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EVALUTION OF ANTIDEPRESSANT ACTIVITY AND BEHAVIORAL ASSESSMENT OF CLITORIA TERNATEA EXTRACT IN RATS

Pharmacology

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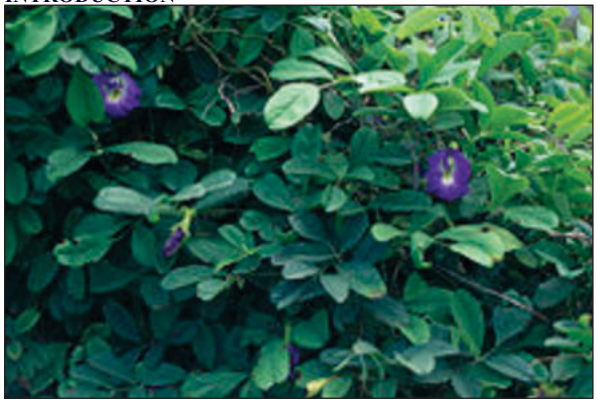
ABSTRACT

The results from the present study confirm the antidepressant activity ofClitoria ternatea,since it reduced the immobility in bothForced swimming test(FST) and Tail suspension test (TST).In the present study, Clitoria ternatea(CT)significantly increased the frequency of 5-hydroxy tryptophan(5-HTP) induced head twitches, Clonidine induced aggression and L-DOPA induced hyperactivity and aggressive behavior indicating its enhanced activity on serotonergic, noradrenergic and dopaminergic pathways respectively. In this work also confirm the involvement of serotonergic, noradrenergic and dopaminergic pathways in depression.Pretreatment with Clitoria ternatea,also significantly increased the levels of Serum superoxide dismutase (SOD) and Catalase with simultaneous decrease in lipid peroxidation (LPO) levels in rat brain, suggesting its strong antioxidant activity. Since oxidative stress is reported to play an important role in depression, the antioxidant activity of CT might be a part of the mechanism for its antidepressant activity.Results from behavioral experiments indicate that the antidepressant activity of Clitoria ternatea , might be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the antioxidant activity.

KEYWORDS

Serum superoxide dismutase, lipid peroxidation, anti oxidant, Clitoria ternatea, Antidepressant activity.

INTRODUCTION



Clitoria ternatea is a perennial herbaceous plant, with elliptic, obtuse leaves. It grows as a vine or creeper, doing well in moist, neutral soil. The most striking feature about this plant is the color of its flowers, a vivid deep blue; solitary, with light yellow markings. They are about 4 cm (1.6 in) long by 3 cm (1.2 in) wide. Some varieties yield white flowers.The fruits are 5–7 cm (2.0–2.8 in) long, flat pods with six to ten seeds in each pod. They are edible when tender. In Southeast Asia the flowers are used to colour food. In Malay cooking, an aqueous extract is used to colour glutinous rice for *kuih ketan*(also known as *pulut tai tai* or *pulut tekan* in Peranakan/Nyonya cooking) and in *nyonya chang*. In Kelantan, it is used to colour white rice for *nasi kerabu*. In Thailand, a syrupy blue drink is made called *nam dok anchan* it is sometimes consumed with a drop ofsweet lime juice to increase acidity and turn the juice into pink-purple. In Burmese and Thai cuisines, the flowers are also dipped in batter and fried. The various Chemical compounds isolated from plant*C.ternatea* includevarious triterpenoids, flavonolglycosides, anthocyanins and steroids. Peptides known as cliotides have been isolated from the heat-stable fraction of *C. ternatea* extract.In traditional Ayurvedic medicine, it is ascribed various qualities including memory enhancing, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing, and sedative properties.[1-2]

AIM AND OBJECTIVES:

Aim of the present study was to evaluate the antidepressant activity of ***Clitoria ternatea*** (*CT*) in experimental models of depression using rats.

