Virudhunagar dist, Tamil Nadu, India.). The plant was authenticated by Dr.Stephen, Department of Botany, American College, Madurai, India.

4.2 Animals used

Adult male albino rats (150 - 200g) were used in this study. They were maintained in clean, sterile, polypropylene cages and fed with commercial pellet rat chow (M/S Hindustan lever limited, Bangalore, India) and water ad libitum. The study was approved by the Institutional Ethical Committee, which follows the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPSCEA).

4.3 Preparation of 50% methanol extracts of *Clitoria ternatea* (L) Leaves.

Clitoria ternatea (L). Leaves were shade dried and coarsely powdered. The powdered materials were extracted with methanol. The last traces of the solvent were removed and concentrated to dryness under vacuum using a rotary evaporator. The dried extract was weighed and then kept at -4ºC until ready for use. The yield of the extract was 56.4 % (w/w). In each experiment, the extract was diluted with water to desired concentration.

4.4Materials used for the studies:

4.4.1Drugs used

Ranitidine	-	Aciloc injection (Cadila)
Sodium Pico sulfate	-	Cremalax (Abbott)
Loperamide	-	Loparet (Retord lab)

4.4.2Chemicals used

Methanol	-	CDH(central drug house, New Delhi)
Petroleum ether	-	CDH(central drug house, New Delhi)
Benzene	-	CDH(central drug house, New Delhi)
Chloroform	-	CDH(central drug house, New Delhi)
Silica gel (TLC grade)	-	CDH(central drug house, New Delhi)
Silica gel (column grade)	-	SD Fine chemicals

4.5 Methodology[48-50]

4.5.1 Preliminary Phytochemical Screening for *Clitoria ternatea* (L).

4.5.1.2 Test for Carbohydrate

4ml of the extract was dissolved separately in 4 ml of dH2o and filtered. The filtrate was subjected to the following testes to detect the presence of carbohydrate.

i) Molisch's test

The filtrate was treated with 2-3 drops of 1% alcoholic α-napthol solution and 2 ml of concentrated H2S04 was added along the sides of the test tubes. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrate.

ii) Fehling's test

The filtrate was treated with 1 ml of Fehling, s solution A and B and heated on the water bath. A reddish precipitate was obtained show the presence of carbohydrate.

4.5.1.3 Test for proteins and free amino acid

3ml of extract was dissolved in few ml of distilled water and treated with following reagents

i) **Million's Reagent:** - Appearance of red color shows the presence of proteins and free amino acids.

ii) **Ninhydrin Reagent**: - Appearance of purple color shows the presence of proteins and free amino acids.

iii) **Biuret test**: - Equal volume of 5% sodium hydroxide solution and 1% copper sulphate solution were added, appearance of pink or purple color shows the presence of proteins and free amino acids.

4.5.1.4 Test for phenolic compounds

3ml of extract was taken in distilled water and test for the presence of phenolic compounds was carried out with dilute ferric chloride solution (5%w/v) - Appearance of violet color indicates the presence of phenolic compounds.

4.5.1.5 Test for flavonoids

i) Aqueous NaOH solution

3ml of methanolic extract, dissolved in aqueous sodium hydroxide. Appearance of yellow color indicates the presence of flavonoids.

ii) Conc. Sulphuric acid

2ml of extract, concentrated sulphuric acid was added. Appearance of Yellow orange color indicates the presence of flavonoids.

4.5.1.6 Test for Alkaloids

Wagner test:

Added 2ml filtrate of extract with 1% HCl and applied steam. 1ml of the solution added with 6 drops of Wagner's reagent. Appearance Brownish-red precipitate indicates the presence of alkaloids.

4.5.1.7 Test for Tannin.

Braemer's test

10% alcoholic ferric chloride is added to 2 of methanolic extract. An appearance of Dark blue coloration of the solution indicates the presence of tannin.

4.5.1.8 Test for reducing sugar.

Fehling test

Added 25ml of diluted sulphuric acid (H2SO4) to 5ml of extract in a test tube and boil for 15mins. Then cool it and neutralize with 10% sodium hydroxide to pH 7 and 5ml of Fehling solution. An appearance of Brick red precipitate indicates the presence of reducing sugar.

4.5.1.9 Test for Glycosides

(a) **Legal test**: Dissolved the extract in pyridine and added sodium nitroprusside solution to make it alkaline. The formation of pink red to red color shows the presence of glycosides.

(b) **Baljet test:** To 1 ml of the test 50% methanolic extract added 1 ml sodium picrate solution and the change yellow to orange color reveals the presence of glycosides.

4.6COLUMN CHROMATOGRAPHY[51-52]

Chromatography is a separation technique of complex mixture. Currently there are many techniques in use. Among them chromatography is a simple technique. The separation of components in column chromatography involves the principle adsorption. i.e. the components of mixture have different affinity towards adsorbent material hence, they gets adsorbed and migrate at different rate. So it is possible to isolate single component by adjusting the solvent systemize by increasing or decreasing the polarity of the solvent.

4.6.1SAMPLE PREPARATION:

The methanolic extract of *Clitoria ternatea* (6gm) was dissolved in a small amount of methanol and mix thoroughly with silica gel and dired to have a free flowing nature. This mixture was taken for column studies.

PREPARATION OF COLUMN:

METHOD: WET PACKING

The adsorbent material,silica gel was mixed with solvent of none poured gently from the top of the column. To a desired lengththen the same solvent was run through the

column for 2-3 min. Time to prevent air entrapment and the solvent used was maintained up to 10cm above the column bed.

The sample mixture was poured from the top of the column with the aid of funnel. The column was allowed to keep overnight,undistributed. In the next day column was eluted with different solvents with gradually increasing the polarity by changing the solvent. The flow rate of solvent system was adjusted between 16-20 drops per min. Each fraction was collected to maximum of 100ml and it was evaporated at low temperature. Then it is identify by TLC and chemical test.

4.7THIN LAYER CHROMATOGRAPHY

Thin layer chromatography is so widely used that is has become an essential technique for analyst and research workers. TLC is the almost universal analytical technique in chemical analysis for organic and in organic matter.

