European Journal of Clinical Pharmacy

Print ISSN: 2385-409X **Online ISSN**: 3105-0409

Website: https://farmclin.com/

Original Research Article

FORMULATION AND EVALUATION OF POLY-HERBAL TABLET FOR THE MANAGEMENT OF ANTIDEPRESSANT ACTIVITY

Article History:

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Received: 11-11-2025
Revised: 26-11-2025
Accepted: 10-12-2025
Published: 30-12-2025

Abstract: ***Objective:*** Evaluation of Clitoria Ternatea Flower extract for antidepressant activity in wistar albino rats. ***Methods***: Thirty rats of both sex were assigned to five groups (n=6). Group 1 served as control group which received normal saline for 21 days. Group 2 served as Resrpine (O.2mg/kg i.p) treated disease for 14 days. Group 3 served as standard drug received 10mg/kg imipramine once daily for 7 days after inducing period. Group 4 served test drug 1 received (200mg/kg of CT in 50% DMSO) By orally for 7 days after inducing period. Group 5 served test drug 2 received (400 mg/kg of CT in 50% DMSO) By orally for 7 days after inducing period. ***Results:*** Rats treated with CT extract showed significant effect on behavioural activity, biochemical parameters and body weight. Histopathological examination of substantia nigra region of rat brain showed decrease in formation of segment bodies in rats treated with CT extract. ***Conclusions:*** Using this CT extract preparation treated antidepressant activity. However, its underlying mechanisms of action need further investigation.

Keywords Clitoria ternatea, Antidepressant activity, Reserpine, Imipramine, Wistar albino rats, Behavioral studies, Biochemical parameters, Neuroprotection

INTRODUCTION

One of the biggest causes of disability in the world is depression, especially its primary representation, major depressive disorder[1]. Clinically, depressive disorders are defined by the persistent presence of particular cognitive and physical abnormalities together with anhedonia, which is a depressed, empty, or irritated mood[2]. To get a better understanding of the pathophysiological and aetiological processes that contribute to the development and maintenance of depressed symptoms, many areas of neuroscientific inquiry have been undertaken in recent decades. These studies have produced significant findings on a number of levels, connecting depression to anomalies in genes, neurotransmitter and neuroendocrine systems, as well as in the anatomical and functional architecture of the brain and cognition.

From a clinical standpoint, neurotransmitter-related (or "neurochemical") disorders have likely been the most significant neurobiological findings pertaining to depression. The monoamines (serotonin, noradrenaline, and dopamine) have drawn the most attention. Numerous neurochemical studies in depression patients were prompted by early findings that tricyclic antidepressants might alleviate depressed symptoms and increase serotonin and

noradrenaline activity[3]. goods like herbs, medicinal plant extracts, and other similar botanicals are being researched for their therapeutic potential in neurological diseases because of the promise that some natural goods have shown in treating a variety of ailments[4]. Numerous plants have been found to be effective neuroprotectants clitoria Ternatea, also known as "shankhpushpi" has traditionally been used in Ayurvedic medicine for its neuroprotective properties, contains compounds that have shown potential in improving cognitive function and reducing oxidative stress in antidepressant disease[5]. Leaves are medium green and broader at the base tapering to a point. The blue flowers are edible and are a natural food coloring in Asian cuisine. They are added to beverages and are a popular ingredient in **"Butterfly Pea Tea."** Butterfly pea plant is widely grown as an ornamental and reclamation plant that fixes nitrogen in soil[6].

2. Material and Method

2.1) Chemicals

Imipramine, Reserpine were purchased from labware chemicals.

2.2) Preparation of ethanolic extract

Clitoria Ternatea were dried and collected. Dried material was coarsely pulverized with a mortar, then reduced to powder with an electric blender before being kept in an airtight glass container. The powdered flower of clitoria ternatea (50gm) were extracted sequentially with ethanol in a soxhlet extractor. After extraction, the extract was dried using a vacuum evaporator. The yield of ethanolic extract was 4 grams.

