Research Article

Int Jou Hea Bio Sci, 4(2), 2023, 63-68

International Journal of Health care and Biological Sciences

Content available at www.saap.org.in Online ISSN: 2582-7499

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STUDY OF CENTRAL NERVOUS SYSTEM DEPRESSANT AND BEHAVIORAL ACTIVITY OF ETHANOLIC EXTRACT OF *CLITORIA ANNUA* IN ALBINO RATS

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Article History

Received on: 22-03-2023
Revised on: 11-04-2023
Accepted on: 14-06-2023

Abstract

Objective: The objective was to evaluate depressant effects on central nervous system (CNS) and behavioral effects of ethanol extract of *clitoria annua* and to find the phytochemical responsible for these activities with possible mode of action. Materials & Method: The central nervous system (CNS) depressant activity of *C. annua* was evaluated by the classical models of depression as Rota rod method, Actophotometer test, tail suspension, and thiopental sodium induced sleeping time tests in Rat. The Rats were divided into Normal, Standard, and two test groups containing five rats each. The test groups received extract at the doses of 200, and 400 mg/kg body weight orally where as the Normal group received distilled water (0.1 ml/mice, p.o.). Diazepam (2 mg/kg, i.p.) was used as standard drug. Results: It is clear that the plant extract significantly decreased the locomotor activity of rats in Rota rod method and Actophotometer test when compared to the normal ($p < 0.05$). It is observed that the extract showed significantly ($p < 0.05$) increased in immobility time in tail suspension test in mice. In addition, the extract produced prolongs the sleeping time with onset of action in contrast to the normal group. Conclusions: The present work depicts the evaluation of possible CNS depressant activity of C. annua in mice models. EECA can be a safe and effective approach/alternative for the treatment of number of CNS depressant disorders. More extensive study is required to fractionate, purify, and identify the exact mechanism of action CNS Depressant activity present in ethanolic extract of *Clitoria annua* with its active ingredients.

Keywords: *Clitoria annua,* CNS depressant, Extract, Diazepam.

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DOI: https://doi.org/10.46795/ijhcbs.v4i2.454

Introduction

Anatomy and Physiology of the Brain

The brain is an amazing three-pound organ that controls all functions of the body, interprets information from the outside world, and embodies the essence of the mind and soul. Intelligence, creativity, emotion, and memory are a few of the many things governed by the brain. Protected within the skull, the brain is composed of the cerebrum, cerebellum, and brainstem.

The brain receives information through our five senses: sight, smell, touch, taste, and hearing - often many at one time. It assembles the messages in a way that has meaning for us, and can store that information in our memory. The brain controls our thoughts, memory and speech, movement of the arms and legs, and the function of many organs within our body.

The central nervous system (CNS) is composed of the brain and spinal cord. The peripheral nervous system (PNS) is composed of spinal nerves that branch from the spinal cord and cranial nerves that branch from the brain [1].

Brain Disorder

Any deformities, dysfunction and disease condition in the brain affect the whole body. The brain is susceptible to neuronal disease and neurons or tissue infection.[2] Damage can be caused by trauma (psychiatric condition), or a loss of blood supply (accidental or environmental factors) known as a stroke. In brain injury, the degeneration of brain cells occurs [3,4]. It depends upon a wide range of internal as well as external factors. Brain damage due to trauma is induced by personal factors or mentally unstable conditions, while neurotoxicity refers to

chemically induced neuronal damage [3]. Broadly human brain disorders are divided into two categories namely, Neurodegenerative diseases and Neuropsychiatric disorders. Both are tough to understand and incurable, but there are medicines, surgery and physical the rapies applied for the treatment or to suppress the symptoms of the diseases.

CNS Depression

The causes of depression are not entirely understood, but are thought to be multi-factorial. Studies indicate that depression is, at least in part, an inherited condition involving abnormalities in neurotransmitter functioning. Although inheritance is an important factor in major depression, it does not account for all cases of depression, implying that environmental factors may either play an important causal role or exacerbate underlying genetic vulnerabilities.

Some of the common causes of depression which have been identified include the following:

Genetics

Research indicates that depression is, at least in part, inherited. Thus far, however, no studies have isolated the specific genes responsible for depression.

Brain Chemistry Imbalance

Depression is believed to be caused by an imbalance in the neurotransmitters which are involved in mood regulation. Neurotransmitters are chemical substances which help different areas of the brain communicate with each other. When certain neurotransmitters are in short supply, this may lead to the symptoms we recognize as clinical depression.