TLC is a simple rapid method carried out using thin layer of adsorbents on plates. TLC not only combines the advantage of paper and column chromatography but in certain aspects it is found to be superior to either method.

TLC is an important tool in the separation, identification and estimation of different classes of natural products. When a mixture containing different components is made to ascend in a TLC plate with the help of a solvent which act as a mobile phase, there will be a preferential adsorption of different components at different places on the plate. The result is the separation of components.

4.7.1Preparation of TLC Plate:

80 gm of silica gel G was weighed and shaken to a homogenous suspension with 85 ml of distilled water for 90 sec. This suspension was poured in TLC applicator which was adjusted to 0.25 mm thickness 20 carriers' transparence of layer disappeared. The plates were dried in hot air oven at 110 c for 30 minutes (activation). The plates were then stored in a dry atmosphere and used whenever required.

4.7.2Application of extracts for separation:

The various diluted extracts spotted on a TLC plate 2 cm above its bottom using capillary tube. Most solution for application was between 0.1-1 % strength. The starting point was equally sized as far possible and spots had diameter ranging from 2-5 mm.

$R_f$value = Distance travelled by the solute / Distance travelled by the solvent

4.8Characterization of Phytoconstituents using spectroscopy techniques:

All the separated compounds from methanol extract of *Clitoria ternatea*leaf extract Curcuma was characterized by FTIR spectroscopy technique.

4.9ANTI ULCER ACTIVITY[53]

4.9.1PYLORIC LIGATION INDUCED ULCER MODEL:

PROCEDURE:

Animals were divided into four groups of five animals each. The dosages of drugs were administered by following

GROUP I	:	Control (Tween 80, 5mg/kg)orally.
GROUP II	:	Ranitidine 30 mg/kgorally.
GROUP III	:	Methanolic extract of Clitoria ternaea 150 mg/kg orally.
GROUP IV	:	Methanolic extract of Clitoria ternatea 300 mg/kg orally.

The animals were deprived of food for 24 hr before the experiment. The pylorus was ligated by means of technique used in (shay et, al.1945). *Clitoria ternatea*leaf extract was administered in a dose of 150,300 mg/kg by orally for 7 days. After last dose of administration one hour, the pylorus ligation was made under anesthesia. The animal were returned to the observation chamber for 4 hr then the animals were sacrificied by decapitation method, the abdomen of each was opened and the stomach was isolated after suturing the lower esophageal end. The gastric juice was collected along the greater curvature, the mucosal layer was washed with distilled water.

Titrations of Acid Concentrations[54]

One ml of filtered gastric contents was pipetted into a small beaker, the washings and the gastric contents were centrifuged at 2000 rpm for 10 min. The supernatant fluid (1 ml) was diluted with 9ml of distilled water and 2-3 drops of Topfer's reagent was added and then titrated with0.01N sodium hydroxide solution till the solution turns to orange color. Free acidity was calculated based on the volume of alkali added. The solution was further titrated with 0.01N sodium hydroxide solution till the solution regained pink color. The total acidity was calculated based on the total volume of alkali added. The appearance of yellow color after the addition of methyl orange indicates that no free acid is present. Each stomach was then examined carefully for scoring the severity of ulcers. The data obtained for pH, volume of acid, secretion of gastric juice

and ulcer index were analyzed. The ulcers were graded as per following methods.The mean ulcer scores of each animal were expressed as ulcer index.

0 - Normal colored stomach.

0.5 - Red coloration.

1 - Spot ulcer.

1.5 - Haemorrhagic streaks.

4.9.2ASPIRIN INDUCED ULCER MODEL[53].

Animals were divided into four groups of five animals each.The dosage of drugs was administered by following.

GROUP I : Control (Tween 80, 5mg/kg)orally

GROUP II : Ranitidine 30 mg/kgorally

GROUP III : Methanolic extract of *Clitoria ternaea* 150 mg/kg orally.

GROUP IV : Methanolic extract of *Clitoria ternatea* 300 mg/kg orally.

The gastric ulcer was induced in each rat by administrating aspirin 500 mg/kg orally.After 45 min methanolic extract of *Clitoria ternatea*and other drugs were administered for seven days. The animals were sacrificed and stomach was excised and cut along the greater curvature, rinsed gently with saline to remove the gastric content and blood clots and the ulcer index was calculated.

4.9.3ETHANOL INDUCED ULCER MODEL[53].

The animals were divided into four groups. The gastric ulcers were induced in rats by administrating absolute ethanol (99%) (1 ml/200 gm) orally, after 45 min methanolic extract of *Clitoria ternatea*and other drugs were administered for seven days. The animals were sacrificedunder anaesthetized conditions, and the stomach was dissected out and ulcer index was calculated.

GROUP I : Control (Tween 80, 5mg/kg)orally.

GROUP II : Ranitidine 30 mg/kgorally.

GROUP III : Methanolic extract of *Clitoria ternaea* 150 mg/kg orally.

GROUP IV : Methanolic extract of *Clitoria ternatea* 300 mg/kg orally.

Ulcer index (UI) was calculated by the following formula

$$\text{Ulcer index} = 10/x$$

X= Total mucosal area / total ulcerated area

The percentage inhibition was calculated by the following formula

$$\% \text{ inhibition} = \frac{\text{UI control} - \text{UI treated}}{\text{UI control}} \times 100$$

4.10 LAXATIVE ACTIVIY [55].

4.10.1LOPERAMIDE INDUCED ANIMALMODEL:

Rats were divided into four groups, each group consist of five rats as following.

GROUP I : Normal saline (5 ml/kg.p.o)

GROUP II : Standard drug Sodium picosulfate (5 mg /kg .p.o)

GROUP III : Methanolic extract of *Clitoria ternatea* (150 mg/kg .p.o).

GROUP IV : Methanolic extract of *Clitoria ternatea* (300 mg/ kg. p.o).

