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Evaluation of Antidepressant, Motor Coordination and Locomotor Activities of Ethanolic Root Extract of *Clitoria Ternatea*

M. Parvathi[1]* and K. Ravishankar[1]

[1] Sri Sai Aditya Institute of Pharmaceutical Sciences and Research, Adb Road, Surampalem, 533437, East Godavari District, India

Abstract

Clitoria ternatea (*CT*), a perennial herbaceous plant commonly known as 'butterfly pea', has been used for centuries as a traditional Ayurvedic medicine. The aim of present study was to investigate the antidepressant (by Tail suspension test and Forced swimming test), motor coordination (by Rota-rod method) and locomotor (with an actophotometer) activities of ethanolic root extract of *Clitoria ternatea* Linn. in experimental animals. The study revealed that the extracts (150 and 300 mg/kg, p.o.) were able to reduce the immobility time of rats in a dose-dependent manner when subjected to both Tail suspension and Forced swim tests, and the results were similar to that of standard drug imipramine (15 mg/kg, p.o.). *Clitoria ternatea* ethanolic root extract (300 mg/kg) showed mild reduction in locomotor and motor coordination activity. The results indicated that ethanolic root extract of *Clitoria ternatea* has significant antidepressant activity (150 mg/kg (p<0.01) and 300 mg/kg (p<0.001)) when compared with standard imipramine (p<0.001) with a mild sedative effect (300 mg/kg only). The results of the sedative effect are not statistically significant, and the sedative effect may be due to higher dose. Therefore, *Clitoria ternatea* may be served as a potential resource for natural psychotherapeutic agent against depression and mood disorders.

Keywords: *clitoria ternatea*, antidepressant, muscle relaxant, locomotor activity, imipramine, diazepam

1. Introduction

Depression is a heterogeneous disorder that affects a person's mood, physical health and behaviour. According to the World Health Report [1], approximately 450 million people suffer from a mental or behavioural disorder, yet only a small minority of them receive even the most basic treatment, accounting for 12.3% of the global burden of the disease. It is expected to rise to 15% by 2020. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models. Patients with major depression have symptoms that reflect changes in their brain neurotransmitters, specifically norepinephrine, serotonin and dopamine [2]. A muscle relaxant is a drug which effects skeletal muscle function and decreases the muscle tone. The sedative action of a drug along with antidepressant action is useful in the treatment of depression associated with anxiety [3]. Since all the synthetic drugs available for the treatment of depression have various adverse effects associated with problematic interactions, our aim was to explore the potential of plants in the management of depression.

**Corresponding author:*
E-mail: parvathi.pharma5@gmail.com

Clitoria ternatea Linn., belonging to the family Fabaceae, is a perennial twining herb found in India, China, Philippines and Madagascar. This plant is known as Aparajit (Hindi), Aparajita (Bengali), Kakkattan (Tamil) and Dintena (Telugu) in Indian traditional medicine. The roots have laxative, diuretic, anthelmintic and anti-inflammatory properties, and they are useful in severe bronchitis, asthma and hectic fever [4, 5] .The root has been used traditionally to induce abortion, and its paste has been used for curing abdominal swellings, sore throats and mucous disorders. *Clitoria ternatea* has been reported for nootropic, anxiolytic, anticonvulsant [6], anti-diabetic [7], antipyretic, anti-inflammatory and analgesic activities [8], among others. It enhances the memory [9] and increases acetylcholine content in rats [10]. The preliminary phytochemical screening of the roots revealed the presence of alkaloids, glycosides, flavanoids, resins, saponins, phenols, triterpenes, proteins and carbohydrates [11, 12]. Nevertheless, the available scientific information about the plant in treating depression is scarce, and there was no clear information whether the drug exhibits strong antidepressant or sedative action. At present, we have focused our study towards the evaluation of these activities.

2. Materials and Methods

2.1 Collection and Extraction of Plant Material

The roots were collected in the months from October to December from Kakinada (India) and authenticated by Taxonomist Dr T. U. Raghuram. The roots were washed, air-dried under shade and coarsely powdered. The powdered material was extracted with 95% ethanol in a Soxhlet apparatus for about 8 hr. The extract was concentrated by distilling the solvent to obtain the crude extract. Finally, the extract was evaporated to dryness and a dark-brownish solid extract was obtained, which was used in the study. The percentage yield of ethanolic extract of *Clitoria ternatea* roots was found to be 9.6%w/w.

2.2 Experimental Animals

Swiss albino mice (18–25 g) and albino rats (80–120 g) of either sex were used for the study. The animals were obtained from the National Institute of Nutrition, Hyderabad (NIN). The animals were housed in colony cages at an ambient temperature of 25±2°C, 12-hr light/dark cycle and 50±5% relative humidity with free access to food and water ad libitum. Food, but not water, was deprived overnight and during the experiment. All the experiments were carried out during the light period (9.00–16.00 hr). Each group consisted of five animals. The institutional animal ethical committee approved the study protocol. The dose which was administered to the experimental animals was based on the literature on acute toxicological studies. According to this study, the LD50 value was 1500 mg/kg body wt [13].