Circadian Rhythm Disturbance

One type of depression, called seasonal affective disorder, is believed to be caused a disturbance in the normal circadian rhythm of the body. Light entering the eye influences this rhythm, and, during the shorter days of winter, when people may spend limited time outdoors, this rhythm may become disrupted.

Poor Nutrition

A poor diet can contribute to depression in several ways. A variety of vitamin and mineral deficiencies are known to cause symptoms of depression. Researchers have also found that diets either low in omega-3 fatty acids or with an imbalanced ratio of omega-6 to omega-3 are associated with increased rates of depression. In addition, diets high in sugar have been associated with depression.

Medical Illnesses

Illness is related to depression in two ways. The stress of having a chronic illness may trigger an episode of major depression. In addition, certain illnesses -- for example, thyroid disorders, Addison's disease and liver disease -- can cause depression symptoms.

Stressful Life Events

Stressful life events, which overwhelm a person's ability to cope, may be a cause of depression. Scientists have theorized that the high levels of the hormone cortisol, which are secreted during periods of stress, may somehow induce depression by affecting the neurotransmitter serotonin [5].

PLANT PROFILE: (*Clitoria annua*)

Fig no 2.1 *Clitoria annua*

Scientific classification

Kingdom: Plantae

Division: Tracheophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: *Clitoria*

Species: *C. Annua*

Common name

Asian pigeonwings, Bombay bean, butterly pea.

Botanical name

Clitoria annua

Synonyms

Clitoria biflora

Geographical distribution

Clitoria annua is a wide ranging plant being native to tropical and subtropical Asia . The native range of this species is W. Central Himalaya to India. It is an annual or perennial and grows primarily in the seasonally dry tropical biome [6].

Chemical constituents

It shows the presence of tannins, saponins, triterpinoids, phenols, flavanoids, flavanol glycosides, alkaloids, steroids and acid compounds.

Health Benefits of Blue *Clitoria annua*

- The plant is used to boosts brain health by increasing level of acetylcholine, known to decrease age-associated memory loss and improve memory.
- The plant is used to Fights against cancers by entering the cancer cells and inhibits their growth [7].

Methods

Chemicals

The following drugs and chemicals were used in this study: Diazepam (GLAND PHARMA LIMITED), Thiopental sodium (ANGEL BIOGENICS PRIVATE LIMITED), Ethanol (LABPRO). Diazepam (2 mg/kg i.p.) was used in Rota rod apparatus, Actophotometer, tail suspension and thiopental sodium-induced sleeping time tests. The drugs were intraperitoneally (i.p.) administered 15 min before the

experiment. The extract was orally administered 30 min before the experiment at the doses of 100, and 200 mg/kg, where as the animal of normall group received distilled water (0.1 mL/mouse, p.o.).

Collection of plant materials

Fresh plant leaves of *Clitoria annua* was collected from talakona forest, Tirupati.

Preparation of extraction

The shade dried leaves of plant *Clitoria annua* was taken, powdered in a grinder mixer to obtain a coarse powder and then passed through 40 mesh sieve. About 200gms of powder was extracted by using by soxhlet apparatus process upto 24 hrs. The solution was filtered through whatman filter paper and the resultant filterate was distilled under reduced pressure for recovery of solvent. The dried extract thus obtained was kept in desiccators and used for the investigation of phytochemical screening and evaluation of central nervous system (CNS) depressant activity in mice models.

Experimental animals

Wister albino rats of (150-230gms) procured from Raghavendra enterprises (Bangalore) were used in the present study. The animals were housed in the clean propylene cages and maintained under standard conditions (25-2 C, relative humidity 44-56% and 12-hours light and dark cycles respectively) and fed with standard rat diet (M Mysore feeds, Bangalore) and purified water ad libitum for 1 week before and during the experiments. Animals were handled with human care. Institutional Animal Ethical Committee (IAEC) of P.Rami Reddy Memorial College of Pharmacy (1423/PO/Re/S/11/CPCSEA) approved the present study.

Acute Toxicity Studies

Acute toxicity study was performed as per OECD-423 guidelines. Wister albino rats (n=5) of either sex selected by rabdom sampling techniques were used for acute toxicity study. The animals were kept fasting for an overnight provided with only water, after which the extract was administered orally at the dose level of 5, 50, 500, and 1000 mg/kg body weight. If mortality was ascertained in one animal, then the same dose was prepared again t confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 1000mg/kg of body weight.