2.3) Animals

Wistar Albino Rat of weight (180-200 g) of both sexes were used in the study. Animals were obtained from the Crystal biological solution, Pune. The animals were housed in polypropylene cages, approximately six per cage, under conditions of controlled temperature (22-26˚C) and humidity (50-60%), with a 12-h light/dark cycle, and free access to water and the specified diet ad libitum of water. All the experimental procedure were approved by Institutional Animal Ethics Committee (IAEC) with proposal no: CPCSEA/CBPL/AH/84.

2.5) Experimental protocol

All the animals were allowed to acclimatize to the housing facility for 15 days before initiation of the protocol. The experiment protocol lasted for 21 days. Thirty rats of both sex were assigned to five groups (*n*=6). Group 1 served as control group which received distilled water (1 ml) 21 days. Group 2 served as a Resrpine (O.2mg/kg i.p) treated disease for 14 days. Group 3 served as standard drug received 10mg/kg imipramine once daily for 7 days after inducing period. Group 4 served test drug 1 received (200mg/kg of CT in 50% DMSO) By orally for 7 days after inducing period. Group 5 served test drug 2 received (400 mg/kg of CT in 50% DMSO) By orally for 7 days after inducing period.

2.6) Behavioral Parameters

a) Tail Suspension test: The tail suspension test has been described by Steru et al. (1985) as afacile 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[7].

b) Forced swim test: Behavioural despair was proposed by Porsolt forantidepressant activity. It was suggested that mice forced to swim in restricted space from which they cannot escape are induced to characteristics behaviour of immobility. This behaviour reflects a state of despair which can reduced by several agents which are therapeutically effective in human depression[8].

c) Elevated Plus Maze: The Elevated Plus Maze has a rich history rooted in the quest to better understand anxiety & depressant and related behaviors in rodents. Since its inception in the 1980s, it has become a cornerstone in behavioral neuroscience, providing invaluable insights into the effects of pharmacological agents and the underlying mechanisms of anxiety & depressant. Its continued evolution and integration with new technologies promise to further enhance its utility and reliability in research[9].

2.7) Statistical analysis

The results were expressed as the mean ± standard error mean (SEM). Statistical analyses were performed using one way analysis of variance (ANOVA)between groups, and unpaired comparisons were analysed using the least significant difference method t-test. A P-value of 0.0001 or less was considered statistically significant. All analyses were conducted using Tukey's test Prism GraphPad software Version 9.2.0.

RESULTS

The phytochemical screening of clitoria ternatea plant extracts revealed the presence of the following phytoconstituents:

When CLITORIA TERNATEA L. were tested for below (Table 1) phytochemical tests, positive results were obained while detecting phenolic acids with extracts and all other pharmacognistic tests (detection test for alkaloids, flavonoids, tannins, saponin, terpenoid and carbohydrates)

Table 1: Phytochemical screening results of Clitoria ternatea

Clitoria ternatea FT-IR spectrum shows peak position 3297.45 at cm^{-1}, indicating stretching of the -OH group in the fruit pulp extract. It confirms the existence of phenolic chemicals and flavonoids in the plants.

