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Studies on Neuropharmacological Effects of *Clitoria ternatea* Linn. Root Extract in Rats and Mice

R. Boominathan[1], B. Parimaladevi Devi[1], Subhash C. Mandal[1] and Subhash. C Mandal[2]*

[1]*Dvision of Pharmacognosy & Phytochemistry*
Department of Pharmaceutical Technology Jadavpur University Kolkata ?2 INDIA
[2]*Regional Institute of Pharmaceutical Science & Technology Agartala, INDIA*

Abstract – Ethanol extract of the root of *Clitoria ternatea Linn* (CTEE) was evaluated for different neuropharmacological actions, such as general behaviour, exploratory behaviour, muscle relaxant activity and phenobarbitone induced sleeping time, in rats and mice. The extract was found to cause reduction in spontaneous activity, decrease in exploratory behavioural pattern by the head dip and Y- maze test, reduction in the muscle relaxant by rotarod, 30° inclined screen and traction tests. In addition CTEE significantly potentiated the phenobarbitone-induced sleeping time. Preliminary tests indicate that the ethanol extract of *Clitoria ternatea* Linn. at the doses of 100 and 150mg/kg showed significant neuropharmacological activity.
Keywords : *Clitoria ternatea*, Roots, ethanol extract, neuropharmacological activity.

Introduction

Clitoria ternatea Linn. (Family; Fabaceae) is perennial twining herb, found abundantly in Indo China, the Phillipiness islands and Madagascar. The plant *clitoria ternatea* is well known as Aparajit (Hindi), Aparajita (Bengali), Kakkattan (Tamil) in Indian traditional medicine. The root has sharp bitter taste; cooling, enhance learning and memory (K.S. Rai *et al.*, 2001) laxative, diuretic, anthelmintic, analgesic, anti-inflammatory and antipyretic (B. Parimaladevi *et al.*, 2003) severe bronchitis, asthma and hectic fever (Kirtikar & Basu 1975). The roots used by tribals to cause abortion; paste applied for curing abdominal swellings, sore throat, mucous disorders and fever (Asolkar *et al.*, 1992 & Nadkarni K.M 1976). The Root juice is given in cold milk to remove phlegm in chronic bronchitis The Root juice is given for the relief of fever by the rural people. Here, we reported some neuropharmacological effects of the ethanol extract of the *Clitoria ternatea* using the animal models rats and mice.

Experimental

Plant material The roots of *Clitoria ternatea* Linn. were collected from the Jhilimili, Bankura, West Bengal, India, during the month of July and august. The Taxonomical identification of the plant was done by The Botanical Survey of India, Shibpur, Howrah, West Bengal.

Preparation of the Extract – The dried roots were pulverised by a mechanical grinder and passed through 40 mesh sieve then extracted with ethanol 90% in a soxhlet extraction apparatus. The solvent from the ethanol extract was completely removed under vacuum, a semisolid material was obtained. (Yield 9.8% w/w with respective to dry starting material). The chemical constituents of the extract (Alkaloids, steroids, triterpenoids, flavonoids, saponins and tannins) were identified by qualitative analysis and confirmed by thin layer chromatography (Stahl, 1969). The extract was stored in a desiccator and weighed amount was dissolved in 2% v/v aqueous Tween 80 for further experiments.

Animals – Albino rats of either sex (180-200 gms) and mice (20-25 gms) were used. The animals were kept at constant room temperature (22°±1°C) and submitted to a 12h light/dark cycle with free access to food and water.

Drugs and chemicals – Diazepam (Ranboxy Labs. New Delhi), Phenobarbitone sodium (National chemicals, Mumbai).

Toxicological study – The LD_{50} determination of the ethanol extract of *C. ternatea* (CTEE) was performed in albino mice by the method of Ghosh MN (1984).

General behavioural effects – The experiment on behavioural profile was performed by the method of Dixit and Varma (1976). Adult albino mice (male) were taken

*Author for correspondence
E-mail: subhashmandal@yahoo.com

and divided into five groups containing ten animals each. The first three groups were injected with the ethanol extract of *Clitoria ternatea* (CTEE) in Tween 80 solution at different doses (50,100 and 150 mg/kg i.p.), the fourth group received Chlorpromazine (CPZ, 5 mg/kg, i.p.) and the fifth group was treated with 2% v/v aqueous Tween 80 (10 ml/kg). The effects were observed at 30- min intervals in the first hour and at hourly intervals for the next 4 h for the following parameters (Subhash *et al.*, 2001).