Methanolic extract of *Clitoria ternatea (*150mg, 300mg*)*and other standard drugs were administered for seven days.On 6th day the rats were fasted for 12 hr before the experiment.After last dosing, one hour later all the animals were received loperamide (5 mg/kg. p.o) by gavage. The faecesproduction in all five groups was monitored for 24 hr.

Chapter 5

EXPERIMENTALRESULTS

5.1 Preliminary phytochemical screening

The phytoconstituents were identified by chemical tests, which showed the presence of various phytoconstituents in 50% methanolic extract of *Clitoria ternatea* (L)Presented in Table no.1.

Table no: 1 Preliminary phytochemical screening of the 50 % methanolic extract of*Clitoria ternatea* (L).

S. No.	Constituents	Tests	*Clitoria ternatea extract*
1.	Carbohydrate	Molish's test	+
2.	Proteins & amino acids	Million's test	+
3.	Flavonoids	Aqueous NaOH test	+
4.	Alkaloids	Dragendroff's test	+
5.	Tannin	$Fecl_3$	+
6.	Glycosides	Baljet test	+

Where, + = Presence, – = Absence

5.2 Table 2 Various Fractions of *Clitoria ternatea* (L) extract on Column Chromatography

S.No	Solvent system	Ratio	Nature of Residues
1.	Petroleum ether	100	Yellow color residue *
2.	Petroleum ether + Benzene	90 + 10	Yellow color residue *
3.	Petroleum ether + Benzene	80 + 20	Colorless residue
4.	Petroleum ether + Benzene	70 + 30	Colorless residue
5.	Petroleum ether + Benzene	60 + 40	Greenish residue
6.	Petroleum ether + Benzene	50 + 50	Greenish residue
7.	Petroleum ether + Benzene	40 + 60	Greenish residue
8.	Petroleum ether + Benzene	30 + 70	Greenish residue
9.	Petroleum ether + Benzene	20 + 80	Colorless residue
10.	Petroleum ether + Benzene	10 + 90	Colorless residue

* = Selected for TLC studies.

5.3TLC studies of *Clitoria ternatea* (L) extract

Table no: 2Analytic TLC using Chloroform: Ethyl acetate as the mobile phase solvent

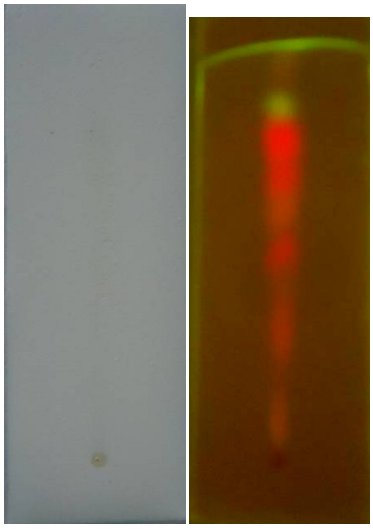
S. No.	Chloroform: Ethyl acetate	No. of bands
1	1:9	04
2	2:8	03
3	3:7	02

The 50 %MEtOH extract of *Clitoria ternatea* (L). TLC plates were visualized under UV254and visible. The plates were spray with anisaldehyde-sulfuric acid and heat at110^{0}C for 10 min.

The plates were scanned densitometrically using CAMAG TLC scanner at 584 & 250 nm.

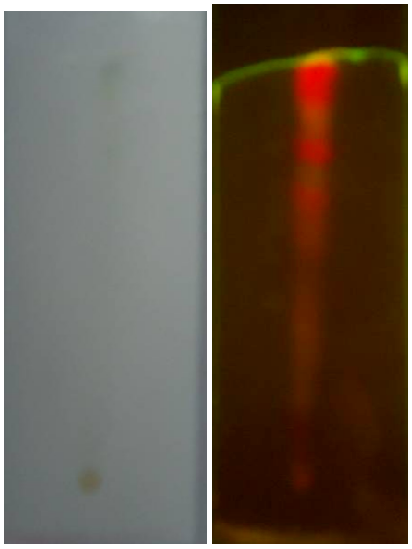
(Fig 1- 3).

Fig-1: 50 %MEtOH extract of *C. ternatea* (L).TLC plates were visualized under UV254and visible.



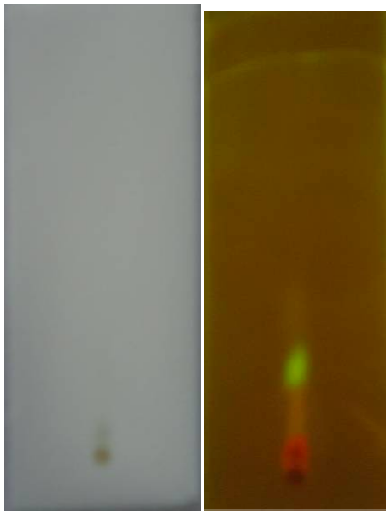
VISIBLE UV254nm

Fig-2: 50 %MEtOH extract of *C. ternatea* (L).TLC plates were visualized under UV254and visible.



VISIBLE UV254nm

Fig-3: 50 %MEtOH extract of *C. ternatea* (L).TLC plates were visualized under UV254and visible.



VISIBLE UV254nm

5.4 FTIR studies of separated compound

The IR spectrum showed an absorption at 2917, 2848, 1734, 1462 and 1271 cm^{-1} indicating the presence of C-H stretching in CH3, C-H stretching in CH2, C=C stretching,CH Bending and C-O stretching. The reports are showed in fig -13page no.

5.5ANTI ULCER ACTIVITY

5.5.1Effect of *C. ternatea* (L) on Pylorus Ligation Induced Ulcer

The alcoholic extract of the*C. ternatea* (L) at a dose of 150 and 300 mg/kg produced a reduction in the ulcer index, gastric volume, free acidity, total acidity and raised gastric pH significantly ($p < 0.05$) in comparison to the control group. The reference drug ranitidine as expected produced a significant reduction in gastric ulcer and total acid output as compared to control group (Table 4).

