Research Article

Evaluation of Antiulcer Activity of *Clitoria ternatea* Flower and Stem-Induced Wister Rats

Eedulakanti Santhosha*, Dr. M Sandya Rani
Centre for Pharmaceutical Sciences, Institute of Science and Technology, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad-500085, Telangana state, India.
***Corresponding author's E-mail:** eedulakantisanthosha95@gmail.com

Received: 10-09-2023; **Revised:** 20-10-2023; **Accepted:** 26-10-2023; **Published on:** 15-11-2023.

ABSTRACT

Medicinal Plants are the replacements of synthetic drugs. Traditional medicine offers us knowledge on biological activity of Medicinal plants. Most of the Nutraceutical's have become popular these days. People started believing herbal medications than synthetic drugs due to their low or none side effects. Obesity is the prevailing situation now-a-days. WHO is expecting about 27million population may become obese until 2030. Therefore, the research of the present study aimed at anti-ulcer activity of Ethanol extract of flowers and stem of C.T. In-vivo antiulcer activity was studied for C.T at different doses of both flowers and stem (100mg/kg, 200mg/kg,) single dose and repeated dose and compared with activity of standard drug Ranitidine (30mg/kg). C.T flower and stem at dose of 200mg/kg showed highest antiulcer activity. This study is possibly advantageous as the bottom line for further study on *Clitoria ternatea* for antiulcer activity.

Keywords: Nutraceutical's, Antiulcer, *Clitoria ternatea*, Ranitidine.

INTRODUCTION

Traditional medicine over the past few decades, eco-friendly, bio-friendly, affordable, and generally safe herbal medications have transitioned from the fringe to the mainstream. The use of medicinal plants is essential in alternative medicine. Indians have historically had a significant impact on the management of biological resources for millennia and have been the guardians of relevant knowledge for generations through trial and error. India has an abundance of medicinal plants and the capacity to rise to the task of satisfying the demand for them on a worldwide scale. The primary healthcare systems in Indian society include Ayurveda, Naturopathy, Unani, Siddha, and Folk medicine, all of which are completely reliant on natural resources[1].

Despite the decline in the incidence of peptic ulcer disease (PUD) in the recent years the economic burden, morbidity, and mortality due to the disease are massive[2]. The most effective classes of drug available to treat PUD include proton pump inhibitor, histamine-2 receptor blockers, and prostaglandin analogues[3]. The efficacy of these agents is marred by their numerous adverse effects which include gastrointestinal dysfunction, mental state changes, and an increased risk of respiratory/enteric infections. Furthermore, the various cytochrome enzyme interactions of these agents can also affect the therapeutic levels of other agents[4]. The limitations of the therapeutic agents available, their interactions, and their adverse effects are leading researchers to investigate various medical plants which could provide an excellent source for never molecules that could be more safe, efficacious, and economical.

The prevalence of peptic ulcer disease in the general population in India. It was determined by endoscopy in a randomly selected samples of population 2763 adults and aged 15 years. In these 239 people with ulcer symptoms, 193 had an oesophagogastroduodenoscopy. Its randomly selected 177 peoples from among that remaining population without ulcer symptoms, they were also endoscoped. The point that prevalence of peptic ulcer was 4.72% and the life time prevalence was 11.22%. The duodenal gastric ulcer ratio was 17.1:1. Duodenal and gastric ulcer both are common in men. The prevalence of peptic ulcer increased with age, with a peak prevalence of 28.8% in the 5th decade of life. The prevalence of complication, such as bleeding, perforation and stenosis were similar to those reported in the west.

Clitoria ternatea, commonly known as butterfly pea, is a perennial herbaceous plant from the Fabaceae family. It has recently attracted a lot of interest as it has potential applications both in modern medicine and agriculture, and as a natural food colorants and antioxidants. *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[5]. Native to the island of Ternate in the *Molluca archipelago*, this species is now widely grown as ornamental, fodder or medicinal plant[6]. The plant originated from tropical Asia and later was distributed widely in south and Central America, East and West indies China and India, where it has become naturalized[7].

