



Evaluation of the Antidepressant Activity of Ethanolic Extract of *Clitorea ternatea* Leaves In Mice.

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ABSTRACT

The study was conducted to evaluate the antidepressant activity of ethanolic extract of *Clitorea ternatea* leaves in mice. This test was done using healthy albino mice weighing 20-25gm each of either sex in the Department of Pharmacology of Gauhati Medical College. They were housed in standard laboratory condition at 25°C and fed on standard diet and water *ad libitum*. Five groups were selected each containing six mice. The groups were Group I (Normal Control), Group II (Disease Control), Group III (Fluoxetine 10mg/kg i.p), Group IV (Ethanolic extract of *Clitorea ternatea* leaves 150mg/kg i.p) and Group V (Ethanolic extract of *Clitorea ternatea* leaves 300mg/kg i.p). Reserpine (2mg/kg i.p) was used to induce depression in all the groups except the Normal Control. After 24 hours, the standard and test drugs were given and the mice were subjected to tail suspension test (TST) after 30 mins and forced swim test (FST) after 1 hour of injecting the drugs. Mean±SEM values were calculated for each group. The data were analyzed using ANOVA and post analysis was done by Dunnett's test. Results were found to be significant ($p < 0.05$). The period of immobility was found to be reduced in the test groups in a dose dependent manner as compared to the Disease Control. However, the episodes and the duration of immobility was minimum in the Standard group. Ethanolic extract of *Clitorea ternatea* leaves has been found to be effective in reversing reserpine induced depression in mice.

Keywords: *Clitorea ternatea*, Reserpine, Antidepressant, Forced Swim Test, Tail Suspension Test .

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INTRODUCTION

According to World Health Report, 450 million people suffer from a mental or behavioral disorder¹. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020². Depression is the most prevalent mental disorder and depression is recognized to be symptomatically, physiologically and biologically heterogeneous³. In spite of the availability of antidepressant drugs like tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), selective reversible inhibitors of monoamine oxidase-A (MAO-A) and selective noradrenaline reuptake inhibitors (SNRIs), depression continues to be a major medical problem⁴. These considerations implicate the search for new antidepressant agents that have a fast onset of action, with less side effects and a wider safety margin. Various plants are being used in complementary and alternative medicines for management of mood disorders.

Clitoria ternatea has been reported for inotropic, anxiolytic, anticonvulsant⁵, anti-diabetic⁶, antipyretic, anti-inflammatory and analgesic activities⁷, among others. It enhances the memory⁸. *Clitoria ternatea* leaves is a member of the family Papilionaceae, commonly known as 'Aparajita' or 'Girikarnika'. It is a perennial climber, widely used in the traditional Ayurvedic system of Indian medicine for treating a wide variety of ailments. It has slender downy stems with leaves having 5-7 leaflets, elliptical to oblong in shape and flowers are usually solitary, bright blue or sometimes white with an orange centre and are a very good source of anthocyanins. In the traditional system of medicine, 'Aparajita' is considered as 'Medhya' drug to improve intelligence and enhance memory function. It is also used in the treatment of chronic bronchitis, dropsy, goiter, leprosy, mucous disorders, sight weakness, skin disease, sore throat and tumours. The plant contains several glycosides eg. Malvidin-3- β -glycoside. Therefore, the present study has been undertaken to investigate the effect of ethanolic extract of *Clitoria ternatea* on depression in mice.

MATERIALS AND METHOD

Ethical review:

The present study was conducted in the Department of Pharmacology, Gauhati Medical College, Guwahati, Assam (India) after getting approval from Institutional Animal Ethics Committee. (MC/05/2015/46)

Experimental Animals:

The animals were taken from the animal house of Department of Pharmacology, Gauhati Medical College, Guwahati, Assam (India). Adult Swiss Albino mice weighing 25-35 gm of

either sex from our breeding stock were used in this study. The animals were housed at $24\pm 2^\circ\text{C}$ with 12:12 hrs light and dark cycle. They had free access to food and water *ad libitum*. The animals were acclimatized for a period of 7 days before the study.

Drugs and Chemicals:

Drugs and chemicals needed for the present study were Fluoxetine (Pfizer), ethanolic extract of *Clitorea ternatea* leaves, Reserpine (Novartis).