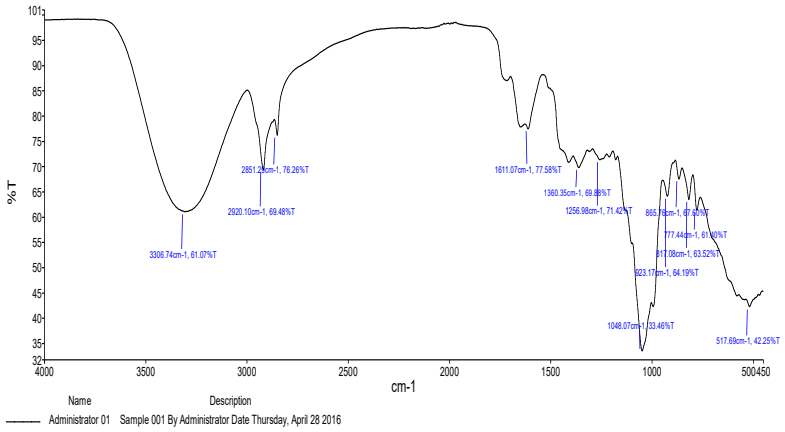

Table 1: Phytochemical screening results of Clitoria ternate

Sr. no.	Phytochemical	CT
1.	Alkaloids	+
2.	Phytosterols	+
3.	Glycosides	-
4.	Fixed oil and fats	+
5.	Protein and amino acids	+
6.	Tannins	+
7.	Saponins	+
8.	Terpenoids	+
9.	Flavonoids	+

Graph 1. FTIR of clitoria ternatea

Forced Swim Test

- The group treated with CT 200 displayed a mean immobility duration ranging from 1.26 to 2.41 seconds, with a mean value of 2.12 seconds and a standard deviation of 0.38 second.
- The group treated with CT400 showed a mean immobility duration ranging from1.43to 2.21 seconds, with a mean value of 2.09 seconds and a standard deviation 0.25.
- The group treated with CT400 exhibited a mean immobility duration ranging from 1.14 to

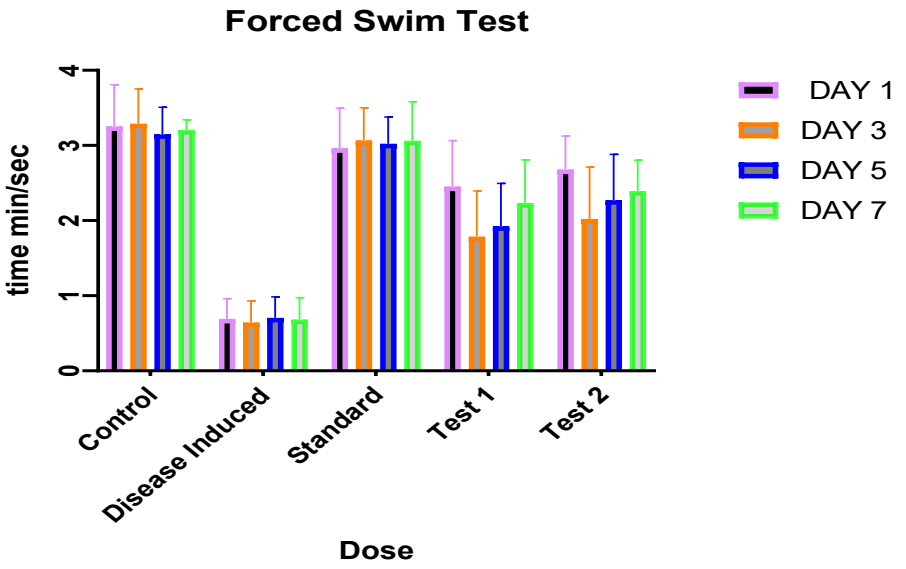

Fig1. Effects of CT extracts on the FST

These findings suggest that the methanolic extract of CLITORIA TERNATEA, particularly at concentrations of CT200 and CT400, possesses significant antidepressant activity as indicated by reduced immobility durations in the FST model. Further investigations are warranted to elucidate the underlying mechanisms responsible for these effects.

Tail suspension Test

- With a mean value of 2.23 seconds and a standard deviation of 0.33 seconds, the CT200-treated group showed mean immobility durations ranging from 1.06 to 2.54 sec A mean immobility time of 2.01 seconds was observed in the CT400-treated group, with a standard deviation of 0.22 seconds and a range of 1.30 to 2.21 seconds. The group treated with CT400 exhibited a mean immobility duration ranging from 1.09 to1.52 seconds, with a mean value of 1.29 seconds and a standard deviation of 0.19 sec.

