

Pharmacological Evaluation of Indian Medicinal *Clitoria Ternatea (L)* in Experimental Animal Models

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ABSTRACT

Nature has bestowed on us very rich botanical wealth and a large number of diverse type of plants grown in different parts of our country. The Materia medica of various indigenous system of medicines practiced in India has become extensive and heterogenous¹.the present study reveals about the Indian medicinal plant *Clitoria ternatea* (L) focussed about the gastroprotective activity. It is commonly known as butterfly pea belonging the family (fabaceae).The useful parts are leaf, root, stem, bark, seeds and flower².the study focussed about various aspects of clitoria ternatea, the preliminary phytochemical studies of *Clitoria ternatea* extract shows the presence of alkaloids, flavonoids, tannins, carbohydrates, and glycosides on experimental basis the methanolic extract of *Clitoria ternatea* posses significant Anti ulcer activity by various methods like Pyloric ligation method, Ethanol induced method, Aspirin induced method and Loperamide induced Laxative activity. All test samples were found to reduced the gastric acid to significant extend (P<0.001) as Rantidine compared to control Group. The extract posses that the methanolic extract of *Clitoria ternatea* can suppress gastric damage induced by various aggressive factors , similarly posses the laxative activity on experimental animal models.

Keywords: CPSCEA, Pyloric Ligation Method, Clitoria Ternatea, Cremalex, Loperamide, Sodium Picosulphate, Aciloc Injection, Methanol

I. 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 ailments has been in vogue from ancient times³.The medicinal use of the plant is found in the Rig Veda , the oldest repository of human knowledge, our knowledge of medicinal plants has mostly been inheritent 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.

II. METHODS AND MATERIAL

Collection and Authentication of Plant and Plant Parts

The plant materials used in this study were leaves of *Clitoria ternatea* (L) family fabaceae is collected from the Krishnan koil, Srivilliputtur (virudhunagar dist), tamilnadu, India. The plant was authenticated by Dr.Stephen, Department of Botany, Americal college, Madurai.

Animals

Adult male albino rats (150-200 g) were used in this study. They were maintained in a 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 institution of ethical committee, which follows the guidelines of committee for the purpose of control and supervision of experimental animals(CPSCEA)

Preparation of 50 % Methanolic extracts

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 -4 °C until ready for use. The yield of the extract was 56.4 % (w/w). In each experiment was diluted with water to desired concentration

Drugs & chemicals used

Ranitidine (Aciloc injection), Sodium Picosulphate (Cremalex), loperamide (loparate), methanol (CDH).

Preliminary Phytochemical Screening^{4,5,6}

Methanolic extract of *Clitoria ternatea* was performed with test for carbohydrates, test for proteins and amino acids, test for Phenolic compounds, test for flavonoids, test for alkaloids, test for tannins, test for glycosides.

Anti Ulcer Activity⁷

Pyloric ligation method

Animals were divided into four groups of five animal each. The dosage of drugs were administered by the following groups. Group-1 (control) receives tween 80, 5 mg/kg. orally. Group-2 (standard) Rantitidine 30 mg/kg orally. Group-3 (test 1) methanolic extract of *Clitoria ternatea* 150 mg/kg orally. GP4 (test 2) methanolic extract of *Clitoria ternatea* 300mg/kg orally.

Titration of Acid Concentration

1 ml of filtered gastric contents was pipette out into a small beaker, and centrifuged at 2000rpm for 10 min. The supernatant was collected and the volume of gastric juice was expressed as ml/100 g body weight. total acidity was determined in the supernatant by titration against 0.01 N NaoH, using 2-3 drops of Topfers reagent as indicator until canary yellow colour was observed. Volume of NaoH required was noted and this corresponds to free acidity. Further 2-3 drops of phenolphthalein was added and titrated with 0.01 N NaoH until pink colour was restored and this gives total acidity. Free acidity and total acidity is expressed in terms of 0.1 N HCL / 100 g of gastric contents. The

data obtained for pH, volume of acids, secretion of gastric juice and ulcer index was analysed. The ulcers were graded by ulcer scores of each animal was expressed as ulcer index.