5.5.2Effect of *C. ternatea* (L)on Aspirin Induced Ulcer

The results obtained in the experimental model of aspirin-induced gastric ulceration in rats are presented in Table 5. The alcoholic extract was found to possess remarkable ulcer-protective properties at150, 300 mg/kg. The maximum effect of ulcer protection (52.23 %) was observed at 300 mg/kg of *C. ternatea* (L)fed animals whereas the standard drug ranitidinegave 66.29% of ulcer protection.

5.5.3 Effect of *C. ternatea* (L)Ethanol Induced Ulcers

The results obtained in the experimental model of ethanol -induced gastric ulceration in rats is summarized in Table 6. The alcoholic extract was found to possess remarkable ulcer-protective properties at 150, 300 mg/kg. The maximum effect of ulcer protection (45.83%) was produced at300 mg/kg and the standard drug (ranitidine) gave 69.94% of ulcer protection.

5.6 Effect of *C. ternatea* (L) Laxative activity

The results obtained in the experimental model of loperamide induced laxative in rats are summarized in Table 7. The alcoholic extract was found to possess remarkable ulcer-protective properties at 150, 300 mg/kg. The maximum effect of Faeces output (73%) was produced at300 mg/kg and the standard drug Sodiumpicosulphate (5mg/kg) gave 77% ofFaeces output.

Fig-1 Control-pyloric ligation induced ulcer model.

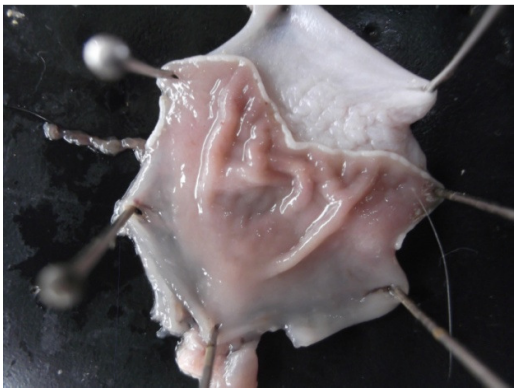


Fig-2 STD Ranitidine treated -pyloric ligation induced ulcer model

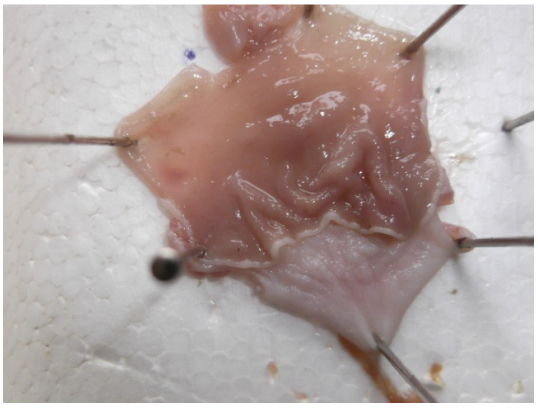


Fig-3Test 1-*C. ternatea* (L) 150mg/kg treatedpyloric ligation induced ulcer model

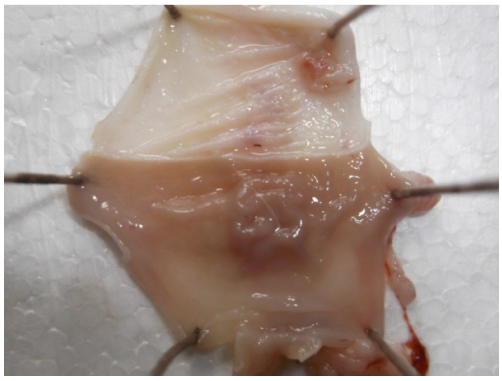
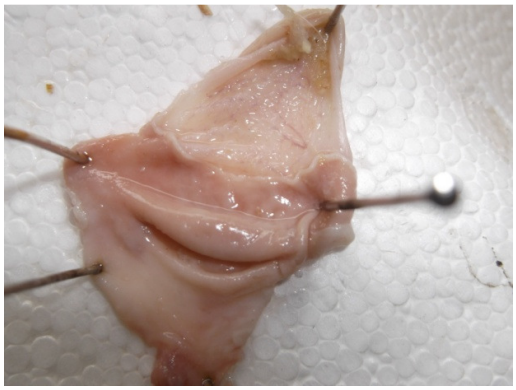


Fig-4Test 3-*C. ternatea* (L) 300mg/kg treatedpyloric ligation induced ulcer model.



Aspirin induced ulcer model

Fig-5 Control-Aspirin induced ulcer model.

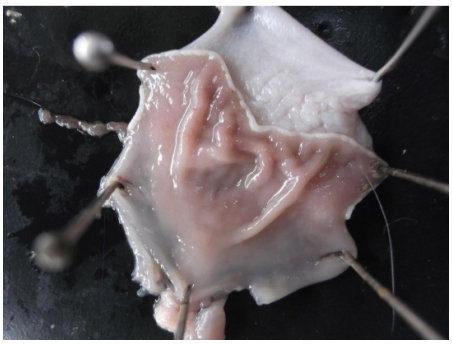


Fig-6 STD Ranitidine treated-Aspirin induced ulcer model.

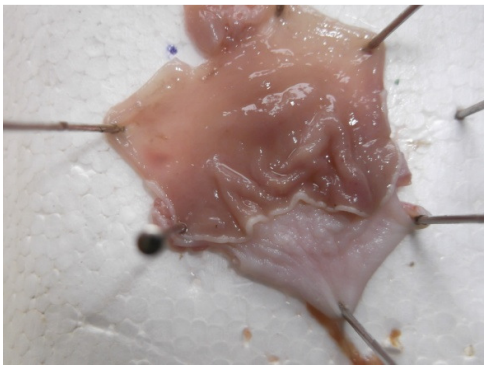


Fig-7Test 1-*C. ternatea* (L) 150mg/kg treatedAspirin induced ulcer model.