Methodology:

The mice were divided into five groups each consisting 6 animals:

Group I: Normal Control, received normal saline at a dose of 10ml/kg.

Group II: Disease Control, Reserpine 2mg/kg i.p.

Group III: Standard, Reserpine 2mg/kg i.p +Fluoxetine 10mg/kg i.p.

Group IV: Reserpine 2mg/kg i.p + ethanolic extract of *Clitorea ternatea* 150mg/kg i.p.

Group V: Reserpine 2mg/kg i.p + ethanolic extract of *Clitorea ternatea* 300mg/kg i.p.

Assessment of depression:

Reserpine (2mg/kg i.p) was used to induce depression in all the groups except the Normal Control. After 24 hours the standard drug i.e fluoxetine (10mg/kg i.p) and two doses of the test drugs-ethanolic extract of *Clitorea ternatea* leaves (150 mg/kg and 300 mg/kg) were given and the mice were subjected to tail suspension test after 30 mins and forced swim test after 1 hour of injecting the drugs.

Tail suspension test:

For the test, mice were suspended on the edge of a shelf 58cm above a table top by adhesive tape placed approximately 1cm from the tip of the tail. The duration of immobility in seconds were recorded for a period of 5mins. Mice were considered immobile when they hang passively and completely motionless.

Forced swim test:

Mice were forced to swim individually inside a vertical plexiglass cylinder (height:40cm; diameter:18cm, containing 15cm of water maintained at 25°C). After an initial period of vigorous activity, each animal assumes a typical immobile posture. A mouse was considered to be immobile when it remains floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility in seconds were recorded during the 5 mins test. The change in immobility duration was studied after administering drugs in different groups of animals⁹.

Acute Toxicity study:

The ethanolic extract of *Clitorea ternatea* leaves was administered in doses of 200, 400, 800, 1600 and 2000 mg/kg i.p. to 4 groups of mice, each consisting of 10 mice and mortality was observed after 24 hours.

Statistical analysis:

Mean \pm SEM of the values were calculated for each group. The data were analyzed using ANOVA and post analysis was done by Dunnett's test. Results were considered significant when *p value* < 0.05. All the data were analyzed using Graph pad prism version 5.

RESULTS AND OBSERVATION**Acute Toxicity study:**

The ethanolic extract of *Clitorea ternatea* leaves was found to be safe in the doses used and there was no mortality upto a dose of 2000mg/kg i.p. after 24 hours.

Table: 1

	Forced swim test , mean +/- SEM (in seconds)	Tail suspension test, mean +/- SEM (in seconds)
Group I	158.6667+/-2.616189	175.1667 +/- 3.015699
Group II	228.5+/-6.08687	236.5+/-2.895398
Group III	84.33333+/-3.190263	72.83333+/-2.600213
Group IV	125+/-1.914854	126.3333+/-2.485513
Group V	107.5+/-4.232808	101.5+/-2.860652
F	207.5	540.8
Df	4, 25	4,25
P value	< 0.0001	< 0.0001

Values are expressed as Mean +/-SEM (n=6);

One Way ANOVA followed by Dunnett's multiple comparison tests were done.

p<0.0001 when compared to the Normal control group.

p<0.0001 when compared to the Disease control group.