(i) Spontaneous activity, awareness and alertness These were evaluated by placing a mouse in a bell jar. It usually shows a moderate degree of inquisitive behavior.

(ii) Sound response Mice normally utter no sound, so that vocalization may point to a noxious stimulus.

(iii) Touch response It was noted when the animal was touched with a forceps (or) pencil at various parts (i.e., on the side of the neck, on the abdomen and on the groin.)

(iv) Pain response and alertness This response was graded when a small artery clamp was attached to the base of tail.

Effects on exploratory behaviour pattern – This was performed by (1) The Head dip test and (2) The Y- maze test.

(i) Head dip test – Adult albino mice were divided into five groups (n=6). Thirty minutes after intraperitoneal injection of 2% v/v aqueous Tween 80 (10 ml/kg), diazepam (10 mg/kg) or the ethanol extract (50, 100 and 150 mg/kg), the mice were placed I on a wooden board with 16 evenly spaced holes. The number of times they dipped their heads into the holes in 3 min was counted. (Dorr *et al.*, 1971).

(ii) Y- maze test – Adult albino rats were placed in symmetrical Y- shaped runway (33×38×13 cm) for 3 min and the number of times a rat entered in the arm of the maze with all four feet was counted (Rushton *et al.*, 1961). Experiments were conducted in groups of six rats at 30, 60, 90, and 120 min after injection of either 2%v/v aqueous Tween 80 (10 ml/kg), CTEE (50, 100 and 150 mg/kg) or diazepam (10 mg/kg).

Effects on muscle relaxant activity – This was studied by (i) the Rotarod test (ii) the Traction test (iii) the 30° inclined screen test.

(ii) Rotarod test – Untreated mice were placed on a horizontal wooden rod (32-mm diameter) rotating at a speed of 5-rev./min. Animals remaining on the rod for 3 min or more in three successive trials were selected for the experiment and were placed in-groups of ten animals each. Each group was then injected with 2% v/v aqueous Tween 80 (10 ml/kg) or CTEE (50, 100 and 150 mg/kg) or diazepam (10 mg/kg) and placed on the rod at an interval of 30, 60, 90, 120 & 150 min. If the animals failed more than once to remain on the rotating rod for 3 min the test was considered to be positive (Kulkarni, 1999).

Traction test – Forepaws of a mouse were placed on a small twisted wire rigidly supported above with a bench top. Normal mice grasped the wire with forepaws and when allowed to hang free, placed atleast one hind foot on the wire within 5 sec. Inability to put up atleast one hind foot constituted failure to traction. The test was conducted in groups of ten previously screened animals. 30 mins after injection of 2% aqueous Tween (10 ml/kg), diazepam (10 mg/kg) or CTEE (50,100 and 150 mg/kg) (Courvoisier, *et al.*, 1956)

(iii) 30° inclined screen test – Male mice after 15 min injection of either 2%v/v aqueous Tween 80 (10 ml/kg) or CTEE (50, 100 and 150 mg/kg) or diazepam (10 mg/kg) were left on the screen for atleast 4 h to observe whether the paralysed effect was severe enough to cause the mice to slide off the screen (Subhash *et al.*,2001) Groups of 10 mice were taken for each group of control and experimental batches.

Effect on phenobarbitone sleeping time – Adult albino mice were divided in to groups of 10 each. The extract (CTEE) at doses of 50, 100 and 150 mg/kg and 2% v/v aqueous Tween 80 were injected intrapertionally to separate groups. 30 min after receiving the same, each animal was injected with phenobarbitone (40-mg/kg i.p,). The sleeping time was noted by recording the interval between the loss and regaining of righting reflux (Dandiya and Collumbine, 1959).

Statistical analysis – In all cases the results were expressed as the mean ± S.E.M Significance was evaluated by students

Table 1. Determination of Median Lethal Dose (MLD) of the extract of *Clitoria ternatea* roots administered Intrapertonially

Treatment	Dose (mg/kg)	No. of animals used	No. of survival	No. of death	MLD
Control (2%Tween 80)	10 ml/kg	20	20	0	
Extract	25	20	20	0	< 1.2 gms/kg
	50	20	20	0	
	100	20	20	0	
	200	20	20	0	
	400	20	20	0	
	800	20	20	0	
	1000	20	20	0	
	1200	20	11	9	
	1600	20	8	12	

Table 2. Behavioural effects of *Clitoria ternatia* .Linn ethanol extract in mice

Behaviour type	CTEE			CPZ	2 % v/v aqueous
	50 mg/kg	100 mg/kg	150 mg/kg	5 mg/kg	Tween 80
Awareness	+	+	++	++	0
Alertness	+	++	+++	+++	0
Spontaneous activity	+	++	+++	++++	0
Sound response	+	++	+++	++++	0
Touch response	+	++	+++	++++	0
Pain response	+	+++	++++	+++	0

0 No effect; + Slight depression; ++ Moderate depression; +++ Strong depression; ++++ Very strong depression; CPZ Chlorpromazine; CTEE *Clitoria ternatea* Ethanol Extract.

t-test (Woodson. 1987).