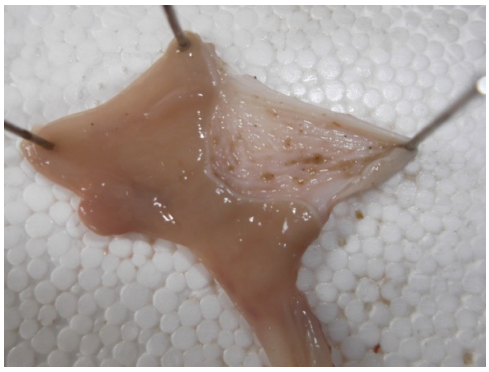


Fig-8Test 2-*C. ternatea* (L) 300mg/kg treatedAspirin induced ulcer model.

Ethanol induced ulcer model

Fig-9 Control-ethanol induced ulcer model.

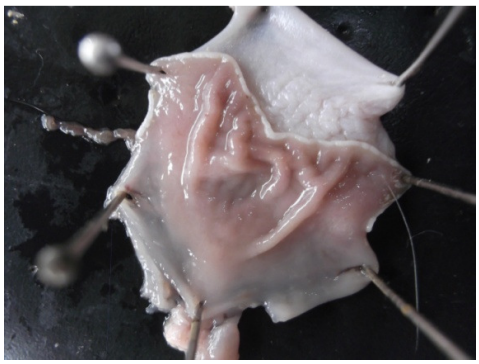


Fig-10 STD Ranitidine treated-ethanolinduced ulcer model.

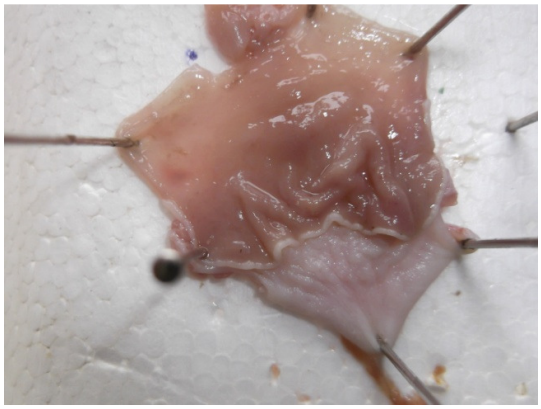
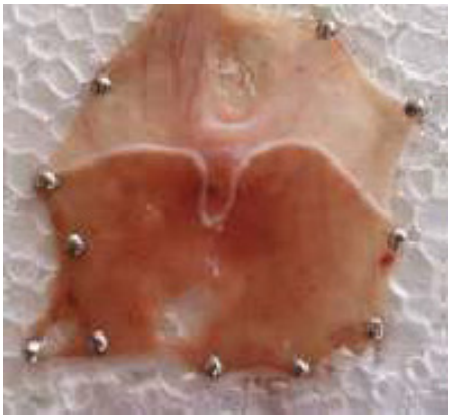


Fig-11Test 1-*C. ternatea* (L) 150mg/kg treatedethanolinduced ulcer model.

Fig-12Test 2-*C. ternatea* (L) 300mg/kg treatedethanolinduced ulcer model.



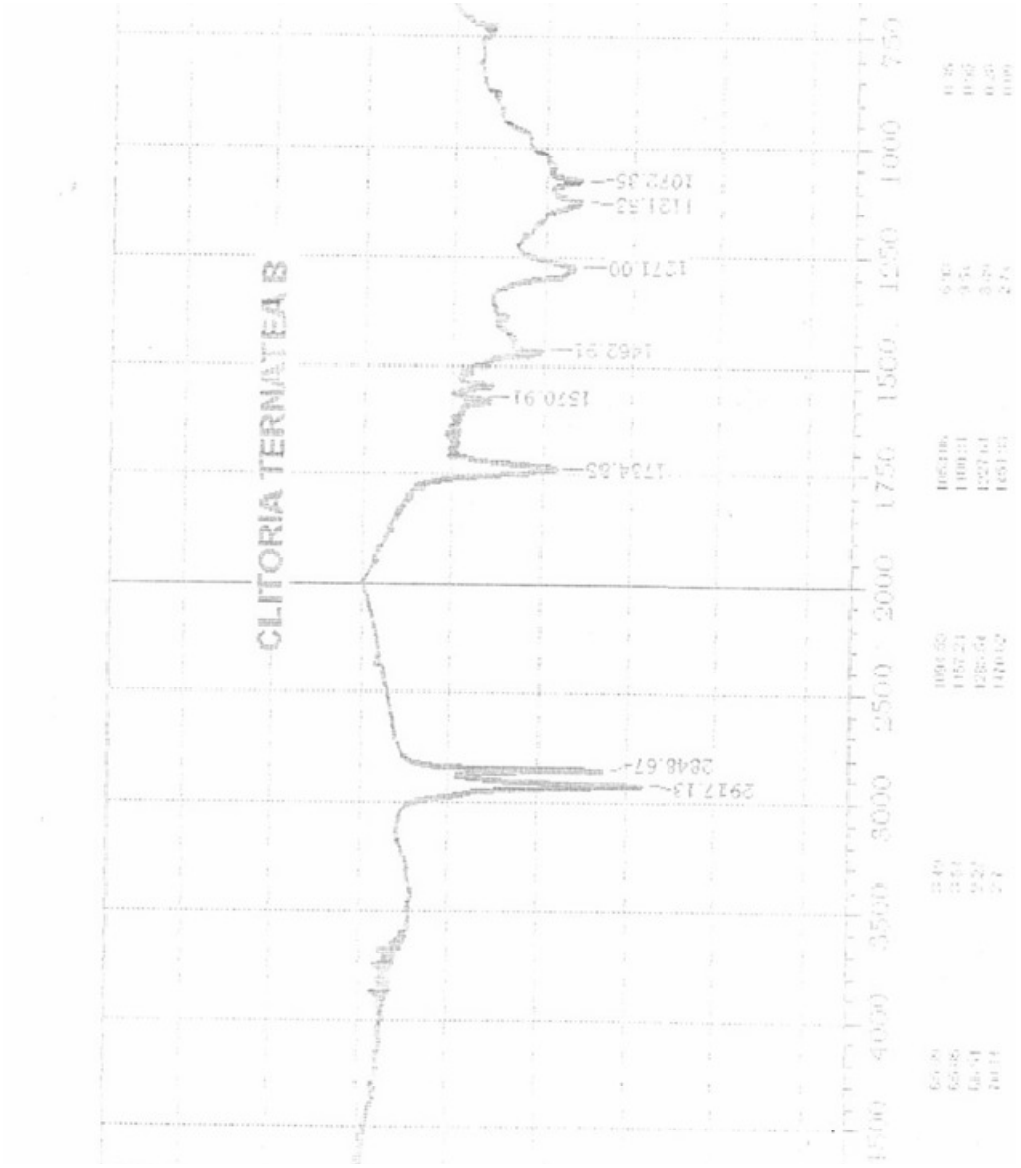

CLITORIA TERNATEA B
1072.35
1121.53
1571.00
1462.91
1570.91
1734.65
2846.67
2917.13

Table: 4Effect of *Clitoria ternatea* leaf extract on pyloric ligated model.