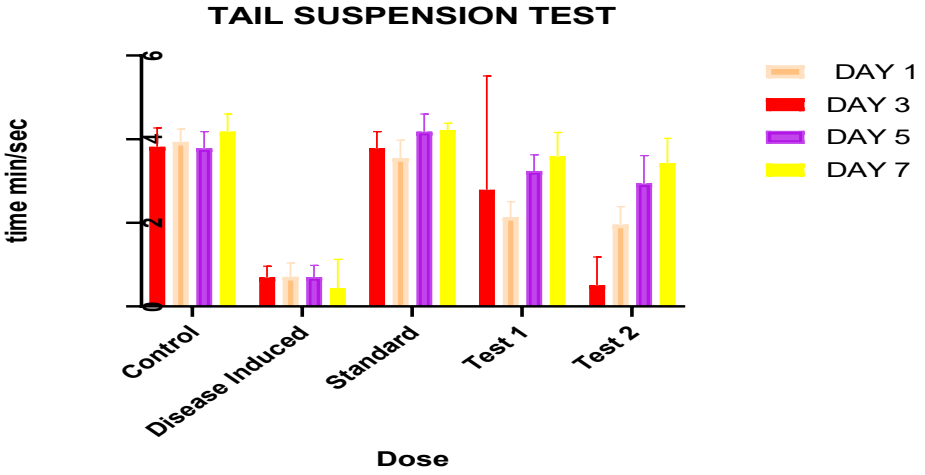

Fig2. Effects of CT extracts on the TST

2.8). Effect of CT extract on transfer latencies in an elevated plus maze

Day 1:

The doses (i.e., test 1 Clitoria ternatea 200mg/kg CT 400mg/kg) decreased the initial transfer latency significantly to 5.33 ±0.482 seconds and 4.66± 0.527 seconds, respectively .total time spend in open/close arm in disease induced dose, more time spend in close arm this is latency significantly 37.33±0.032, 90±0.53 respectively.

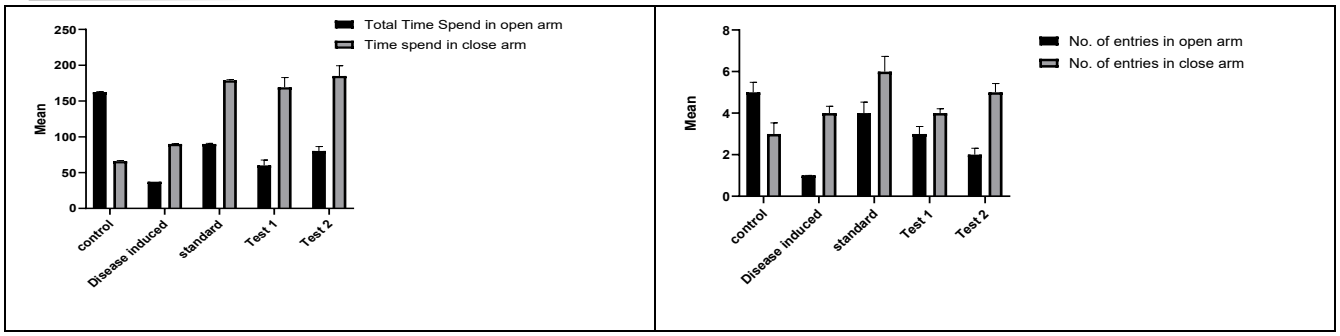

Fig 3 :No. entries in open /close arm on day 01 Effect Clitoria Ternatea extract on the spatial of reserpine induced depression in rats in FST and TST. A) Escape latency of rats in acquisition phase; B) Escape latency of rats in retention phase; reserpine 0.2mg/kg, DPZ- imipramine 10mg/kg, CT 200mg/ kg, Retention phase. The columns represents mean ± SEM values

DAY 3 :

After the inducing period of days completion time duration than the transfer latency time in increase day by day 0.333±0.218, standard dose 112±16.289, respectively. Time taken by the rat to enter the closed arm, with its all the four paws inside, was recorded as transfer latency (TL). TL was again recorded after 24h. more time spend in close arm this is latency significantly 40.33±0.035, 90±0.53 respectively.