- To study the effect of *CT*on behavior models of depression like forced swim test, tail suspension test.
- To study the effect of *CT*on mechanism based models of depression like 5-HTP induced head twitches, clonidine induced aggression and L-Dopa induced hyperactivity and aggressive behavior.
- To study the effect of *CT*on anti-oxidant levels of brain.

MATERIALS AND METHODS

Thiobarbituric acid and DTNB reagent (HiMedia Laboratories Ltd., Mumbai), Trichloroacetic acid (Qualigens Fine Chemicals, Mumbai), Riboflavin (Astra IDL, Bangalore), Sodium dihydrogen phosphate and Disodium hydrogen phosphate (S.D. Fine Chemicals, Mumbai), Lorazepam (Ranbaxy, India), 1,1,3,3,-Tetraethoxy propane, O-Dianisidine, Imipramine hydrochloride, 5-Hydroxy Tryptophan (5-HTP), Clonidine and L-DOPA (Sigma, St. Louis, USA) were used in the study. The other chemicals and solvents used were of analytical grade and purchased from commercial suppliers. Imipramine (IMP), 5-HTP, clonidine, L-DOPA, Lorazepam was administered intraperitoneally by dissolving in normal saline.

METHODOLOGY

Collection And Authentification Of Plant Material

The Aerial Parts of *Clitoria ternatea* were collected and authenticated

Extraction Of Plant Material

The plant is grinded in to a coarse powder with the help of suitable grinder.

Cold Extraction (Methanol Extraction)

In this work the cold extraction process was done with the help of methanol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of ethanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.

Evaporation of Solvent

The filtrates (methanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vaccum dissecator for 7 days.

% Yield value of Methanol Extract from Aerial Parts of *Clitoria ternatea* Plant.

Powder taken for extraction = 250gm
Weight of the empty china dish = 50.0gm
Weight of the china dish with extract = 162.5gm

Weight of the extract obtained = (162.5-50.0) gm
= 112.5
% yield of methanol extract = (weight of extract)/(powder taken for extraction) × 100
= 112.5/200 ×100 = 56.3 %.

Preliminary Phytochemical Screening
Preliminary phytochemical screening of the *Clitoria ternatea* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, and Flavonoids[3-7].

1. Detection of Alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.
a).Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
b).Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
c).Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
d).Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of Carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.
a).Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.
b).Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
c).Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars[8-9].

3. Detection of saponins
a).Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.
b).Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

4. Detection of steroids.
a).Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
b).Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

5. Detection of Phenols
Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of Tannins
Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was addexd. Formation of white precipitate indicates the presence of tannins.

7. Detection of Flavonoids
Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids[10-14].

TOXICITY STUDIES IN ANIMALS
Healthy Adult Male mice of 5 weeks old with Average weight in the range of 40-60gms were selected. Animals are housed 4 per cage in temperature controlled (27 ^{0}C ±3 ^{0}c) room with light/dark cycle in a ratio of 12:12 hrs is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water *ad libitum*. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

Acute Toxicity Studies
The Acute oral toxicity test of the extracts was determined prior to the experimentation on animals according to the OECD (Organization for Economic Co-operation and Development) guidelines no 423. Female Albino wistar mice (30-40 g) were taken for the study and dosed once with 2000 mg/kg of the extract. The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. No mortality was observed till the end of the study revealing the 2000 mg/kg dose to be safe. Thus, 1/5, 1/10 and 1/20 doses of 2000 mg/kg i.e. 100 mg/kg and 200 mg/kg 400mg/kg were chosen for subsequent experimentation.

***IN VIVO* MODELS OF DEPRESSION EMPLOYED IN THE STUDY**
1. Forced swimming test (FST)
2. Tail suspension test (TST)
3. 5-HTP induced head twitches in mice
4. Clonidine-induced aggression in mice
5. L-DOPA-induced hyper activity and aggressive behavior in mice (LHA)

Forced Swimming Test (FST)
Principle: Behavioral despair was proposed as a model to test for antidepressant activity. It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression.
Advantages of the method are the relative simplicity and the fact that no interaction with other drugs is necessary. Like in other behavioral tests, e.g. the catalepsy test in chicken, not only antidepressants and monoamine oxidase inhibitors but also central stimulants give positive results[15-17].