S.No	Treatment	Dose (mg / kg)	Gastric volume (ml)	pH	Free acidity (mEq/L/100g)	Total acidity (mEq/L/100g)	Ulcer index	Protection %
1	Control tween80	5ml / kg	3.17±0.14	3.15±0.18	32.20±1.75	72.12±1.62	4.10±0.11	---
2.	Ranitidine	30mg / kg	5.82±0.81**	5.85±0.61	62.12±0.45**	54.34±0.56**	1.59±0.12**	61.21 %
3.	CT extract-I	150mg/kg	4.02±0.06	4.07±0.02	31.45±0.72	43.09±0.42	2.32±0.14	43.41%
4.	CT extract- II	300mg/kg	4.62±0.08**	4.45±0.09**	47.70±0.8**	49.65±1.33**	1.96±0.10**	52.19%**

Data are expressed as Mean SEM from five observations as compared to Control group and analyzed by one way analyses of variance (ANOVA). P value is less than 0.0063. (**P< 0.05) By Dennett's Multiple Comparison Test; this difference is considered to be statistically significant.

Table 5 Effect of *Clitoria ternatea* leaf extract on Aspirin induced ulcer model.

S.No	Treatment	Dose (mg / kg)	Ulcer index (mm^2/rat)	Protection %
1	Control tween 80	5mg / kg	4.45 ± 0.18	---
2.	Ranitidine	30 mg / kg	1.50 ± 0.11**	66. 29 %
3.	CT extract I	150 mg / kg	2.12 ± 0.13	46.96%
4.	CT extract II	300 mg / kg	2.36± 0.16**	52.23 %

Results are expressed as mean ± SEM from five observations as compared to Control group the two-tailed paired*t* test. Graph Pad's software method, (**P< 0.001) by conventional criteria; this difference is considered to be extremely statistically significant.

Table 6Effect of *Clitoria ternatea* leaf extract on ethanol induced ulcer model.

S.No	Treatment	Dose (mg / kg)	Ulcer index (mm^2/rat)	Protection %
1	Control tween 80	5mg / kg	4.32 ± 0.01	---
2.	Ranitidine	30 mg / kg	1.32±0.09**	69.94%
3.	CT extract I	150 mg / kg	2.98 ± 0.19	31.10%
4.	CT extract II	300 mg / kg	2.34 ± 0.04**	45.83%

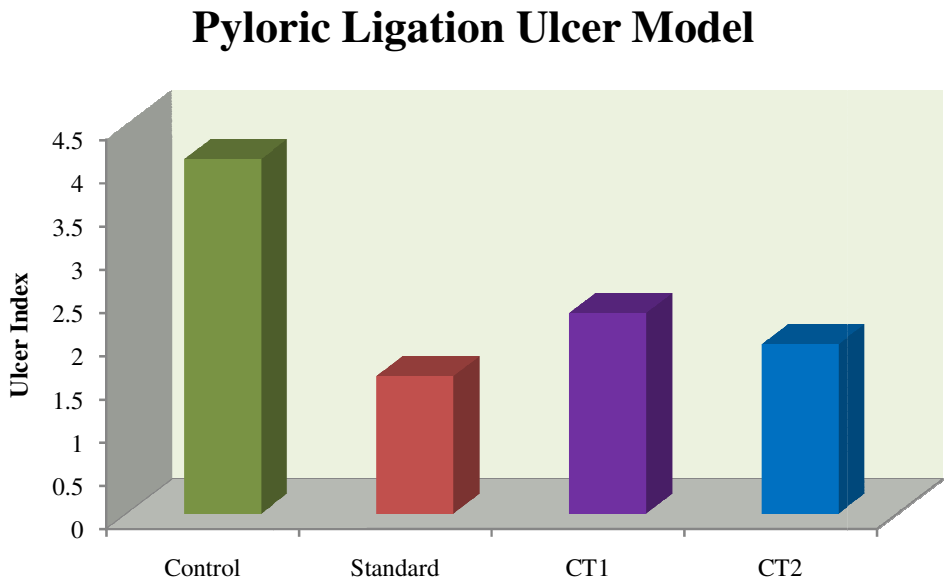
Results are expressed as mean ± SEM from five observations as compared to Control group the two-tailed paired*t* test. Graph Pad's software method, (**P< 0.05) by conventional criteria; this difference is considered to be extremely statistically significant.

Table 7Effect of *Clitoria ternatea* leaf extract on Laxative activity.

