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# Analgesic and Neuropharmacological Activities of Methanolic Leaf Extract of *Clitoria ternatea* Linn.

**Shammy Sarwar, Md. Rashidur Rahman, Kamrun Nahar, Muhammad Ashikur Rahman**

**Abstract**

The present study was carried out on methanolic extract of *Clitoria ternatea* Linn. for pharmacological investigations. In this study, analgesic activities of the methanolic extract of *Clitoria ternatea* Linn. leaves were examined at the doses of 200 mg/kg and 400 mg/kg of body weight on mice. The analgesic activities were investigated using acetic acid induced writhing test. The plant extract's Central Nervous System (CNS) depressant activity was evaluated by using hole cross and open field tests. Acetic acid induced writhing test revealed that the extract at the lower dose inhibited 82.67% and at the higher dose produced a maximum of 87.87% inhibition of writhing that is comparable to the reference drug Diclofenac Sodium. The results of 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.

**Keywords:** *Clitoria ternatea* Linn., Open Field Test, Hole Cross Test, Analgesic and Neuropharmacological Activities.

## 1. Introduction

*Clitoria ternatea* Linn. is a strangling and climbing herb with a strong base, ovate-oblong leaves and beautiful blue flowers, has been grown as an ornamental plant in the garden. This plant belongs to the family Papilionaceae and is commonly known as Butterfly pea plant in English and locally known as Aparajita, Nila in Bengali [1]. *Clitoria ternatea* Linn. is a vine native to tropical and equatorial Asia, but has been introduced to Africa, Australia and the rest. Availability in India is either as wild or cultivated plant. The rest worlds are Egypt, Syria, Iraq, Iran, Afghanistan etc. It grows well in moist neutral soil and requires little care. It is grown as a revegetation species. It fixes nitrogen and is therefore also used to improve soil quality. It requires summer rainfall of 500 mm over 3 months but grows best between 700-1,500 mm AAR. This plant is drought tolerant and will survive in years which have only 400 mm rainfall and a dry season of 5-6 months or longer even if heavily grazed. The useful parts are leaf, root, bark, seeds and flowers. The plant used in colic gonorrhea and skin disease. Root is used as laxative and demulcent, aperients. Roasted and powdered seeds are used in the treatment of ascites, enlargement of abdominal viscera, weakness of sight, sore throat, tumors, and dropsy and skin diseases [2]. It has been claimed to have antihyperglycaemic effect in rats with alloxan-induced diabetes mellitus [3]. This plant has also been studied to find out antibacterial activities [4]. An experiment showed that *Clitoria ternatea* Linn (Fabaceae) root possesses significant anti-diarrheal activity [5]. Anti-oxidant activities has been proven by *Clitoria ternatea* Linn. extract in in-vitro method [6]. Plant have been used for the treatment of diseases all over the world before the advent of modern clinical drugs and are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs [7]. Thus over 50% of these modern drugs are of natural products origin and as such these natural products play an important role in the drug development in the pharmaceutical industry [8]. Therefore, we have been done this investigation to know about analgesic and Neuropharmacological activities of *Clitoris ternatea* Linn. extract in animal behavioral model.

## 2. Materials and Methods

### 2.1 Collection and Identification of plants

The leaves of *Clitoria ternatea* Linn. were collected from Boldha Garden, Dhaka, Bangladesh and were identified by the expert at the Bangladesh National Herbarium, Mirpur, Dhaka where the Voucher specimen number 35202 has been deposited. The collected plant parts were separated and dried for one week.

### 2.2 Preparation of Methanolic Extract

The dried leaves of *Clitoria ternatea* Linn. were coarsely powered by a milling machine and extracted with a mixture of methanol**:** water (7/3, v/v) by Soxhlet apparatus at 50 $^{o}$C for 72 hours. When the powders became exhausted of its chemical constituents as evident from cycles of colorless liquid siphoning in the Soxhlet apparatus, extraction was considered to be complete. After completion of the extraction from leaves of *Clitoria ternatea* Linn., the extracts were filtered using a sterilized cotton filter. Then solvent was completely removed and obtained dried crude extract which were used for investigations.

### 2.3 Animals

Swiss-Albino mice of either sex (20-25 gm body weight) were collected from animal resources branch of the International Center for Diarrheal Disease Research, Bangladesh (ICDDR, B) and used for the experiments. The animal were kept in the standard polypropylene cages and provided with standard diets (ICDDR, B formulated). The animals were acclimatized in animal house (Department of Pharmacy, Stamford University Bangladesh) under standard laboratory conditions (relative humidity 55-60%, room temperature 25±2 °C and 12 hours light: dark cycle) for period of 14 days prior to performing the experiments [9]. All experimental protocols were approved by the Institutional Ethics committee of the Stamford University Bangladesh.