Behavioural Profile

Alertless, restlessness, irritabilty and fearfulness.

Neurological profile

Spontaneous activity, touch, response and gait

Autonomic profile

Defecation and urination

The mortality and morbidity were observed after 24 hours.

Phytochemical screening

Ethanolic extract of C. annua was qualitatively tested for the detection of alkaloids, flavonoids, saponins, tannins, cardiac glycosides, carbohydrates, reducing sugars, proteins, glucosides, terpenoids, and steroids e.t.c.

Pharmacological tests

Rota-rod performance

Five animals at a time were placed on rod rotating at 20–25 rpm speed. Only the mice that demonstrated their ability to remain on the revolving rod (20–25 rpm) for 5 min after training sessions during pre-test screening were selected for studies. The fall off time was recorded in all the groups before and 30 min after drug administration. Decrease in fall off time is suggestive of depression of the central nervous system **[8].**

Actophotometer test

The animal locomotor behaviour was monitored using actophotometer. Animals were placed in actophotometer individually, and basal activity score was recorded over the period of 5 min. Each animal was treated with respective drug, and activity score was recorded after 30 min and 1 h. Decreased activity score was taken as index of CNS depression **[8].**

Tail suspension test

This behavior displayed in mices subjected to unavoidable and inescapable stresses during tail suspension test reflects behavioral despair, which reflects depression in humans. Mice were divided into four groups. Thirty minutes later, the extract (200, and 400 mg/kg, p.o.) was used as test groups. The diazepam (2 mg/kg, p.o.) was used as standard drug when the normal group received distilled water (0.1 ml/mouse, p.o.). Mice were suspended 50 cm above the floor using adhesive tape placed approximately 1 cm from the tip of their tails. The duration of immobility time was recorded for 6 min. The mice were considered immobile when they passively hung or stayed motionless [9].

Thiopental sodium-induced sleeping time test

In this test, the animals were assigned for four groups comprising of five mice in each group. The test groups expected the extract at the doses of 200, and 400 mg/kg when the normal group received distilled water (0.1 ml/mouse, p.o.). The standard drug diazepam (2 mg/kg, i.p.) was used as positive standard group. After passing thirty minutes, each mouse was treated with thiopental sodium (40 mg/kg, i.p.) to induce sleep. The mices were monitored by placing them on different chambers for the latent period (time between thiopental sodium administrations to loss righting reflex) and duration of sleeping time (time between the loss and recovery of righting reflex) [9].

Results

Phytochemical screening

Phytochemical screening of the Ethanolic extract of *C. annua* revealed the presence of alkaloids, flavonoids, saponins, tannins, triterpenoids, and steroids (Table 1).

Table 1 : Preliminary phyto-chemical screening of ethanolic extract of *Clitoria annua*

Acute toxicity

S.No	Phytochemical tests	Ethanolic Extract of Clitoria annua
01.	Test for Alkaloids	+
02.	Test for Saponins	+
03.	Test for Triterpenoids	+
04.	Test for Steroids	+
05.	Test for Reducing sugars	-
06.	Test for Proteins	-
07.	Test for Glucosides	-
08.	Test for Flavonoids	+
09.	Test for Tannins	+
10.	Test for Carbohydrates	-

The results of acute toxicity study showed no clinical signs of toxicity and mortality in the EEAA treated animals even after administration of 1000 mg/kg dose. Hence, as per OECD guidelines lethal dose was assigned to be more than 1000 mg/kg. One-fifth of this lethal dose (400 mg/kg) was taken as effective dose for the study.

ROTA-ROD METHOD & ACTOPHOTOMETER TEST

There was a significant increase in the CNS Depression in rats treated with Diazepam when compared to the normal group. EECA (200 mg/kg and 400 mg/kg) showed significant increase in CNS depression levels when compared to normal group suggesting to treat CNS depression related diseases.

Rota Rod Apparatus

GROUP	TREATMENT	DOSE	Basal	30Mins
I.	Normal	Distilled water	255.23±4.89	206.32±4.23
II.	Standard	Diazepam (2mg/kg)	219.7±1.91	86.3±0.78**
II.	Test 1	EECA (200mg/kg)	236.9±3.52	114.2±2.06*
V.	Test 2	EECA (400mg/kg)	232.53±8.50	102.3±5.03**

Values indicate mean ± SEM (n=6). (analysis of variance test followed by Dunnett's t-test).Significant variation against control at *$P < 0.001$.