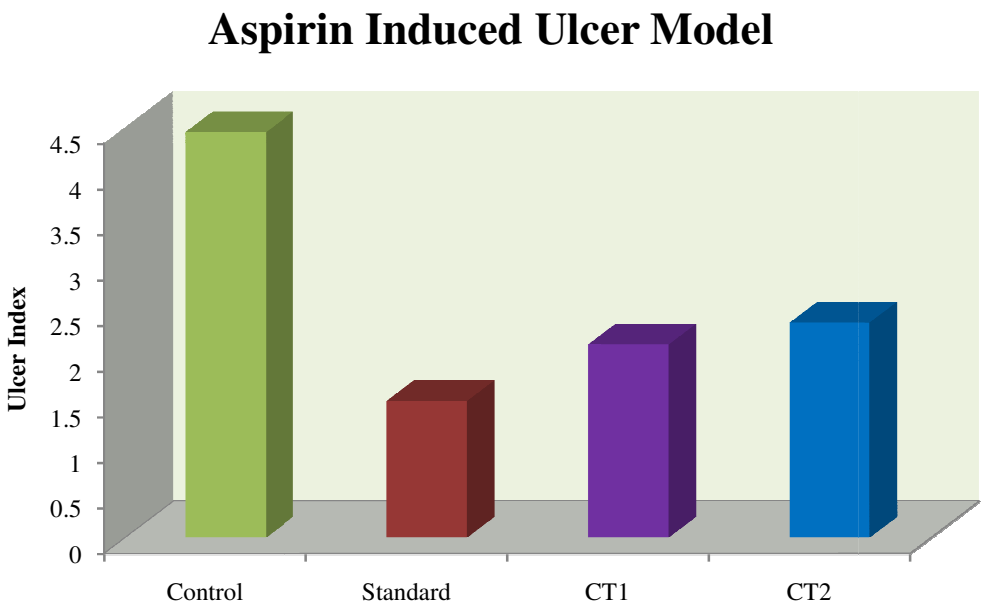
S.No	Treatment	Faeces output (gm)		% of Faces output
		Average (0-8 hrs)	Average (8-16 hrs)	
1.	Normal saline 5ml / kg	0.23 ± 0.62	1.45 ± 0.56	-
2.	Sodiumpicosulphate 5mg/kg	4.02 ± 0.69	6.52 ± 0.14**	77
3.	CT extract I	3.72 ± 0.12	4.12 ± 0.26	64
4.	CT extract II	4.42 ± 0.77	5.38 ± 0.22**	73

Results are expressed as mean ± SEM from five observations as compared to Control group the two-tailed paired*t* test. Graph Pad's software method, (**P< 0.02) by conventional criteria; this difference is considered to be extremely statistically significant.

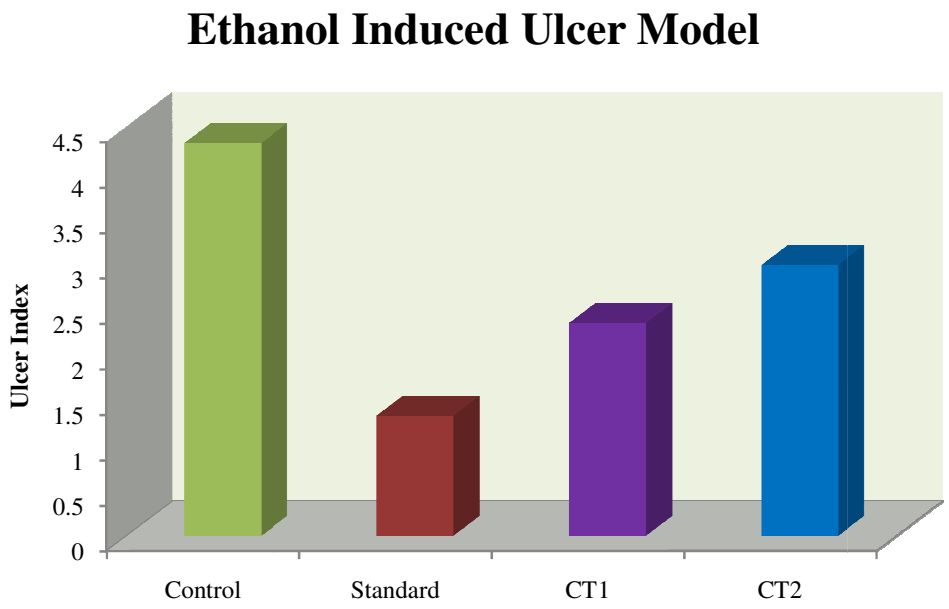
Graph-1 Effect of *Clitoria ternatea* leaf extract on pyloric ligated model



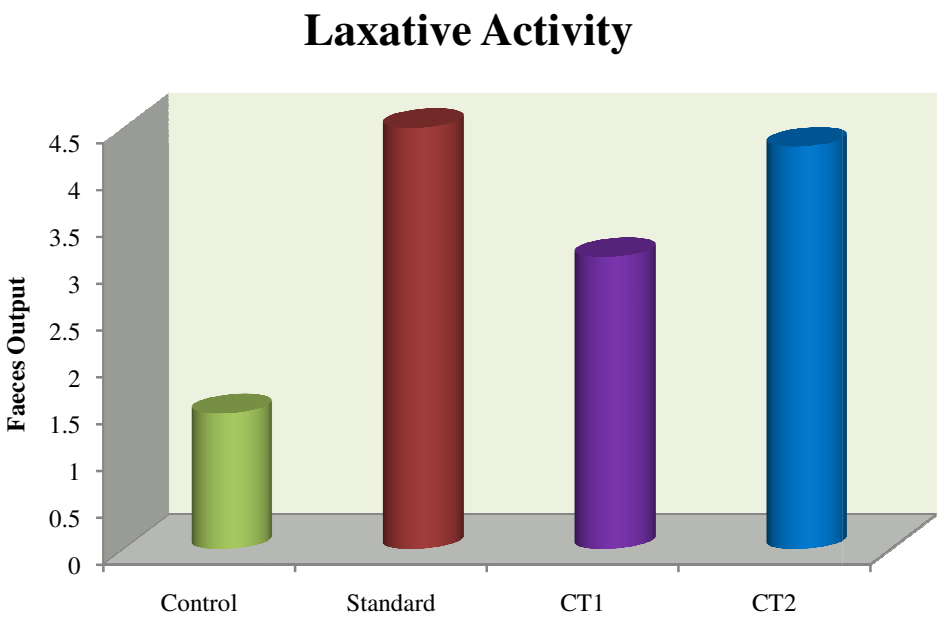
Graph-2Effect of *Clitoria ternatea* leaf extract on Aspirin induced ulcer model