Clitoria ternatea is commonly also called Clitoria, blue-pea (sudan), cunh (Brazil or pokindon (Philippines). This plant

is known as Aparajit (Hindi), Aparajita (Bengali), and kokkattan (Tamil) in Indian traditional medicine[8]. It has several synonyms in Ayurvedic scriptures like: Sanskrit names: Aparajita girikarnu, Aspota and Vishnukranta. English names: Butter-fly pea, Mazerion and Winged leaved Cliroria. Local names: Aparajita (Hin) Aparajita (Beng), (Guj), Gokarna (Mar) and Buzrula(Arabic). The juice of flowers is reported to be used in insect bites and skin diseases[9]. The roots are useful in asthma, burning sensation, ascites, inflammation, leucoderma, leprosy, hemicrania, amentia, pulmonary tuberculosis, ophthalmology and reported as a bitter, refrigerant, ophthalmic, laxative, diuretic, cathartic, aphrodisiac, tonic[10]. Consequently they are used in the treatment of a number of aliments including body-aches, infections, urinogenital disorders and as antihelmintic and antidote to animal stings. Seeds are cathartic and useful in visceralgia. They are considered safe for colic, dropsy and enlargement of abdominal viscera[11]. The roots stem and flower are recommended for the treatment of snakebite and scorpion sting in India[12].

MATERIALS AND METHODS

Collection and identification of plant materials

Clitoria ternatea were collected from local areas in Hyderabad, Telangana, India. The plant was morphologically identified and Authenticated by Dr.K. Madhava chetty. Professor and head Department of Botany bearing voucher number 379 belonging to the family Fabaceae. This sample was shade dried as it contains volatile oils for 2-3 weeks. Then size reduced to fine powders and stored in an airtight container for further use.

Chemicals and kits used

Ethanol, Ethyl acetate, Chloroform, Hexane, Topfers reagent.

Extraction Procedure

180gm of powder of flowers of *Clitoria ternatea* were transferred to 500ml conical flask containing 400ml of Ethanol for simple Maceration technique. Intermittent shaking is done for about 1 week. Further extract is filtered and filtrate is kept for evaporation in Rota evaporator.

Institutional Ethical Committee Approval

The Institutional Animal Committee (IAEC) of CMR College of Pharmacy has approved the experimental protocols for evaluation of Antiulcer Activity of *Clitoria ternatea* and approval number is CPCSEA/1657/IAEC/CMRCP/COL-21/102.

Experimental animals

The animals were kept in a continuous environment with 12/12 hours of light and darkness, a humidity of 55%, and a temperature of 22±2 c. They were kept in polypropylene cages and provided with a regular pellet feed as well as unlimited access to water. The CMR College of Pharmacy's (Hyderabad) institutional animal ethics committee gave its approval for the tests and methods employed in the study. Healthy Male Wister albino rats weighing (200-250 g) were divided into 6 groups containing 6 animals each as mentioned in the table 1.

Table 1: Experimental design of Ethanol extract of Flowers and stem of *Clitoria ternatea.*

Groups (N=6)	Treatment and route of administration	Dose and duration
Normal control	Normal water	14 days
Standard control	Ranitidine	30mg/kg (14 days)
Low dose (flower)	Ethanol extract of Flower of *Clitoria ternatea*	100mg/kg (14 days)
High dose (flower)	Ethanol extract of Flower of *Clitoria ternatea*	200mg/kg (14 days)
Low dose (stem)	Ethanol extract of stem of *Clitoria ternatea*	100mg/kg (14 days)
High dose (stem)	Ethanol extract of stem of *Clitoria ternatea*	200mg/kg (14 days)

EVALUATION OF ANTIULCER ACTIVITY

Ulcer index:

After the incision of the stomach at the greater curvature the ulcers were observed. And the number of ulcers was counted using a magnifying glass and the diameter of the ulcers were measured using vernier calipers. The following arbitrary scoring system was used to grade the incidence and severity of lesions.