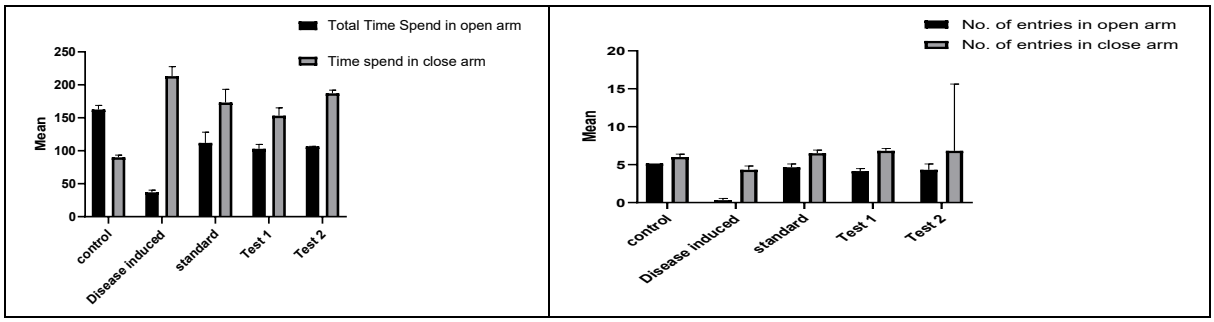

Fig 4 No. of entries open /close arm day 03 clitoria ternatea 1) Initial transfer latency (in seconds) on Day 3; on . Each column represents mean ± SEM of six animals. The data analysis was performed using one way ANOVA followed by least significant difference. or less was considered statistical factor reserpine induce alone group. reserpine o.2mg/kg, imipramine 10mg/kg, CT 200mg/kg, CT400mg/kg

Day 5:

After the inducing period of days completion time duration than the transfer latency time is increase day by day 0.333±0.218, standard dose 112±16.289, respectively. Time taken by the rat to enter the closed arm, with its all the four paws inside, was recorded as transfer latency (TL). More time spend in close arm this is latency significantly 45.33±0.038, 90±0.62 respectively.

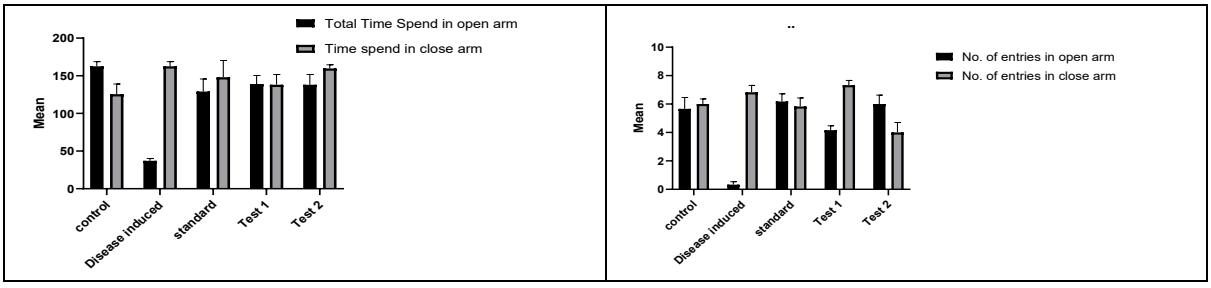

Fig 5 No.of entries open /close arm day 03 clitoria ternatea 1) Initial transfer latency (in seconds) on Day 5; on. Each column represents mean ± SEM of six animals. The data analysis was performed using one way ANOVA followed by least significant difference. or less was considered statistical factor reserpine induce alone group. reserpine o.2mg/kg, imipramine 10mg/kg, CT 200mg/kg, CT400mg/kg

Day 7:

After the inducing period of days completion time duration than the transfer latency time is increase day by day 0.333±0.218, standard dose 112±16.289, respectively. Time taken by the rat to enter the closed arm, with its all the four paws inside, was recorded as transfer latency (TL). More time spend in close arm this is latency significantly 48.33±0.039, 90±0.65 respectively.