Procedure: The procedure was described by Porsolt et al. (1978) was used. Swimming sessions were conducted by placing rats in individual glass cylinders (45 cm high×20 cm in diameter) containing (25±2 °C) water 38 cm deep, so rats could not support themselves by touching the bottom with their feet. Two swimming sessions were performed between 12:00 h and 19:00 h, an initial 15 min pretest followed 24 h later by a 6 min test.

Doses were given once daily for 7 days. On the 7th day rats were subjected to 15 min pretest. After 15 min, in the water the rats were removed and allowed to dry in a heated enclosure (32 °C) before being returned to their home cages. They were again placed in the cylinder 24 h later and the total duration of immobility was measured during a 6 min test. Floating behavior during this 6 min period had been found to be reproducible in different groups of rats. An animal was judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. The total immobility time for the period of 6 min was recorded with the help of stopwatch.

Tail Suspension Test (TST)
Principle: The "tail suspension test" has been described by Steru et al. (1985) as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail.

Doses are given once daily for 7 days. On the 7th day, 1hr after the administration of the test and standard drugs, mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Animal was considered to be immobile when it did not show any movement of body and hanged passively[18-20].

5-HTP Induced Head Twitches In Mice

Principle: According to the monoamine hypothesis of depression compounds exert antidepressant activity because they are capable of enhancing central noradrenergic and/or serotoninergic functions. Several antidepressant agents potentiate serotonin effects by a block of the re-uptake of serotonin. DL-5-Hydroxytryptophan is used as the precursor of serotonin. Enzymatic breakdown is inhibited by the MAO-inhibitor pargyline. In mice the characteristic symptom of head twitches is observed.

Doses were given once daily for 7 days. On the 7th day, 1hr after the administration of the test and standard drugs, mice were treated with 5-HTP (100 mg/kg i.p.) and the numbers of head twitches performed by each mice was counted by staggering method using three 2 min periods (19–21 min), (23–25 min), (27–29 min) after 5-HTP administration and number of head twitches were scored live by a blind observer.

Clonidine-induced Aggression In Mice

The method of Morpurgo (1968) was used. Mice were divided into 5 groups of 8 each (n=8), each group contain 4 pairs of mice, two pairs from each sex (each pair contained same sex of mice).

Doses were given once daily for 7 days. On the 7th day, Clonidine was given 1 h after the administration of the test and standard drugs. The animals were then caged in bell shaped glass jar with a floor area of approximate 16 cm^2. The biting/fighting episodes were recorded live by a blind observer over a period of 30 min, in each pair[21-23].

L-DOPA Induced Hyper Activity And Aggressive Behavior In Mice (LHA)

Mice were treated with L-DOPA (100 mg/kg i.p.) and the experiment was performed according to the method;Mice were divided into 5 groups of 8 each (n=8), each group contain 4 pairs of mice, two pairs from each sex[24] (each pair contained same sex of mice).

Doses were given once daily for 7 days. On the 7th day, L-DOPA was given 1 h after the administration of the test and standard drugs, Stages of activity and aggressive behavior were recorded live every 10 min for 30 min after L-DOPA administration by the blind observer. The different parameters of observation were piloerection, salivation, increase in motor activity, irritability, reactivity, jumping squeaking, and aggressive fighting. The scores were graded in the following manner:

0—No effect; 1—Piloerection, slight salivation, slight increase in motor activity; 2—Piloerection, salivation, marked increase in motor activity and irritability; 3—Piloerection, profuse salivation, marked increase in motor activity, reactivity, jumping, squeaking and aggressive fighting.

Statistical Analysis

Results were expressed as mean ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall *P*-value was found statistically significant ($P < 0.05$)

RESULTS AND DISCUSSION

%Yield value of Ethanolic Extract from Aerial Parts of *Clitoria ternatea* was found to be **56.3%**.