Normal coloration-0

Red coloration- 0.5

Spot ulcers-1

Hemorrhagic streaks-1.5

Ulcer-2

Perforation-3

Ulcer index =10/x

X =Total mucosal area/total ulceration area

The percentage inhibition was calculated by the following formula

% inhibition = UI control – UI treated/UI control

Determination of Free Acidity and Total Acidity:

The gastric contents were centrifuged at 1000rpm for 10mins. 1ml of supernatant was diluted with 9ml distilled

water. A volume of 2 ml diluted gastric juice was treated with 0.1 N sodium hydroxide run from a micro burette using 3-4 drops of Topfer's reagent as indicator until a canary yellow colour was observed. The volume of NaOH run down was noted. This corresponds to free acidity. Further, 2-3 drops of phenolphthalein were added and titrated with NaOH until pink colour was restored. This gives total acidity. Free acidity and Total acidity are expressed in terms of ml of o.1N HCl per 100gms of gastric contents. This is the same as mEq/lit. Acidity may be calculated by using the following formula:

Acidity = Volume of NaOH × Normality of NaOH × 100mEq/Lit/0.1

Statistical Analysis

Results were expressed as Mean ± standard error of the mean (SEM). Differences between the control and treatment groups in the experiments were tested for significance using Turkey's comparison test. Values of $P<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Extractive Values

Following tables 2,3 determines the extractive values flower and stem of *Clitoria ternatea* in different solvents.

The extract was prepared with flowers of *Clitoria ternatea* powder by simple Maceration technique for 7 days with three different solvents. The obtained crude extract 42.4%w/w, 24%w/w, 24%w/w of Ethanol, Ethyl acetate, and Chloroform respectively. As a result, it was found that the Ethanol solvent quantities was higher, at 42.4g. Ethanol solvent produced high quality of extract so the ethanol extract was advised for use going forward.

The extract was prepared with stem of *Clitoria ternatea* powder by simple Maceration technique for 7 days with three different solvents. The obtained crude extract 28%w/w, 13.6%w/w, 0%w/w of Ethanol, Ethyl acetate, and Hexane respectively. As a result, it was found that the Ethanol solvent quantities was higher, at 28g. Ethanol solvent produced high quality of extract so the ethanol extract was advised for use going forward.

Table 2: Below table 2 describes the values of *Clitoria ternatea* flower extracted using various solvents.

S.No	Nature of extract	Colour	Consistency	Extractive values (gm)
1	Ethanol soluble	Greenish brown 	Semi solid	42.4%w/w
2	Ethyl acetate soluble	Greenish yellow 	Semi solid	24%w/w
3	Chloroform ether soluble	Greenish yellow 	Semi solid	24 %w/w

Table 3: Below table 3 describes the values of *Clitoria ternatea* stem extracted using various solvents.

S.NO	Nature of extract	Colour	Consistency	Extractive values (gm)
1	Ethanol soluble	Green 	Semi solid	28%w/w
2	Ethyl acetate soluble	Green 	Semi solid	13.6%w/w
3	Hexane soluble	None 	Semisolid	0 %w/w

Table 4: Below Table 4 Determines Phytoconstituents in various extracts of *Clitoria ternatea flower*

S.NO	Test		Ethanol	Ethyl acetate	Chloroform
1.	Alkaloids				
		a) dragendoff' s test	present	present	absent
		b) mayer's test	present	present	absent
2.	Carbohydrates	Molisch's test	absent	absent	present
3.	Aminoacids		absent	absent	present
4.	Saponin glycosides	Frothing test	present	absent	absent
5.	Tannins	Ferric chloride test	present	absent	absent
6.	Steroids	Liebermann- burchard's test	present	absent	present
7.	Proteins	Biurette test	absent	absent	absent

Preliminary Phytochemical Analysis

The Preliminary Phytochemical investigation was estimated by standard analytical procedures. Ethanol extract, Ethyl acetate extract, Chloroform extract of flowers and stem of *Clitoria ternatea* showed the presence of alkaloids, carbohydrates, amino acids, saponin glycosides, tannins, steroids. Below Tables 4,5 determines the presence of various constituents in following solvents Ethanol, Ethyl acetate, Chloroform and Hexane.