### 2.4 Chemicals and drugs

All chemicals and drugs were obtained commercially and were of analytical grade. Diclofenac Sodium and Diazepam was collected from Square Pharmaceuticals Ltd., Bangladesh. Acetic acid was purchased from Merck, Germany.

### 2.5 Analgesic activity test of the extract of *Clitoria ternatea* Linn

The study of analgesic activity of the extract of *Clitoria ternatea* Linn. was performed in animal models. For the screening of analgesic activity against peripheral mechanism of pain, acetic acid induced writhing test was considered.

#### 2.5.1 Acetic acid induced Writhing test

The antinociceptive activity of the samples was studied using acetic acid-induced writhing model in mice [9, 10]. The animals were divided into control, positive control and test groups with five mice in each group. The animals of test groups received test samples at the doses of 200 and 400 mg/kg body weight. Positive control group received standard drug Diclofenac Sodium at the dose of 10 mg/kg body weight and vehicle control group was treated with 1% Tween 80 in water at the dose of 10 ml/kg body weight. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac Sodium was administered 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min [11]. After inducing the plant extract and control every mice of all groups was observed carefully to count the number of writhing which made within 10 minutes.

### 2.6 Neuropharmacological activity test of *Clitoria ternatea* Linn.

The effects of drugs on the CNS with reference to the neurotransmitters for specific circuits, attenuation should be developed to general organizational principles of neurons. The concept is that synapses represent drug-modifiable control points within neuronal networks. It requires explicit delineation of the sites at which given neurotransmitters may operate and the degree of specificity with which such site that may be affected [12]. Open field and hole cross tests were performed to determine the Neuropharmacological activity of *Clitoria ternatea* Linn. extracts.

#### 2.6.1 Open Field Test

The Open Field Test is clearly the most frequently used of all behavioural tests in pharmacology and neuroscience. Despite the simplicity of the apparatus, however, open field behaviour is complex. Consequently, it has been used to study a variety of behavioural traits, including general motor function, exploratory activity and anxiety-related behaviours. Open field behavioral assays are commonly used to test both locomotor activity and emotionality in rodents.

##### 2.6.1.1 Experimental Design

In open field test, the animals were divided into control, positive control, and test groups containing five mice each. The test groups received methanolic extract at the doses of 200 and 400 mg/kg body weight orally whereas the control received vehicle (1% Tween 80 in water). Animals in positive control group received diazepam (1 mg/kg body weight). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90, and 120 min after oral administration of the test drugs and the standard [13].

#### 2.6.2 Hole Cross Test

The method was carried out as described by Takagi *et al.* (1971) [14]. A steel partition was fixed in the middle of a cage having a size of (30 × 20 × 14) cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the centre of the cage. The animals were divided into control, positive control, and test groups containing five mice each. The test groups received extract of 200 and 400 mg/kg body weight orally whereas the control and positive control groups received vehicle (1% Tween 80 in water) and the standard drug diazepam (1 mg/kg body weight) respectively. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test drugs and the standard.

## 3. Results and Discussion

### 3.1 Test for Analgesic Activity of *Clitoria ternatea* Linn.

#### 3.1.1 Acetic Acid-Induced Writhing Test

The analgesic effect of the methanolic extract on acetic acid-induced writhing in mice was determined. The extract significantly inhibited writhing response induced by acetic acid in a dose dependent manner. The result was comparable to the reference drug Diclofenac Sodium.

**Table 1:** Results of Acetic acid induced writhing test

| Administered Substance | SEM | Mean ± SEM | % of Inhibition |
|---|---|---|---|
| Control | 2.12 | 40.4±2.12 | 0 |
| Positive control | 2.00 | 9±2.00 | 77.72 |
| Group-1 | 1.60 | 7±1.60 | 82.67 |
| Group-2 | 1.13 | 4.9±1.13 | 87.87 |

Values are expressed as Mean ± SEM (n=5)
Here, Control = 1% Tween 80 in Water (0.2 ml/Mouse), Positive Control = Diclofenac Sodium (10 mg/Kg), Group 1 and Group 2 (200 mg /Kg and 400 mg/Kg body weight respectively)