Graph-3Effect of *Clitoria ternatea* leaf extract on ethanol induced ulcer model



Graph-4Effect of *Clitoria ternatea* leaf extract of laxative activity



Chapter 6

DISCUSSION

Globally, there is a positive trend in favor of traditional and integrative health sciences both in research and practice. Screening of plant extracts and herbal formulations in pharmacological and toxicological studies will give new findings about medicinal plants long familiar to mankind. In aerobic organisms, reactive oxygen species (ROS) are continuously produced as a by-product of metabolisms and are also produced on exposure to tobacco smoke, ozone, radiations, organic solvents, pesticides, auto exhaust and other environmental pollutants[56]. In in-vivo, ROS play a positive role such as energy production, phagocytosis, regulation of cell growth and intracellular signaling[57]. On the other hand, over production of ROS are also capable of damaging a wide range of essential cellular bimolecular such as proteins, enzymes, DNA, RNA, lipids and carbohydrates through oxidative modification, consequently may adversely affect immune functions and contributing to the pathological conditions including, aging, gastric ulcer, diabetes, carcinogenesis, neurodegenerative diseases, rheumatic joint inflammation and AIDS. Natural antioxidants such as flavonoids, phenolics, tannins, curcumin and terpenoids are found in various plants. They can reduce the access of oxidants and other deleterious molecules due to their ability to scavenge oxygen nitrogen derived free radicals by donating hydrogen atom or an electron, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases[58].

Peptic ulcer occurs when there is an imbalance between the damaging effects of gastric acid and pepsin, and the defense mechanisms, which protect the gastric and duodenal. The etiology of peptic ulcer is unknown in most of the cases, yet it generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanism. To regain the balance, different therapeutic agents including plant extracts may be used. Methanolic extract of *Clitoria ternatea*is one of the traditional drug used in the present study to evaluate the anti ulcer activity in Pylorus ligation, ethanol induced, aspirin induced ulcer model and laxative activity in albino rats. Aspirin, phenylbutazone, indomethacin, and some other NSAID are also known to cause duodenal and peptic ulceration. Prostaglandins are predominantly synthesized by secretion of gastric acid and stimulate the secretion of mucus and bi carbonate. Hydrophobic surfactant like phospholipids secretion in the gastric epithelial cells is also stimulated by the

prostaglandin. It is also showed volume of gastric ulcers in pyloric ligation model. Volume of gastric secretion is an important factor in the production of ulcer due to exposure of unprotected lumen of the stomach to the accumulating acid.

Ethanol is also has been reported to cause disturbance in gastric secretion, damage to the mucosa, alteration in the permeability, gastric mucus depletion and free radical production. This is attributed to the release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol as oxygen derived free radicals has been found to be involved in the mechanism of acute and chronic ulceration in gastric mucosa. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the hemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to intracellular membrane permeability to sodium and water.

The preliminary phytochemical studies analysis of *Clitoria ternatea* extract showed the presence of alkaloids, flavonoides, tannins, carbohydrate and glycosides. The significant increase in the anti ulcer activity of *Clitoria ternatea* extract could be attributed to the presence of alkaloids, tannins and flavonoides.

The results of the study reveal that extract Clitoria *ternatea* posses significant anti ulcer activity. In Pyloric- ligated, aspirin induced ulcer , ethanol induced ulcer models all the test samples were found to reduced the gastric acid to significant extent ($p<0.001$) as ranitidine compared to control group. The total acidity and free acidity also registered significant decrease in a similar manner. The ulcer index was significant reduced with all the test samples. Similarly the extract of *Clitoria ternatea* posses significant laxative activity on loperamide induced method all the test samples were found that increase in feces content using sodium picosulphate as a standard to compare to control group. It is suggested that methanolic extract of *Clitoria ternatea* can suppress gastric damage induced by various aggressive factors, similarly posses laxative activity on animal models.

The findings of the present study confirmed that the leaf extract of *Clitoria ternatea*possess gastro protective effects against experimentally induced gastric ulcer models. These data corroborate with the earlier observations on *Centella asiatica*[59] in reducing the experimentally induced gastric ulceration. Therefore, the anti-ulcer effects of the leaf extract of *Clitoria ternatea*may be due to its anti-secretary and anti- oxidant properties.

Chapter 7

SUMMARY AND CONCLUSION

There has been global resurgence of interest in herbal drugs in the recent past. Though herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited or improperly used. Therefore, these herbal drugs deserve detailed studies in the light of modern medicine. In spite of synthetic drugs, herbal drugs have their place in therapy. Their effectiveness, low-cost and comparative freedom from serious toxic effects makes these medicines not only popular but also an acceptable mode of treating diseases even in modern times.

Based on accumulative evidence in recent decades, tremendous interest has considerably increased in finding natural substances (i.e. antioxidants) present in foods or medicinal plants to replace synthetic antioxidants, which are being restricted due to their side effects. Natural antioxidants are gaining importance due to their health benefits for humans, decreasing the risk of cardiovascular and degenerative diseases by reduction of oxidative stress and counteraction of macromolecular oxidation.

The theories of herbal formulation have the synergistic, potentiative, agonists/ or antagonistic pharmacological agents within themselves due to incorporation of plant medicines with diverse pharmacological actions. These pharmacological principles work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects. Based on the above theory, the potential plants selected for investigation were*Clitoria ternatea*extracts on gastric ulcer andlaxative in rats. In general there is very little biological knowledge on the specific modes of action in the treatment of selected diseases, but most of the plants have been found to contain substances like flavonoids, glycosides, alkaloids, terpenoids etc that are frequently implicated as having potential biological effects.

On the basis of the present study results and available reports, it can be concluded that the anti ulcer activity and laxative activity elucidated by *Clitoria ternatea* leaf extract could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to suppression of gastric juice. This may be anticipated inhibition of H2 receptor blockers and by stimulation of prostaglandin synthesis in the gastric mucosal membrane.

In the traditional system of Indian medicine, plant formulation and combined extracts of plants are used as drug of choice rather than single drug. In this context, the present studies have been designed to scientifically validate the traditional claims of*Clitoria ternatea*and formulate a potent antiulcer and laxative herbal formulation.

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