Ethanol extract revealed the presence of alkaloids, saponin glycosides, tannins, and steroids. Ethyl acetate extract revealed the presence of alkaloids. Chloroform extract

revealed the presence of Carbohydrates, Amino acids and steroids.

Ethanol extract revealed the presence of alkaloids, Carbohydrates, tannins, and steroids. Ethyl acetate extract revealed the presence of steroids. Hexane extract revealed the presence of alkaloids and Carbohydrates.

In-Vivo Anti-Ulcer Activity

Ethanol extract of flowers and stem of *Clitoria ternatea* were explored for its anti-ulcer activity in induced male Wister rats. After the treatment with Ethanol Extracts of flowers and stem of *Clitoria ternatea* of 14 days, Ulcer was evaluated by Biochemical parameters. All the results obtained in this study were represented in the table 4.

The ethanol extracts of *Clitoria ternatea* flower and stem at dose of 100mg and 200mg/kg produced ulcer 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 6).

Table 5: Below Table 5 Determines Phytoconstituents in various extracts of *Clitoria ternatea stem*

S.No	Test		Ethanol	Ethyl acetate	N-hexane
1.	Alkaloids				
		a) dragendoff' s test	present	absent	present
		b) mayer's test	present	absent	present
2.	Carbohydrates	Molisch's test	present	absent	present
3.	Amino acids		absent	absent	present
4.	Saponin glycosides	Frothing test	present	absent	absent
5.	Tanins	Ferric chloride test	present	absent	absent
6.	Steroids	Liebermann-burchard's test	present	steroids	absent
7.	Proteins	Biurette test	absent	absent	absent

Table 6: Effect of *Clitoria ternatea* flower and stem pylorus ligation induced ulcers

Groups	Volume of gastric secretion	pH	Total acidity	Ulcer score	Reduction in Ulcer score	Ulcer index	% Inhibition of Ulceration
Normal Control tween 80 (5ml/kg)	3.92±0.24	3.32±0.75	84.45±1.74	5.84±0.74	-	5.71±0.85	-
Ranitidine 30mg/kg	2.31±0.84***	5.74±0.47***	51.92±1.96***	3.21±0.85***	50.15±1.54***	2.17±0.98***	48.15±1.45***
C.T Flower extract 100mg/kg	3.75±0.85	3.76±0.84	81.56±3.52	4.75±0.86	18.42±1.74	5.14±0.96	25.75±2.57
C.T Flower extract 200mg/kg	2.74±0.24**	4.25±0.78**	81.24±4.25**	3.54±0.78**	37.12±2.45**	3.07±0.74**	39.45±1.85**
C.T stem extract 100mg/kg	3.35±0.84**	4.25±0.74**	77.56±4.85**	4.15±0.26**	30.42±2.96**	3.14±0.74**	26.75±1.96**
C.T stem extract 200 mg/kg	2.74±0.74***	5.24±0.27***	59.24±2.69***	3.54±0.79***	41.12±1.84***	3.07±0.45***	45.45±1.74***

Data were expressed as mean ± standard error of mean and statistically evaluated using one-way analysis of variance, followed by Turkey's multiple comparison tests. $P<0.05$**, $P<0.005$***was considered to be significant.