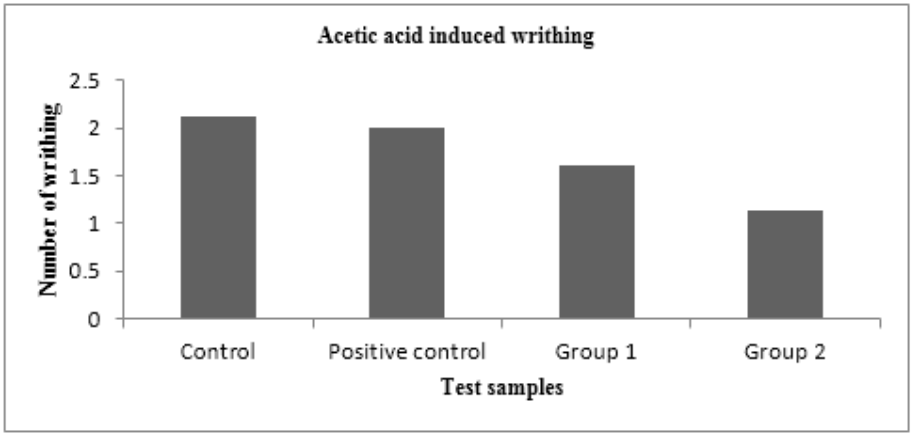


**Fig 1:** Graphical Representation of effect of leaves parts of *Clitoria ternatea* Linn. on acetic acid induced writhing test in mice

### 3.2 Tests for Neuropharmacological activity

In both open field and hole cross tests, the extract significantly decreased the locomotor activity of the mice (Table 2 and 3). The locomotor activity lowering effect (depressant effect) was evident at the 2nd observation time (30 min) and continued up to the 5th observation period (120 min) at the doses of 200 & 400 mg/kg body weight and the results were dose dependent, the maximum being at the dose of 400 mg/kg body weight. In the open field test, the number of squares travelled by the mice at all doses of the extract (200 and 400 mg/kg body weight) was reduced significantly from the initial score. The results were comparable to those of the reference drug, Diazepam. The maximum reduction was exhibited at 90 and 120 min after administration of the drug.

**Table 2:** Effect of *Clitoria ternatea* Linn. extract on Hole Cross Test

| Group | Route of Administration | Observation | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min | 120 min |
| **Positive control** | Oral | 15.4±0.91 | 6±0.94 | 2±0.79 | 1.6±1.04 | 1.2±0.42 |
| **Control** | Oral | 17±1.97 | 17.8±1.92 | 17.6±2.28 | 17.2±1.56 | 18.4±0.91 |
| **Group-1** | Oral | 12.4±1.44 | 5.2±1.14 | 4±0.78 | 2.6±0.76 | 0.8±0.55 |
| **Group-2** | Oral | 8.6±1.26 | 5.4±0.76 | 2.2±0.65 | 0.8±0.65 | 0.4±0.27 |

Values are expressed as Mean ±SEM (n=5)
Here, Control = 1% Tween 80 in Water (0.2 ml/Mouse), Positive control = Diazepam (1 mg/Kg), Group 1 and Group 2 (200 mg /Kg and 400 mg/Kg body weight respectively)

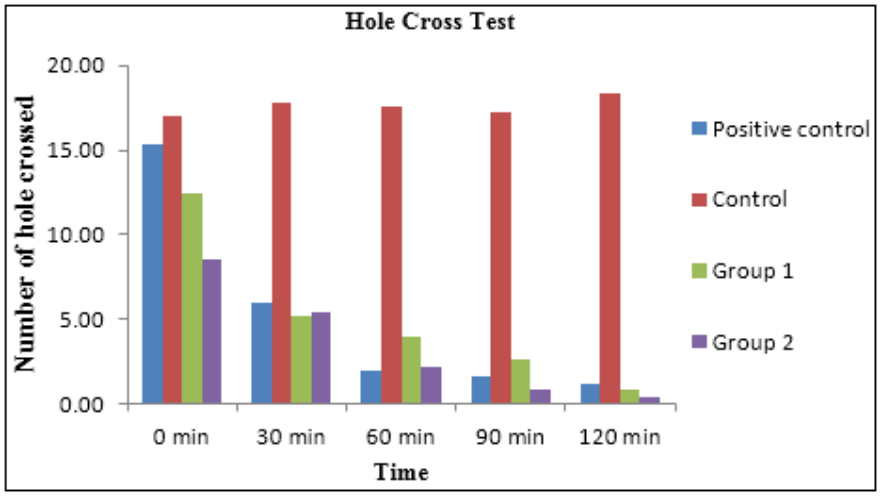


**Fig 2:** Graphical Representation of effect of *Clitoria ternatea* Linn. on Hole cross test in mice

**Table 3:** Effect of *Clitoria ternatea* Linn. extract on Open Field Test

| Group | Route of Administration | Observation | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min | 120 min |
| **Positive control** | Oral | 118.4±7.04 | 67.8±5.63 | 41.6±3.65 | 19.6±2.61 | 10.8±2.04 |
| **Control** | Oral | 121.6±5.35 | 117.8±3.94 | 116.6±2.61 | 110.8±7.53 | 117.2±4.62 |
| **Group-1** | Oral | 48.2±10.3 | 23.8±7.9 | 19.0±7.6 | 13.4±5.3 | 7.2±2.9 |
| **Group-2** | Oral | 64.4±8.3 | 18.8±6.9 | 9.8±2.2 | 11±1.6 | 2±1.2 |