Preliminary Phytochemical Screening

Investigation revealed the presence of steroid, Alkaloid, Tannins, phenols& Flavonoid in Ethanolic Extract of *Clitoria ternatea* (*CT*).

Acute Toxicity Studies

As per (OECD) draft guidelines 423 Female albino mice were administered *Clitoria ternatea* and doses was be selected in the sequence (1.75- 5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days,. In all the cases, no death was observed within 14 days. Attention was also given to observation of tremors and convulsions, salivation, diarrhoea, lethargy, sleep and coma. Overall results suggested the LD_{50} value as 2000 mg/kg. Hence therapeutic dose was calculated as 1/10th and 1/20th i.e. 100mg/kg and 200 mg/kg of the lethal dose for the purpose of antidiabetic investigations.

Forced Swim Test (FST)

The results (Table. 1) showed that both *CT* (100, 200 and 400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in FST model. Post-hoc analysis showed that the *CT* (100, 200 and 400 mg/kg) and Imipramine (IMP) treated groups were significantly different ($p<0.001$) from the vehicle treated group (Fig. 1).

Table. 1. Effect Of *CR* And Imipramine (IMP) On Forced Swim Test (FST) In Rats.

Group no.	Treatment (dose in mg/kg)	Immobility period (sec) Mean ± SEM
I	Control (0.3% CMC) + FST	149.2±1.9
II	*Clitoria ternatea* (100 mg/kg, p.o.) + FST	120.8±3.7
III	*Clitoria ternatea* (200 mg/kg, p.o.) + FST	88.6±1.6*
IV	*Clitoria ternatea* (400 mg/kg, p.o.) + FST	66.6±1.4*
V	Imipramine (15 mg/kg, i.p.) + FST	77.6±1.8*

Each column represents mean ± S.E.M. of immobility period (sec), n = 6. * = $p<0.001$ compared to control

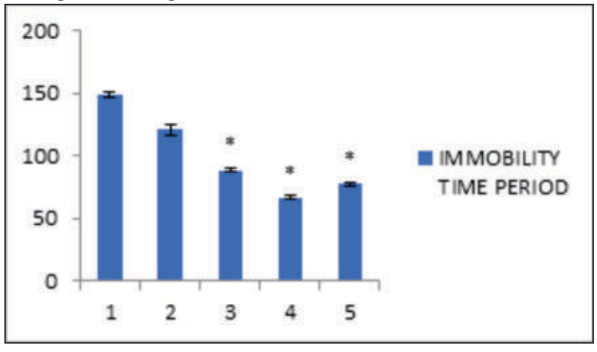


Figure. 2 Effect of *CT* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on forced swim test (FST) in rats. Each column represents mean ± S.E.M. of immobility period (sec), n = 6. *= $p<0.001$ compared to control

2) Tail Suspension Test (TST)

The results (Table. 2) showed that both *CT* (100,200,400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in TST model. Post-hoc analysis showed that the *CT*(100, 200 and 400 mg/kg) and IMP treated groups were significantly different ($p<0.001$) from the vehicle treated group (Fig. 2).

Table.2. Effect Of *CT* And Imipramine (IMP) On Tail Suspension Test (TST) In Mice.

Group no.	Treatment (dose in mg/kg)	Immobility period (sec)
I	Control (0.3% CMC) + TST	148.5±4.3
II	*Clitoria ternatea* (100 mg/kg, p.o.) + TST	113.5±4.3[a]
III	*Clitoria ternatea* (200 mg/kg, p.o.) + TST	92.5±2.63[a]
IV	*Clitoria ternatea* (400 mg/kg, p.o.) + TST	81.0±3.01[a]
V	Imipramine (15 mg/kg, i.p.) + TST	72.5±2.75[a]

Each column represents mean ± S.E.M. of immobility period (sec), n = 6. a = $p<0.001$ compared to control