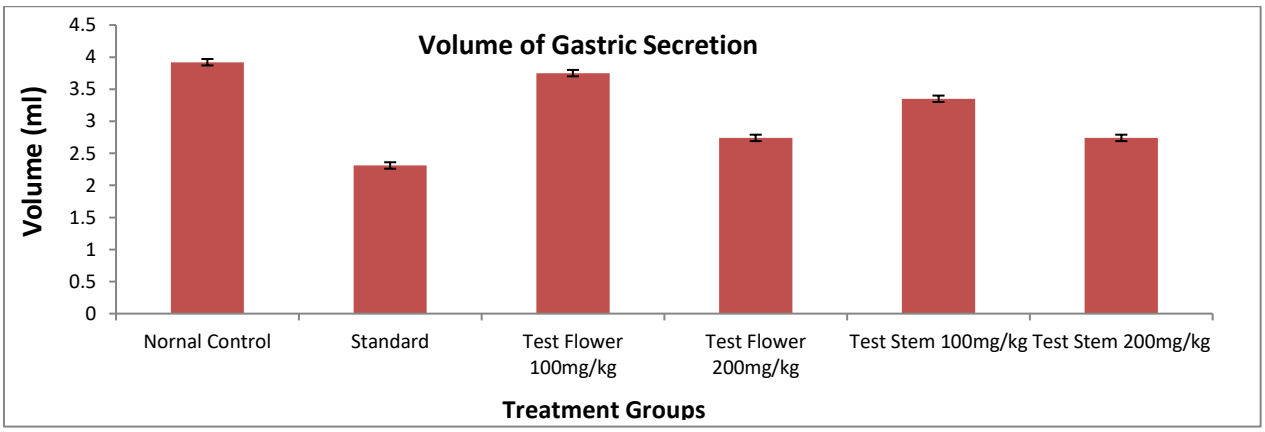


Figure 1: Effect of Volume of gastric secretion on plant extraction

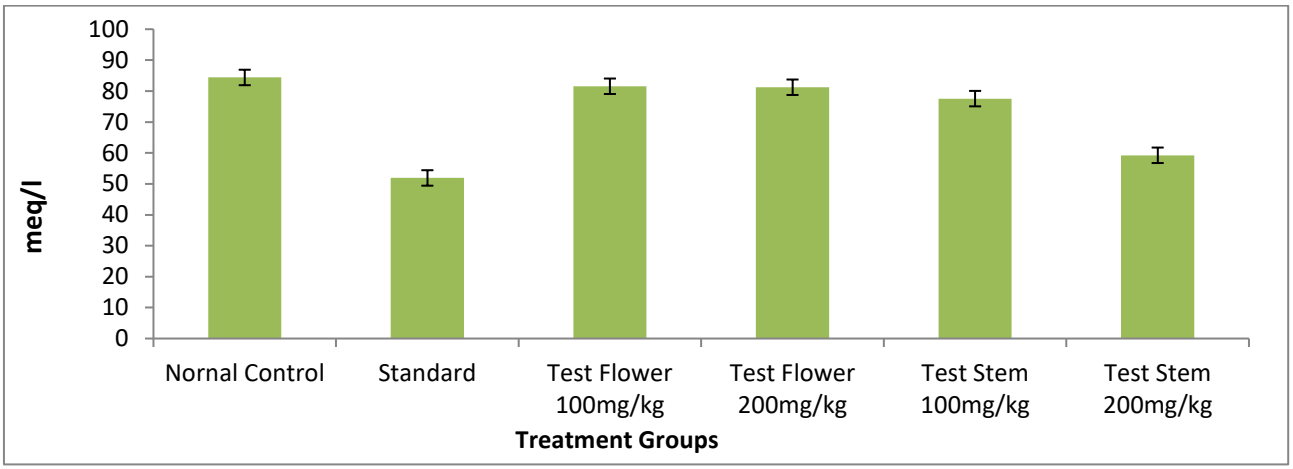