2.3 Drugs

Imipramine hydrochloride (Sigma-Aldrich, St Louis, USA) and diazepam injection I.P (Ranbaxy Laboratory Ltd., New Delhi, India) were used in this study. All drugs were dissolved in distilled water and administered either intraperitoneally (i.p.) or orally (p.o.). Distilled water was used as the vehicle.

2.4 Experimental Design

2.4.1 Evaluation of antidepressant activity

2.4.1.1 Tail suspension test (TST)

The total duration of immobility by the Tail suspension test was measured according to the method of Steru et al[14]. Rats were isolated both acoustically and visually and suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was observed during a 6-min test for animals of all groups. Rats were considered to be immobile when they hung passively and were completely motionless.

2.4.1.2 Forced swim test (FST)

The development of immobility when the rats are placed in an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behaviour. The cylindrical container (diameter 10 cm, height 25 cm) was filled to a 19-cm depth with water at (25±1°C). The duration of immobility during the 6-min test was scored. Each rat was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water [15].

Table 1: Effect of ethanolic root extract of *Clitoria ternatea* on duration of immobility time in the Tail suspension test and Forced swim test

Treatment	Tail suspension test Duration of immobility(in sec)	Forced swim test Duration of immobility(in sec)
Vehicle (water)	170.48±10.42	148.55±9.38
Clitoria ternatea (150 mg/kg, p.o.)	112.14±7.28**	97.84±5.82**
Clitoria ternatea (300 mg/kg, p.o.)	79.17±8.93***	58.63±10.46***
imipramine (15 mg/kg, p.o.)	76.92±6.85***	65.22±8.19***

Test solutions were administered orally 60 min prior to the test. Values represented mean±SEM (n=5), **p<0.01, ***p<0.001 vs control (group 1).

2.4.2 Evaluation of motor coordination activity

The motor coordination and performance of each mouse was evaluated 1 hr after the extract oral treatment or 30 min after i.p. administration of standard diazepam (4 mg/kg) in a Rota-rod apparatus. This equipment has a bar 2.5 cm in diameter and divided into six parts, and it is placed at a height of 50 cm, rotating at 20 rpm. Latency to fall from the rotating bar was registered [16].

2.4.3 Evaluation of locomotor activity

The spontaneous locomotor activity of each rat was recorded individually for 10 min using an actophotometer. The movement of the animal cuts off a beam of light falling on the photocell, and the count is recorded digitally. Two doses of *Clitoria ternatea* (150 and 300 mg/kg p.o.) were administered 60 min before the test and diazepam (3 mg/kg i.p.), used as standard, was given 30 min before the test. The control group was treated with water orally, 60 min before test.

2.4.4 Statistical analysis

Data were analysed by GraphPad InStat® version 3.0software and presented as mean±SEM values. The statistical tests used were one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The levels of statistical significance ranged from $p<0.05$ to $p<0.001$.

3. Results

3.1 Antidepressant Activity

The antidepressant effects of *Clitoria ternatea* (150 and 300 mg/kg) and imipramine were studied by observing the changes in the duration of immobility in the two models, Forced swim test (FST) and Tail suspension test (TST). In both TST and FST, *Clitoria ternatea* 150 and 300 mg/kg, p.o. produced significant reduction ($p<0.01$ and $p<0.001$ respectively) in the immobility period when compared with that of control group animals that received only the vehicle. The extract (300 mg/kg) was found to be effective and it exhibited activity similar to that of the conventional drug imipramine ($p<0.001$). The results are tabulated in Table 1.

3.2 Muscle Coordination Activity

In this test, *Clitoria ternatea* (300 mg/kg only, p.o.) (p value not significant) slightly reduced the time spent by the animals on the revolving rod when compared with control, indicating mild muscle relaxant activity. The standard drug (diazepam) showed a significant effect ($p<0.001$) when compared to control. The results are tabulated in Table 2.

3.3 Locomotor Activity

Clitoria ternatea (300 mg/kg only, p.o.) exhibited slight reduction in locomotor activity when compared with the control animals that received the vehicle only. But the results were not significant statistically (p value not significant). The diazepam-treated group exhibited statistically significant ($p<0.001$) decrease in locomotor activity when compared with the control. The results are tabulated in Table 3.