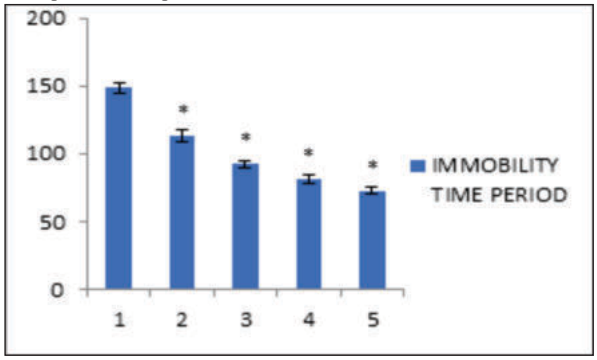


Figure. 2 Effect of *CT* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on tail suspension test (TST) in mice. Each column represents mean ± S.E.M. of immobility period (sec), n = 6. a = $p<0.001$ compared to control.

3) 5-HTP induced head twitches in mice

Table.3. illustrates the effect of *CT* and IMP on 5-HTP-induced head twitches in mice. Post-hoc analysis revealed that three doses of *CT* (100, 200 and 400 mg/kg, p<0.01, p<0.001) significantly increased the 5-HTP-induced head twitches in comparison to control group. Further, the dose of 400 mg/kg was more effective than 100, 200 mg/kg. Similarly, IMP treated group showed significant increase (p<0.001) in the 5-HTP-induced head twitches compared to control. However, the effect of 400 mg/kg of *CT* was significantly higher than IMP (p<0.001) (Fig. 3).

Table. 3. Effect Of *CR* On 5-HTP-Induced Head Twitches In Mice.

Group no.	Treatment (dose in mg/kg)	Head twitches Mean ± SEM
I	Control (0.3% CMC)	13.67±0.8
II	*Clitoria ternatea* (100 mg/kg, p.o.)	19.83±1.0[a]
III	*Clitoria ternatea* (200 mg/kg, p.o.)	24±1.36[b]
IV	*Clitoria ternatea* (400 mg/kg, p.o.)	31.5±1.3[b]
V	Imipramine (15 mg/kg, i.p.)	21.5±1.2[b]

Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.01, b = p<0.001 compared to control

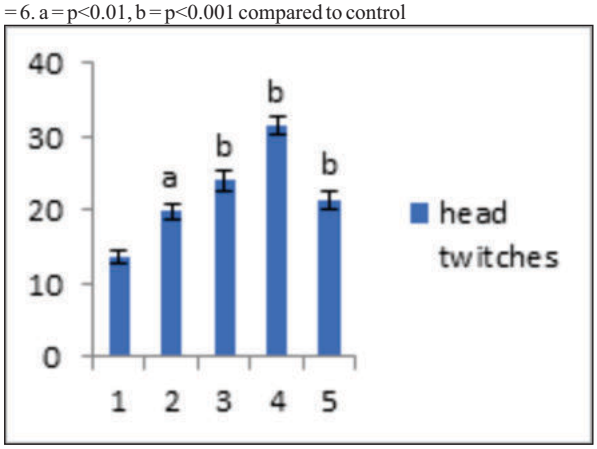


Figure. 3. Effect of *CT* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on 5-HTP-induced head twitches in mice. Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.01, b = p<0.001, compared to control.

4) L-DOPA Induced Hyperactivity And Aggressive Behavior In Mice

The effect of *CT* and lorazepam on L-DOPA-induced hyperactivity and aggressive behavior is shown in Table 4. Post-hoc analysis revealed that three doses of *CT* (100,200 and 400 mg/kg, p<0.001) significantly increased the L-DOPA-induced hyperactivity and aggressive behavior (LHA) in comparison to control group (Fig. 4).

Table. 4. Effect Of *CT* And Lorazepam On L-DOPA-induced Hyperactivity And Aggressive Behavior In Mice.