Figure 2: Effect of Total acidity on plant extraction

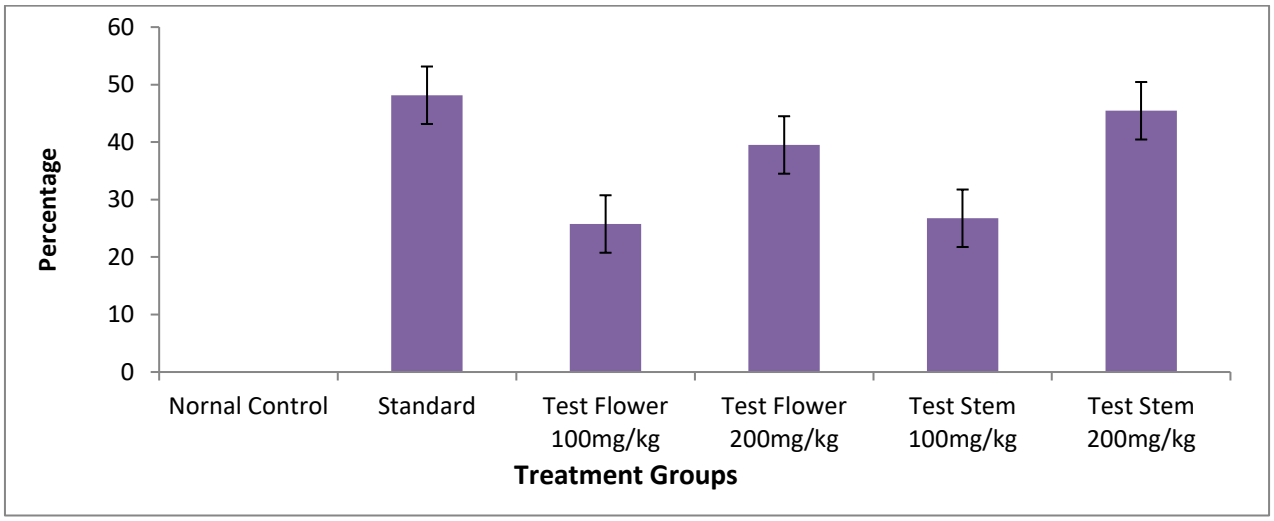
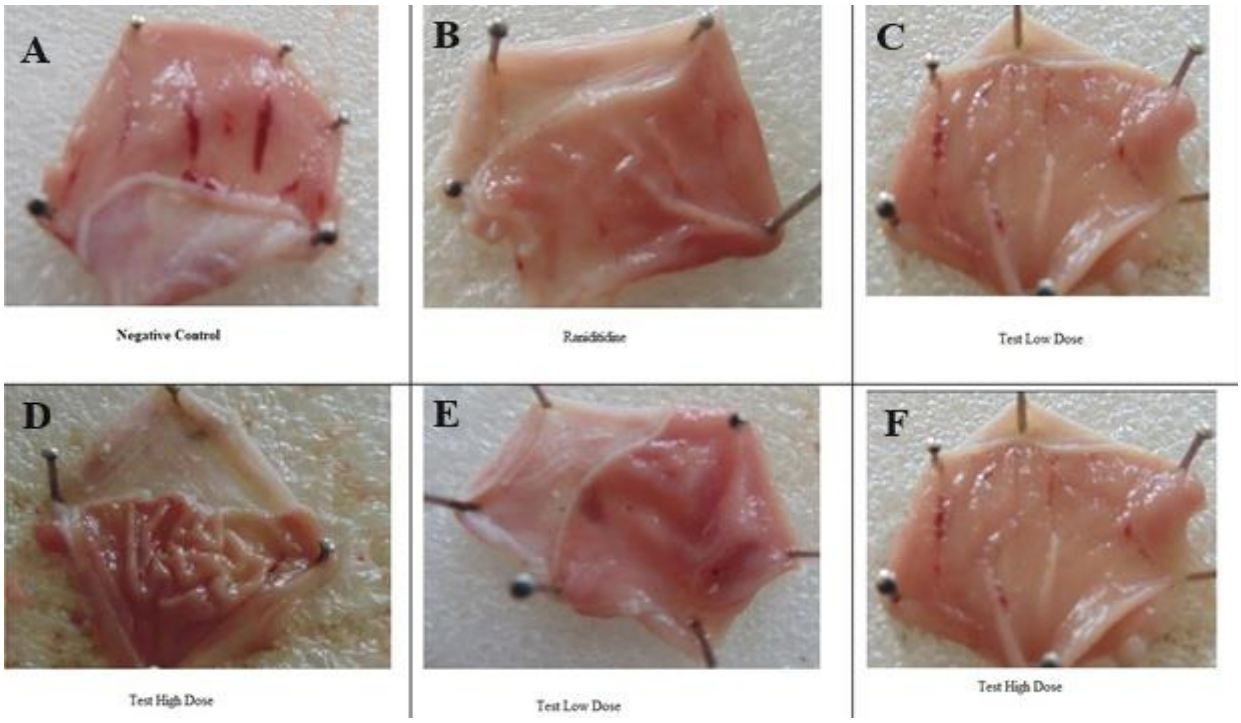