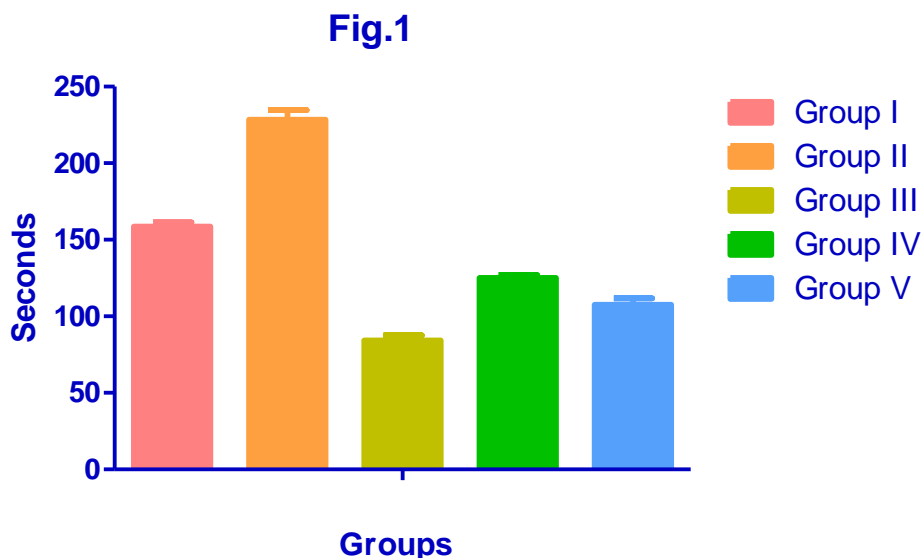


Figure 1: Forced Swim Test

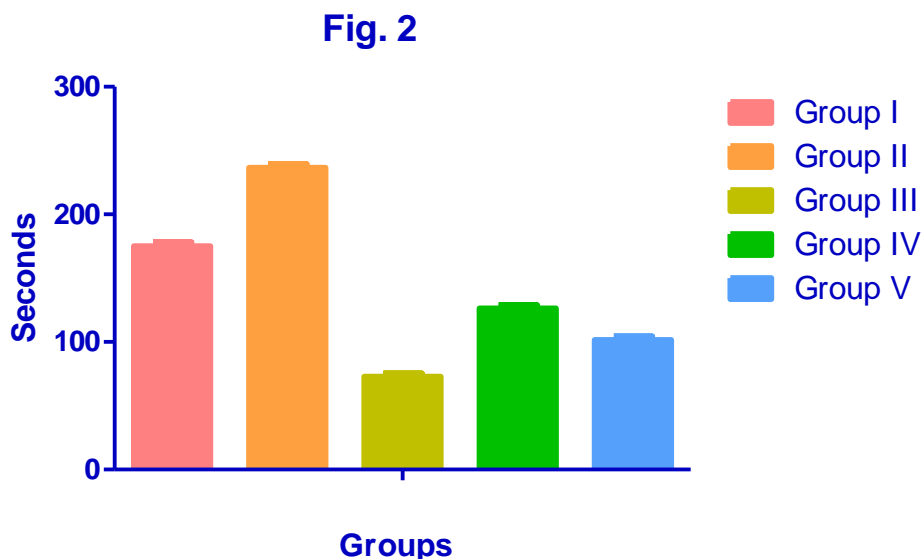


Figure 2: Tail Suspension Test

DISCUSSION:

On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors. The two most widely used animal models for antidepressant screening are the forced swimming and tail suspension tests. These tests are quite sensitive and relatively specific to all major classes of antidepressants. In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Similarly in the FST, mice are forced to swim in restricted space from which they cannot escape. This

induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression. It has been seen that the TST is less stressful and has higher pharmacological sensitivity than FST¹⁰.

In the present study, we have tried to demonstrate that ethanolic extract of *Clitorea ternatea* leaves is able to reverse the depression in mice induced by reserpine. The duration of immobility expressed in Mean \pm SEM in FST and TST respectively (Table 1) was found to be highest in Group II i.e. the group which received only reserpine (228.5 \pm 6.08687, 236.5 \pm 2.895398) and it was least in Group III, i.e. the group which received standard antidepressant, fluoxetine following reserpine administration the previous day (84.33333 \pm 3.190263, 72.83333 \pm 2.600213). However, the duration of immobility was seen to reduce substantially in Group IV (125 \pm 1.914854, 126.3333 \pm 2.485513) and Group V (107.5 \pm 4.232808, 101.5 \pm 2.860652), which were given ethanolic extract of *Clitorea ternatea* in the doses of 150mg/kg and 300mg/kg i.p. respectively as compared to the animals which received reserpine alone. Thus, the result found in our study shows that further research can be done to investigate the use of this plant extract which might be beneficial to mankind especially since the incidence of anxiety and depression in the community is very high and is associated with lot of morbidity.

CONCLUSION:

In the above study, the duration of immobility of mice in forced swim test (Figure 1) and tail suspension test (Figure 2), which was increased by reserpine has been found to be reversed by ethanolic extract of *Clitorea ternatea* in a dose dependent manner.

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