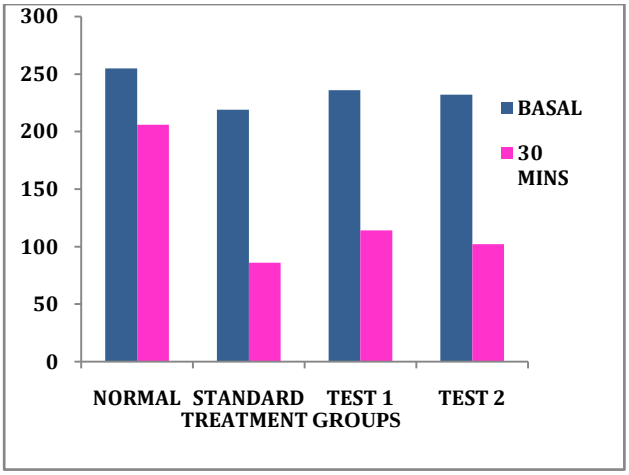

Figure 1 : Mean fall off time in rota-rod method

Actophotometer

GROUP	TREATMENT	DOSE	Basal	30Mins	60Mins
I.	Normal	Distilled water (0.1ml/mouse)	346±12.5	209.64±3.81	196.33±2.08
II.	standard	Diazepam (2mg/kg)	244.2±36.8	61.87±1.53**	48.1±4.05**
III.	Test 1	EECA (200mg/kg)	324±11.07	132.23±1.27*	118.36±1.08*
IV.	Test 2	EECA (400mg/kg)	287.06±9.08	82.91±0.74**	73.42±0.98**

Values indicate mean ± SEM (n=5). (Analysis of variance test followed by Dunnett's t-test).

Significant variation against control at *$P < 0.001$.

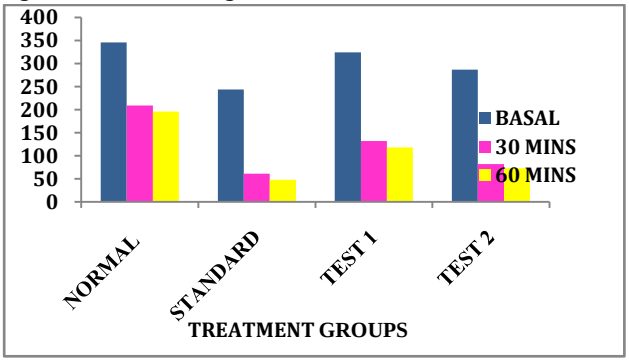

Figure 2: Activity score in Actophotometer

Tail Suspension Method & Thiopental Sodium Induced Sleeping Time

Administration of Diazepam induced a significant increase in CNS Depression levels, in standard group when compared to normal group. EECA of (200 mg/kg and 400 mg/kg) showed significant increase in CNS depression levels, when compared to normal.

Tail Suspension Method

GROUP	TREATMENT	DOSE	IMMOBILITY TIME (s)
I.	Normal	Distilled water (0.1ml/mouse)	87.63±2.34
II.	Standard	Diazepam (2mg/kg)	236.60±3.87**
III.	Test 1	EECA (200mg/kg)	137.16±0.30*
IV.	Test 2	EECA (400mg/kg)	186.60±0.57**

Values are presented as mean ± SEM (n = 5). EECA = Ethanolic extract ofC. annua.* p < 0.05, vs. Normal (Dunnett's test)

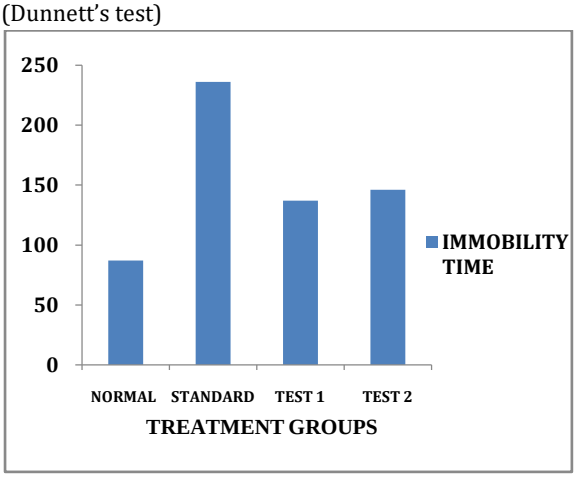

Figure 3: Immobility time in Tail Suspension Method

Thiopental Sodium Induced Sleeping Time Test

GROUP	TREATMENT	DOSE	Onset of action (min)	Duration of sleeping time (min)
I.	Normal	Distilled water (0.1ml/mouse)	5.04±0.63	67.43±8.06
II.	Standard	Diazepam (2mg/kg)	2.73±0.20*	134±8.32**
III.	Test 1	EECA (200mg/kg)	4.46±0.22	98.73±13.97*
IV.	Test 2	EECA (400mg/kg)	3.10±0.34*	130±13.58**