Group o.	Treatment (dose in mg/kg)	Behavioral score
I	Control (0.3% CMC)	1
II	*C. ternatea* (100 mg/kg, p.o.)	2.2 ± 0.1[a]
III	*C. ternatea* (200 mg/kg, p.o.)	2.3 ± 0.1[a]
IV	*C. ternatea* (400 mg/kg, p.o.)	2.7 ± 0.1[a]
V	Lorazepam (2.5 mg/kg, i.p.)	2.2 ± 0.1[a]

Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.001, compared to control.

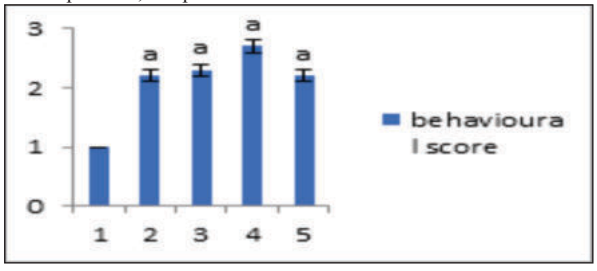


Figure. 4 Effect of *CT* (100, 200 and 400 mg/kg, p.o.) and Lorazepam (2.5 mg/kg) on L-DOPA-induced hyperactivity and aggressive behaviorin mice. Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.001, compared to control

5) Clonidine Induced Aggression In Mice

Table. 5. Indicates the effect of *CT*(100, 200 and 400 mg/kg, p.o.) and lorazepam (LA; 2.5 mg/kg) on the latency to first attack and the number of bouts in the clonidine induced aggressive behavior in mice. Post-hoc analysis showed that *CT* (p<0.001) significantly increased the latency to first attack and decrease the no. of bouts compared to control (Fig. 5)

Group no.	Treatment (dose in mg/kg)	% Response(MEAN ± SEM)	
		Latency to 1st attack	Fighting response
I	Control (0.3% CMC)	100 ± 12.7	100 ± 1.1
II	*Clitoria ternatea* (100 mg/kg, p.o.)	109.8 ± 4.4[a]	81.395 ± 0.6[a]
III	*Clitoria ternatea* (200 mg/kg, p.o.)	115.5 ± 1.1[b]	72.09± 0.6[b]
IV	*Clitoria ternatea* (400 mg/kg, p.o.)	139.2 ± 8.2[b]	56.976 ± 1.2[b]
V	Lorazepam (2.5 mg/kg, i.p.)	146.0 ± 3.9[b]	53.48± 0.6[b]

Each column represents mean ± S.E.M, n = 6. a = p<0.01, b = p<0.001 compared to control

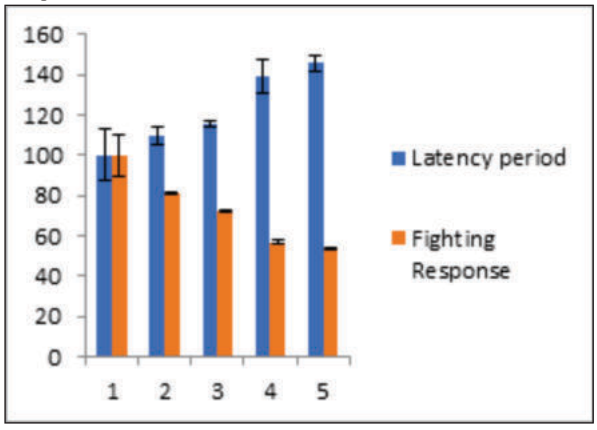


Figure. 5 Effect of *CT* (100, 200 and 400 mg/kg, p.o.) and Lorazepam (2.5 mg/kg) on clonidine induced aggression in mice. Each column represents mean ± S.E.M, n = 6. a = p<0.01, b = p<0.001 compared to control

DISCUSSION

Depression constitutes the second-most common chronic condition in clinical practice, exceeded only by hypertension. Despite recent progress achieved in the development of clinically relevant antidepressant drugs in recent years, the currently available antidepressant therapy is not at totally effective and it is associated with many undesirable collateral effects. In addition, only 60% of patients are responsive to the treatment with the available antidepressants. For this reason, the search for new drugs for the control of the symptoms associated with depressive disorders is still desirable.