Values are expressed as Mean ±SEM (n=5)
Here, Control= 1% Tween 80 in Water (0.4 ml/Mouse), Positive control = Diazepam (1 mg/Kg), Group 1 and Group 2 (200 mg /Kg and 400 mg/Kg body weight respectively)

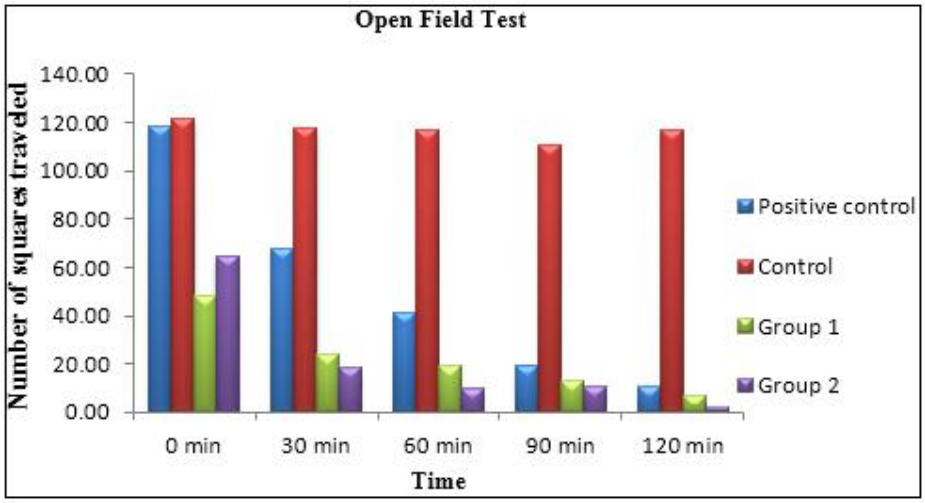


**Fig 3:** Graphical Representation of effect of *Clitoria ternatea* Linn. on Open field test in mice

The present study has established analgesic potential of *Clitoria ternatea* Linn. using acetic acid-induced writhing test for visceral pain. Acetic acid-induced writhing in mice is a model of visceral pain which is highly sensitive and useful for screening peripherally acting analgesic drugs. *Clitoria ternatea* Linn. plant caused dose-dependent antinociception against chemical induced pain in mice. Methanolic extract of the leaves parts of *Clitoria ternatea* Linn. were treated in test animals at a dose of 200 & 400 mg/kg body weights. The leaves parts of *Clitoria ternatea* Linn. extracts at the dose of 400 & 200 mg/kg body weight respectively, was found to exhibit the highest (87.87%) & (82.67%) writhing response inhibitory effect respectively, where the reference drug Diclofenac Sodium shown about 77.72% writhing inhibitory response at a doses of 10 mg/kg. Acetic acid-induced writhing method is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. This model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of

free arachidonic acid from tissue phospholipids [15]. So, the observed analgesic activity may be attributed to these compounds. An important step in evaluating drug acting on CNS is to observe its effect on locomotor activity of the animal. The activity is a measure of the level of excitability of the CNS and this decrease may be closely related to sedation resulting from depression of the central nervous system [16]. The extracts significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests. The locomotor activity lowering effect was evident at the 2nd observation (30 min) and continued up to 5th observation period (120 min). Both hole cross and open field tests showed that the depressing acting of the extracts was evident from the 2nd observation period in the test animals at the doses of 200 & 400 mg/kg body weight. Maximum depressant effect was observed from 3rd (60 min) to 5th (120 min) observation period. From the result this is observed that, *Clitoria ternatea* Linn. has CNS depressant activity by using both open field & hole cross tests, which is comparable to the reference drug Diazepam at doses of 1 mg/kg.

## 4. Conclusion

Based on the results of the present study, it can be concluded that the leaves parts of crude methanolic extract of *Clitoria ternatea* Linn. possesses remarkable CNS depressant and analgesic potential in animal behavioral model. Hence, further studies are suggested to be undertaken to pinpoint the exact compounds and to better understand the mechanism of such actions.

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