Figure 3: % Inhibition of Ulceration



A-(Normalcontrol) **B-(**Ranitidine 30mg /kg) **C-(**100mg /kg flower)
D-(200mg/kg flower) **E**-(100mg/kg stem) **F**-(200mg/kg stem)

Figure 4: Effect of Ulcer score on different doses pre-treatment in pylorus ligation induced ulcers

DISCUSSION

Ulceration happens when either more aggressive Behaviour or lowered mucosal resistance break the natural balance. The stomach mucosa is continuously exposed to elements that can be hazardous, such as medications, dietetic components, alcohol, microbial by products, acid, pepsin, and bile acids. These elements have been connected to the aetiology of gastric ulcers, and they include increased secretion of pepsin and stomach acid, decreased blood flow and motility, suppression of prostaglandin synthesis, and altered cell proliferation.

Prostaglandin synthesis, stress resistance, the production of antioxidant enzymes, and wound healing characteristics are all enhanced by phenolic compounds and flavonoids. Flavonoids also improve microcirculation and capillary resistance. Tannins directly shield the mucosa's top layer and alter its structure to make it more resistant to chemicals and mechanical harm. Compounds linked to saponins and triterpenoids promote the production of mucus. As a result, the presence of active secondary metabolites such as polyphenolics, terpenes, saponins, and tannins may be related to their strong anti-ulcer activity. The phytochemical investigation of the current study indicated that ethanol and aqueous extracts of *Clitoria ternatea* were sources of alkaloids, carbohydrates, polyphenols, saponins, tannins, amino acids and glycosides.

Ligation of pyloric part is one of the approaches to induce the gastric ulcer. Ligation of the pyloric portion causes collection of gastric acid and pepsin activation, which leads to the formation of ulcers. Additionally, mucosal digestion affects the production of prostaglandins E2 and I2, which are critical regulators of mucus, bicarbonate, and phospholipids, as well as the suppression of gastric acid secretion and stimulation of mucus secretion in the stomach epithelial cells.

Pylorus ligation is a crucial technique that demonstrates potential modifications to the factors governing stomach content, such as the volume of gastric juice, overall acidity, and pH. The increased production of pepsin and gastric acid, which results in the auto-digestive process of the gastric mucosa, is what causes ulcers brought on by pyloric ligation. Since high stomach acidity overwhelms the mucosal defence mechanisms and causes ulcer formation, inhibition of raised gastric acidity is one of the key preventive factors.

The present study assessed all of the potential parameters that may be used to degree the overall anti-gastric ulcer activity of *Clitoria ternatea* flower and stem such as gastric volume, pH, free and total acidity, ulcer index, and percentage of inhibition. A significant increase in the pH of gastric juice and decrease in free and total acidity, and ulcer index after treatment with both extracts were observed. The results were correlated with the results of Ahmed et al, 2023 and a significant protective effect was observed with *Clitoria ternatea.*

CONCLUSION

In this study, an attempt was made to find the potentiality of *Clitoria ternatea* against ulcer induced male Wister rats. For the studies Ethanol extract of *Clitoria ternatea* flower and stem were selected from local areas in Hyderabad and stored for further use.

Extractive values of *Clitoria ternatea* were determined in different solvents (Ethanol, Chloroform, Ethyl acetate and Hexane), among the 4 solvents Ethanol is found to be show better yield of flower 42.4w/w and stem 28w/w.