Thus, a growing number of herbal medicines have been introduced to psychiatric practice and they have been chosen as alternative therapies to alleviate severe symptoms of depression.The antidepressant effect of herbs has been paid more attention gradually because of increasing incidence of depression and predominance of traditional herbs in therapy.

The effective components of herbs that have antidepressant- like effect include flavonoid, oligosaccharide, polysaccharide, alkaloid and organic acid, etc. Herbal drug used in depression are Centella asiatica, Hypericum perforatum, Rhodiola rosea, Pfaffia paniculata, Rauwolfia serpentine, Rhododendron molle, Shizandra chinesis, Thea sinensis, Uncaria tomentosa, Valeriana officinalis and Withania somnifera.

Clitorea ternatea has anti-oxidant property as it contains carotenoids and also rich in amino acids like Tryptophan, Phenylalanine, and Tyrosine. These amino acids essential for the synthesis of Serotonin, Noradrenaline, Dopamine in the body. Normally depletion of neurotransmitters like Serotonin, Noradrenaline leads to depression.

In the present study, 7 days pretreatment with*Clitorea ternatea* at the doses of 100, 200 and 400 mg/kg showed antidepressant activity in the forced swim test (FST) and tail suspension tests (TST). The FST is the tool most widely used for assessing antidepressant activity preclinically. The widespread use of this model is largely a result of its ease of use, reliability across laboratories, and ability to detect a broad spectrum of antidepressant agents. Most clinically active antidepressants are effective in the FST, while neuroleptics and anxiolytics produce different effects.In the forced swim test, it is significantly reduced immobility period suggesting anti-depressant activity and the activity was comparable to the reference drug Imipramine. Immobility is a state of lowered mood or hopelessness, which the rats experience when they are allowed to swim in a restricted space from which they cannot escape. This is thought to reflect either a failure to persist in escape directed behavior after persistent stress or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli. The tail suspension test (TST) is widely used as a dependable animal model of depression to screen new antidepressants, as well as to investigate the mechanism of action of new antidepressants. In the TST, mice are forced to hang in a confined space from which they cannot escape, and exhibit a characteristic behavior of immobility. This immobility, referred to as behavioral despair in animals, is believed to reproduce a condition similar to human depression. Thus, a reduction in the total duration of immobility indicates an antidepressant effect. Additionally, many studies have shown that the test is highly sensitive to the major classes of the clinical antidepressants, including the selective serotonin reuptake inhibitors (SSRIs), the tricyclic antidepressants (TCAs), and the monoamine oxidase inhibitors (MAOIs).

*Clitorea ternatea has*significantly reduced immobility period in the forced swimming test and tail suspension tests indicating antidepressant activity. Both these 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, and monoamine oxidase (MAO) inhibitors. In FST, rats are forced to swim in a restricted space from which they cannot escape, and are induced to a characteristic behavior of immobility. This behavior reflects a state of despair that can be reduced by several agents, which are therapeutically effective in human depression. The TST also induces a state of immobility in mice. This immobility, referred as behavioral despair in animals, which is claimed to reproduce a condition similar to human depression. It has been argued that the TST is less stressful than FST and has greater pharmacological sensitivity. Thus, the combined observations from both FST and TST indicate the antidepressant activity of *CT*.