4. Discussion

Mood disorder is one of the most common mental illnesses with a lifetime risk of 10% in the general

Table 2: Effect of ethanolic root extract of *Clitoria ternatea* on locomotor activity by actophotometer

Treatment	Mean activity score before treatment	Mean activity score after treatment	Reduction in locomotor activity (%)
Control	332.0±25.0	314.6±17.35	5.24%
C.ternatea (150 mg/kg, p.o.)	271.2±17.30	238.4±21.40	12.09%
C.ternatea (300 mg/kg, p.o.)	296.8±15.50	234.4±25.72	21.04%
diazepam (3 mg/kg, i.p.)	325.21±9.80	86.21±4.30***	73.49%

Test solutions were administered orally 60 min prior to the test and standard 30 min prior to the test. Values represented mean±SEM (n=5), ***p<0.001 vs control (group 1).

Table 3: Effect of ethanolic root extract of *Clitoria ternatea* on muscle coordination activity by Rota-rod

Treatment	Mean fall-off time before drug treatment	Mean fall-off time after drug treatment	% reduction in fall-off time
Vehicle	505.46±11.85	492.72±16.15	2.52%
C.ternatea (150mg/kg, p.o.)	484.91±22.08	457.55±27.88	5.64%
C.ternatea (300mg/kg, p.o.)	552.23±16.40	432.28±12.52	28.17%
diazepam (4mg/kg, i.p.)	535.17±18.34	14.76±1.96***	97.2%

Test solutions were administered orally 60 min prior to the test and standard 30 min prior to the test. Values represented mean±SEM (n=5), ***p<0.001 vs control.

population. Most of the drugs that are currently being used in the treatment of depression adversely affect the quality of life of the patient. This leads to patients' non-compliance with medication, which further complicates the problem[17, 18]. Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders [17, 18] and are acclaimed to have a lower side-effect profile than conventional drugs.

Earlier reports on the chemical constituents of various plants and their pharmacology suggest that plants containing flavanoids and tannins possess activity against many Central nervous system disorders [19]. Flavanoids present in the *Hypericum perforatum* [20] and tannins present in the *Emblica officinalis* [18] are responsible for antidepressant action. So these components present in the extract may be responsible for the antidepressant action.

The FST and TST models of depression are widely used to screen new antidepressant drugs. These tests are quite sensitive and relatively specific to all major classes of antidepressant drugs, including tricyclics, serotonin specific reuptake inhibitors, monoamine oxidase inhibitors and atypical [21]. In the present study, *Clitoria ternatea* (150 and 300 mg/kg) produced significant dose-dependent antidepressant effect in behavioural despair tests. Animals subjected to the antidepressant drug treatment struggle more even in desperate situations and they spend less time with immobility [2]. It has been previously suggested [22] that an increase in both swimming and climbing behaviours in the FST occurs when the animal is treated by a drug which increases serotonin, norepinephrine and dopamine levels in the nerve terminals. An increase in all the three neurotransmitters could be effected by inhibition of monoamine oxidase (MAO) activity in the brain. Tannic acid being a non-selective inhibitor of monoamine oxidase causes an increase in the levels of monoaminergic neurotransmitters in the brain [18]. As the plant *Clitoria ternatea* contains tannin, the antidepressant activity may be due to MAO inhibition, thereby increasing norepinephrine and dopamine levels in the brain.

Locomotor activity and muscle coordination are an index of alertness and muscle relaxation. Reduction indicates that it may possess a sedative and skeletal muscle relaxant effect. Decrease in motor activity and

muscle relaxation is an indication of CNS depressant property [23]. At a higher dose (300 mg/kg), the extract exhibits a mild sedation and muscle relaxation which may be due to the presence of flavanoids in *Clitoria ternatea* and their interaction with the Benzodiazepine site of GABA $_A$ Receptors[24,19]. The mild sedative action of the extract at the higher dose (i.e. 300mg/kg) may be attributed to the presence of some constituents at an optimal concentration to induce sedation. Apart from this, it is of interest to note that several established antidepressants decrease locomotor activity [25]. So the sedative effect was not significant when compared with antidepressant activity.

Psycho stimulants also reduce immobility in FST and TST models, but in contrast to antidepressants, these cause marked motor stimulation in locomotor activity test. In case of minor or major tranquilisers, immobility was not affected but there was a reduction in motor activity [15, 21]. So it was concluded that antidepressant effect of *Clitoria ternatea* was not associated with any motor effects. It confirms the assumption that the antidepressant-like effect of *Clitoria ternatea* is specific. Therefore, it was concluded that *Clitoria ternatea* may be served as a potential resource for natural psychotherapeutic agent against depression.

Conclusion

Since ancient times, people have been using plants in various ways as a source of medicine. From the above preclinical study, we can conclude that ethanolic root extracts of *Clitoria ternatea* show a significant antidepressant activity in TST and FST models of depression. We believe that *Clitoria ternatea* has the potential to be used as an adjuvant in the treatment of depression and other mood disorders. Further research is required to gain closer insights into the exact mechanism of its action.

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