Results

In the LD_{50} determination no animal death or any other abnormalities were observed at the dose range of <1.2 g/kg (i.p.) The result has been shown in Table 1.

Experimental results obtained by studying the general behavioural effects are explained in Table 2. It was observed that CTEE affected the spontaneous activity, sound, touch and pain responses at the dose of 150 mg/kg and above and produced moderate/slight depression in tests concerned with awareness and alertness. However, Chlorpromazine hydrochloride produced a profound depression of these responses in comparison with the root extract of the plant C*litoria ternatea* Linn.

On the head dip test with mice treated with different doses of CTEE, it was observed that there was a significant decrease in head dip responses in mice at doses of 100 and 150 mg/kg and above as compared to control. In the case of Y-maze test there was remarkable decrease in the exploratory behaviour of rats treated with CTEE in doses of 150 mg/kg and above as compared to control. (Table 3).

The extract (CTEE) significantly potentiated the phenobarbitone induced sleeping time in mice at doses of 100 and 150 mg/kg and above as compared to control. (Table 4).

In the rotarod test, CTEE in dose of 150 mg/kg and above produced significant motor discoordination in animals. Significant loss of coordination and tone of muscle were also found to occur with different doses of CTEE, as evident from 30° inclined screen test. The extract also produced significant failure in traction studied by traction test at different doses used. (Table 5).

Table 3. Effect of ethanol extract of *Clitoria ternatea* Linn. on exploratory behaviour (Head dip test) in mice

Treatment	Dose	Head dips
Aqueous Tween 80	10 ml/kg	108±4.0
Diazepam	10 mg/kg	35±2.9**
CTEE	50 mg/kg	87±1.7*
CTEE	100 mg/kg	56±1.6*
CTEE	150 mg/kg	41±1.0**

Values are the number of head dips in 3 min (mean ± S.E M). n=10. **P < 0.001 *, P < 0.01, compared with control.
CTEE; *Clitoria ternatea* Ethanol Extract.

Table 4. Effect of ethanol extract of *Clitoria ternatea* Linn on phenobarbitone Induced Sleeping time

Treatment	Dose (mg/kg)	Sleeping time (min)
AqueousTween 80	10 ml/kg	36±1
CTEE	50	54±2*
CTEE	100	72±1*
CTEE	150	78±2*

Values are mean SE, n =10 * *P*<0.001, compared with control.

Discussion and Conclusion

The result reveals that the ethanol extract influences general behavioural profiles, as evidenced in the spontaneous activity, touch, sound and pain responses. The extract (CTEE) significantly potentiated the phenobarbitone sleeping time, suggesting probable tranquilizing action (Rudzik 1973). The possible effects of CTEE were further examined on some other common pharmacological effects of neuroactive agents, e.g. exploratory behaviour (i.e. the head dip and Y-maze tests), muscle relaxant activity (i.e.the rotarod test,

Table 5. Percentage effect of the ethanol extract of *Clitoria ternatea*. Linn on muscle relaxant activity in mice

Treatment	Dose (mg/kg)	Traction test	30° Inclined screen test	Rotarod test
2% Tween 80 solution	10 ml/kg	0	0	0
Diazepam	10	100	100	100
CTEE	50	68*	46*	81.2*
CTEE	100	76*	67*	73.6*
CTEE	150	65*	71*	61.4*

Values are the percentage of animals showing a negative result, n = 10.
*$P < 0.05$ compared with control (Chi-squared test).
CTEE: *Clitoria ternatea* Ethanol Extract.

30°inclined screen and traction tests). Reduction in the exploratory behaviour on treatment with CTEE is in conformity with the actions known to occur with other CNS depressant drugs.

It was observed from these experiments that CTEE suppressed spontaneous movements, reduced initiative and interest in environment and there was some slowness in response to external stimuli. Therefore, it may be suggested that, the plant extract possess many of the pharmacological activities characteristic of neuroleptics.

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