Depression has been linked to perturbations in the neurotransmission involving brain 5-HT, norepinephrine (NE) and dopamine activity. Serotonergic neurotransmission in the central nervous system is believed to be involved in the pathogenesis and therapy of depression. Studies implicating serotonin in the pathogenesis of depression have been widely studied both in preclinical and clinical investigations. Clinical findings showed that most therapeutic agents in use induced increased availability of serotonin, which was in line with their antidepressant response. In present study, the immediate serotonergic activation of *CT* was demonstrated in 5-HTP-induced head-twitch test. Meanwhile, it was also suggested the integrity of 5-HT neural system might be requisite in the antidepressant-like effect of *CT*. In the present study, *CT* significantly increased the frequency of 5-HTP-induced head twitches indicating increase in serotonergic activity in rodent brain in vivo. Increase in stereotypical behavior by serotonin precursor 5-HTP is reported to involve increase in extracellular serotonin and subsequent activation of 5-HT2A receptors. Although, it can be assumed that there is a favorable disposition of 5-HT in CT treated animals, the exact mechanism of *CT* -induced changes in serotonin levels can only be confirmed after ex vivo or in vivo experiments involving 5-HT depletion studies and/or direct 5-HT measurements.

Clinical studies show that combined 5-HT and NE reuptake inhibitor is more effective than used alone. Based on these observations, we evaluated the role of NE in the antidepressant effect of *CT*. *CT* significantly increased the clonidine-induced aggressive behavior indicating increased activity of noradrenergic system. Hence, the antidepressant activity of *CT* may involve both serotonergic and noradrenergic systems. Several studies have shown that the antidepressant effect involves augmentation of dopaminergic neurotransmission. Such interference with the dopaminergic system could explain at least in part the acute effects of some of the antidepressants. However, *CT* also alters L-DOPA induced aggressive behavior indicating effect on the dopaminergic system. Hence, it is possible that this may be the reason for significant effect of *CT* on L-DOPA-induced aggression [25-28].

The above results indicate that *CT* has antidepressant activity by virtue of its action on serotonergic, noradrenergic and dopaminergic systems based on behavioral experimental evidence. Generally, repeated treatment with antidepressants has been reported to facilitate both serotonergic and/or noradrenergic transmission. The dual action of *CT* may have several advantages over SSRIs. Although, SSRIs are widely used, side effects such as nausea, sexual dysfunction and sleep disorders may limit their potential use. Moreover, clinical studies indicate that they may not offer too many advantages over classical antidepressants. Further, clinical studies have shown that mixed 5-HT and NE reuptake inhibitors (SNRIs) are effective and well-tolerated antidepressants. Although, the specific mechanism of action of *CT* needs to be explored before coming to any conclusions on its mechanism of action; preliminary investigations indicate that *CT* may potentially have the more desirable dual action on 5-HT and NE.

It is pertinent to note that although serotonergic and adrenergic drugs have been successfully used in the treatment of depression, never the less there exist no casual relationship between reduced serotonin and depression. Clinically, a decrease in monoamine levels including serotonin does not necessarily lead to depression in undepressed individuals nor does further depletion of monoamines worsen behavioral symptoms in depressed patients.[23]However, depletion of 5-HT or NE leads to rapid relapse in SSRI and NRI treated patients respectively implying that monoamines may not have a critical role in development of depressive symptoms, but however are important for response of antidepressant drugs. It is therefore important to evaluate other factors involved in development of depression.

The present study establishes the antidepressant activity of *Clitorea ternatea* in mice models of depression. Further, results from behavioral experiments indicate that this activity may be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic system.

CONCLUSION

The results from the present study confirm the antidepressant activity of*Clitoria ternatea ,* since it reduced the immobility in both FST and TST.In the present study, *CT*significantly increased the frequency of 5-HTP induced head twitches, Clonidine induced aggression and L-DOPA induced hyperactivity and aggressive behavior indicating its enhanced activity on serotonergic, noradrenergic and dopaminergic pathways respectively. Our results also confirm the involvement of serotonergic, noradrenergic and dopaminergic pathways in depression. Pretreatment with *Clitoria ternatea*,also significantly increased the levels of SOD and Catalase with simultaneous decrease in LPO levels in rat brain, suggesting its strong antioxidant activity. Since oxidative stress is reported to play an important role in depression, the antioxidant activity of *CT*might be a part of the mechanism for its antidepressant activity. Results from behavioral experiments indicate that the antidepressant activity of *Clitoria ternatea*might be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the antioxidant activity.

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