Aspirin Induced Method

The animals were divided into four groups as described above. The gastric ulcer was induced in each rat by administering aspirin 500 mg/kg orally. After 45 min methanolic extract of *Clitoria ternatea* and other control and standard drug were administered for seven days. The animals were sacrificed and the stomach was excised and cut along the greater curvature, rinsed gently with saline to remove the gastric content and the blood clots & ulcer index was calculated.

Ethanol Induced Method

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

Ulcer index (UI) was calculated by the following formula

$$\text{Index ulcer} = 10/X$$

$$X = \text{Total mucosal area} / \text{total ulcerated area}$$

The percentage of inhibition was calculated by the following formula

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

Laxative activity⁸

Loperamide Induced Animal Model

Rats were divided into four groups, each group consists of five rats as described above methods. Methanolic extract of *Clitoria ternatea* (150 ,300 mg/kg) and other standard drug (sodium picosulfate), normal saline were administered for seven days. On 6 th day the rats were fasted for 12 hr before the experiment. After last dosing, one hour later all the

animals were received Loperamide (5mg/kg/p.o) by Gavage. The faeces production in all five group was monitored for 24 hrs.

Glycosides, Tannins, Alkaloids, Flavonoids, Proteins and Amino acids and Carbohydrates.

III. RESULTS AND DISCUSSION

Experimental Results

Phytochemical screening

Preliminary phytochemical screening of methanolic extract of *Clitoria ternatea* revealed presence of

Anti-ulcer activity

Pyloric ligation method

Pretreatment with methanolic extract of *Clitoria ternatea* produced significant protection (**P<0.05) by pyloric ligation method as compared to control animals. The extract showed protective effect of 52.19%, 43.41% inhibition. free acidity, total acidity and ulcer index were expressed in tabular column

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 Tween 80	5mg/kg	3.17±0.14	3.15±0.18	32.20±1.75	72.12±1.62	4.10±0.11	---
2.	Ranitidine	30 mg/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	150 mg/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%

Aspirin induced method

S.No	Treatment	Dose (mg/kg)	Ulcer index (mm ² /rat)	Protection %
1.	Control tween 80	5mg/kg	4.45±0.18	---
2.	ranitidine	30mg/kg	1.50±0.11**	66.29%
3.	CT extract I	150mg/kg	2.12±0.13	46.96%
4.	CT extract II	300mg/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. The percentage of ulcer inhibition 46.96% and 52.23%.

Ethanol induced method

S.No	Treatment	Dose (mg/kg)	Ulcer index (mm ² /rat)	Protection %
1.	Control tween 80	5mg/kg	4.32±0.01	---
2.	Ranitidine	30mg/kg	1.32±0.09**	66.94%
3.	CT extract I	150mg/kg	2.98±0.19	31.10%
4.	CT extract II	300mg/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<0.005) by conventional criteria; this difference is considered to be extremely statistically significant. The percentage of ulcer inhibition 31.10% and 45.83%.

Laxative Activity

S.No	Treatment	Faeces output (gm)		% of faeces output
		Average (0-8 hrs)	Average (8-16 hrs)	
1.	Normal Saline (5ml/kg)	0.23± 0.62	1.45 ±0.56	---
2.	Sodium Picosulphate 5mg/kg	40.2± 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.the percentage of ulcer inhibition 64% and 73%.

IV. CONCLUSION

Globally, there is a positive trend in favour of traditional and integrative health science 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. The preliminary Phyto-chemical studies shows the presence of alkaloids, flavonoids, tannins, carbohydrates, glycosides. The result of the study reveals that extract *Clitoria ternatea* posses significant anti ulcer and laxative activity. It could be attributes to the presence of alkaloids, tannins and flavonoids. It is suggested that methanolic extract of *Clitoria ternatea* can suppress gastric damage induced by various aggressive factors, similarly possess laxative activity on animal models.

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

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