The Preliminary Phytochemical investigation was estimated by standard analytical procedures. Excess phytochemical constituents were observed in Ethanol extract, revealed the presence of Alkaloids, saponin glycosides, tannins, carbohydrates and steroids in flower and stem.

Further the extracts were prepared by simple soxhlation technique using Ethanol as a solvent in 180gms of *Clitoria ternatea* flower and 120gms of *Clitorai ternatea* stem powders to obtain Ethanol extract of *Clitoria ternatea* 24gms of yield is achieved in flower and 16gms of yield is achieved in stem.

Ethanolic extract of flower and stem of *Clitoria ternatea* were explored for its anti-ulcer activity in induced male Wister rats. After the treatment with Ethanol extracts of flower and stem of *Clitoria ternatea* of 14 days, Ulcer was evaluated by Biochemical parameters.

The ethanol extracts of *Clitoria ternatea* flower and stem at dose of 100mg and 200mg/kg produced ulcer 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.

The above results are the clear evidence for the potent anti-ulcer effect of ethanol extract of *Clitoria ternatea* and the effect is completely dose dependent in nature. Another important benefit possessed by the plant is, It did not shown any effect on animals which were treated with the high doses used, where the high dose of drug shown decreasing the acidity level.

REFERENCES

1. Dr. Susan Sam, Importance and effectiveness of herbal medicines, Journal of Pharmacognosy and Phytochemistry, 2019;8(2):354-357.

2. Yuan Y, Padol IT, Hunt RH: Peptic ulcer disease today. Nat Clin Pract Gastroenterol Heptol 2006;3:80-89.

3. Wallace JL, Sharkey KA: Pharmacotherapy of gastric acidity, peptic ulcers, and gastroesophageal reflux disease; in Brunton LL (ed): Goodman and Gilman's The Pharmacological Basis of Therapeutics. New York, McGraw-Hill, 2012, 1307-1351.

4. McQuaid KR: Drugs used in the treatment of gastrointestinal disease; in Katzung BG, Trevor AJ (eds):

Basic and Clinical Pharmacology. New Yark, McGraw-Hill, 2015, 1536-1579.

5. Gomez, S., M., Kalamani, A., Butterfly Pea (*Clitoria ternatea*): A Nutritive Multipurpuse Foraga Legume for the Tropics-An Overview, Pakistan Journal of Nutrition, 2003;2(6):374-379.

6. Jain, N., N., Ohal, C., C., Shroff, S., K., Bhutada, R., H., Somani, R., S., Kasture, S., B., *Clitoria ternatea* and the CNS, Pharmacology, Biochemistry and Behavior, 2003;75:529-536.

7. Barik,D., P., Naik, S., K., Mudgal, A., Chand , P., K., Rapid plant regeneration though in vitro axillary shoot proliferation of butter-fly pea (*Clitoria ternatea L*) - a twinning legume, *In Vitro* Cell. Dev.Biol. - Plant, 2007;43: 144-148.

8. Parimaladevi, B., Boominathan, R., Mandal, S., C., Anti-inflammatory, analgesic and anti-pyretic properties of *Clitoria ternatea* root, Fitoterapia, 2003;74:345-349.

9. Agrawal, P., Deshmukh, S., Al,i A., Patil, S., Magdum,C.,S., Mohite, S., K.and Nandgude, T., D., Wild Flowers as medicines, International Journal of Green Pharmacy, 2007;1(1):20-26.

10. Nadkarni, K., M., Indian Materia Medika, Popular Publications, Bombay, 1976;14:354-355.

11. Morris, J., B., Legume genetic resources with novel value added industrial and pharmaceutical use. In: Janick, J.(Ed.), Perspectives on Newcrops and Uses. ASHS Press, Alexandria, VA, USA,1999, 196-201.

12. Kazuma, K., Noda, N., Suzuki, M., Malonylated flavonol glycosides from the petals of *Clitoria ternatea*, Phytochemistry, 2003;62:229-237.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.