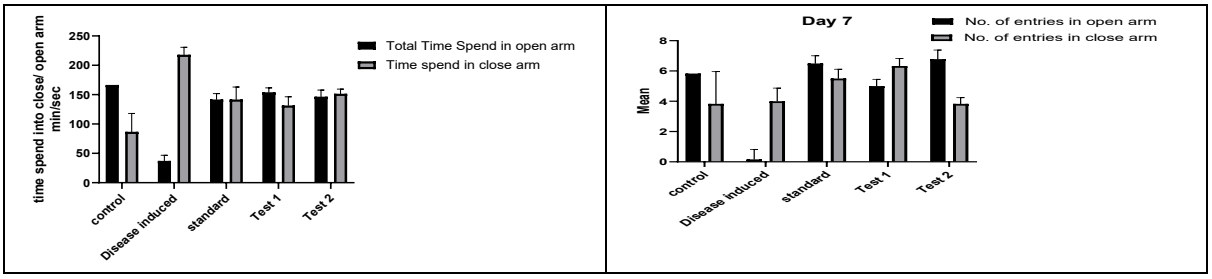

Fig 6 No. of entries open /close arm day 03 clitoria ternatea 1) Initial transfer latency (in seconds) on Day 5; on. Each column represents mean ± SEM of six animals. The data analysis was performed using one way ANOVA followed by least significant difference. or less was considered statistical factor reserpine induce alone group. reserpine o.2mg/kg, imipramine 10mg/kg, CT 200mg/kg, CT400mg/kg

Effect of CT on body weights of Rats:

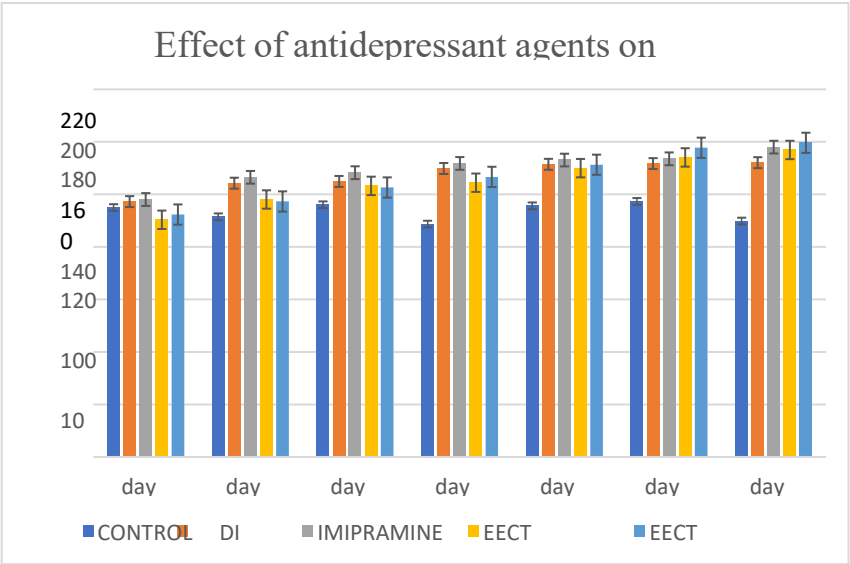

Fig 7: Effect of CT on body weight

DISCUSSION

Apart from the emotional distress and bereavement linked to sadness, the prevalence and long-lasting character of depressive disorders lead to a noteworthy impact on public health10. The medical potential of clitoria ternatea has been investigated in this study in compliance with WHO and other international agency norms, clitoria ternatea a tropical tree species commonly referred to as kadam, is indigenous to South and Southeast Asia, encompassing Indonesia According to clitoria ternatea has been used in medicine to cure a variety of 11conditions, including skin disorders, , dysentery, sugar control, fever, analgesic, antimicrobial Despite being highly valuable medicinally and having analgesic, anti-inflammatory, antipyretic, anthelmintic, and wound-healing properties12. The phytochemicals included in herbal medications are crucial in demonstrating the pharmacological effects of that particular plant. clitoria ternatea has been shown to include alkaloids, flavonoids, proteins, and amino acids; thus, these compounds have anti-inflammatory, antidiabetic, and antioxidant properties13. In this instance, mice were given CT extracts and their antidepressant efficacy was assessed via TST and FST tests. The behavioral tests used to assess the antidepressant efficacy of medications or herbal remedies are called TST and FST. Rat's immobility is comparable to human sadness in that it reflects a feeling of hopelessness or depressed mood. The rat had given up on their hope of escaping the small space. It has been shown that

depressive medications can shorten the duration of immobility 14. The Rat have surrendered the expectation of running away from the limited area. It has been informed that the antidepressant medicines can reduce the immobility period in the animal model. The CT extract administered to the Rat at medium and higher doses could degrade significantly the immobility period based on the FST and TST testing compared to the stress control animals15.The two most popular animal models of depression for assessing antidepressant efficacy are the tail suspension test and the forced swim test16. Antidepressant medications were said to be able to reverse the immobility caused by forced swimming and tail suspension in animals, which was thought to be comparable to depression in humans17. These animal models, which are susceptible to different antidepressant medications, were inspired by the hopelessness or helplessness that animals exhibit in some unavoidable and restricted spaces. Given that the TST has a higher sensitivity than the FST and is frequently used to identify and assess the effectiveness of antidepressant medications 18. Mice exposed to FST developed a condition of "hopelessness" and/or "abandonment". Antidepressant medications have been shown to decrease this abandoning behavior in rat 19 Furthermore, this model has been used to test a number of plant extracts, with encouraging outcomes. When compared to control groups, animals placed in an unavoidable circumstance exhibit antidepressant-like activity as seen by a reduction in immobility. In the FST, a rat submerged in a cylinder of water to the extent that its hind feet are unable to contact the bottom. An average animal will first become very active, attempt to flee, and then assume a "immobile" position in which it will only move in order to maintain its head above the water. Prior exposure to the test facilitates the development of immobility, and preexposure to the exam 24 hours beforehand is frequently employed 20.

In this test, the degree of immobility versus active movement is monitored while a mouse is hung by its tail 21. The TST is predicated on adopting a passive response in a stressful scenario, just as the FST. Acute antidepressant medication administered before the test is thought to have strong predictive validity since it shortens the TST's immobility period22 Tannic acid and polyphenols, however, may have some of the antidepressant properties due to their ability to lessen the oxidative stress that depression causes. Since that CT had strong antidepressant efficacy and contains no flavonoids, this further eliminates out the major function of flavonoids. Tannic acid concentration was found in NC to be 69.42± 0.20 mg/g.

Therefore, it is clear that Clitoria Ternatea functions as an antidepressant medication when taken as prescribed due to the presence of alkaloids and flavonoids in CT extract and substantial outcomes in FST and TST 23. Third model is The EPM exam was employed to assess the study of antianxity activity on drugs. The EPM was initially intended to evaluate anxiety and is based on rats' apparent innate aversion to open, elevated areas 24. stress assessment uses certain EPM metrics, such as retention transfer latency (the time it takes the animal to shift from open arms to enclosed arms). Additionally, in the retention experiment, an animal's transfer latency is lowered when it has previously entered the open arms 25. The one plant showed a significant anxiolytic activity in comparison to control. CT (200mg/kg) and CT (400mg/kg) showed activity comparable to IMIPRAMINE. CTE was the least active among. A similar pattern of decrease in the anxiolytic activity at doses higher than showing maximum activity was observed in all the three plants. This decrease in the activity at higher dose levels could be due to the CNS depressant activity of the plants. Recently These results suggest that this combination has a nootropic effect because it ameliorates the retention of information in absence of any anxiety, stress impairment inducer. Furthermore, CLITORIA TERNATEA significantly reduced the retention transfer latency after 48 hours 26.

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