Values are presented as mean ± SEM (n = 5). EECA = Ethanolic extract of*C. annua.* * p < 0.05, vs. Normal (Dunnett's test)

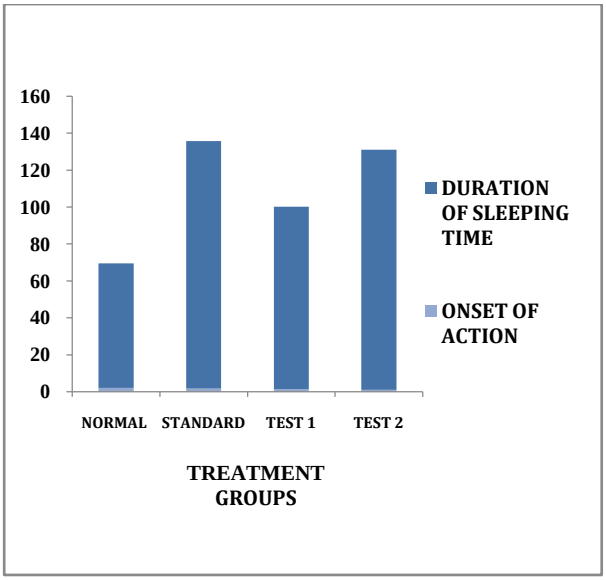

Figure 4: sleeping time in thiopental sodium induced sleeping time test

Discussion

Anxiety and hypnosedation are principally mediated in the CNS by the GABAA receptor complex, Gamma amino butyric acid is the major inhibitory neurotransmitter in the central nervous System.[10] which is also involved in other physiological functions related to behavior and in various psychological and neurological disorders such as epilepsy, anxiety, depression, Parkinson syndrome, and Alzheimer's disease [11].

CNS depressants such as including diazepam, clonazepam, Phenobarbital, mephobarbital and zolpidem act on the brain by increasing activity of gamma-aminobutyric acid (GABA) a chemical that inhibits brain activity which causes the drowsy and calming effects that make the medicine effective for anxiety and sleep disorders but they are not promising enough due to their limited effectiveness and low tolerability.

Medicinal plants such as Anacyclus pyrethrum, Argemone mexicana, Avicennia officinalis, Clitoriaternatea, Rutachalepensiswith anxiolytic,epileptic and CNS depressant activities exert inhibitory effects, making them useful for treatment of CNS depression.

Clitoria annua belonging to the family Fabaceae having medicinally important active constituents.The preliminary phytochemical screening of the extract of *Clitoria annua* leaves showed the presence of saponins, falvonoids, tannins and steroids. The alkaloids, tannins and triterpenoids are present in the extract which may possibly responsible for the CNS depression action *Clitoria annua.*

Diazepam (2 mg/kg b.wt) was used as a standard drug in inhibiting CNS depression, due to its potent anxiolytic, muscle spams and sedative effect, which is beneficial in relieving CNS depression.

Phytochemical investigation suggests that flavonoids and neuroactive steroids are ligands for GABAA receptors

in the CNS which indicates that they can act as benzodiazepine-like agents[12]. It has also been reported that some flavanoids exhibit high affinity binding to the benzodiazepine site of GABAA receptors. Therefore, the CNS depressant activity may be due to the phytoconstituents present in the extract of C.annua. Triterpenoids, and saponins are reported to have agonistic activities at GABAA receptor complex [13].

There is no strict evidence which substances are exactly responsible for the CNS depressant effects [14]. The CNS depressant effect of C. annua was studied using five neuropharmacological models namely Rota rod method, Actphotometer test, tail suspension method, and thiopental sodium induced sleeping time tests.

Conclusion

From the results obtained, we can conclude that EECA possesses considerable CNS depressant activity which is comparable with the standard. Triterpenoid, saponins may be the phytochemicals responsible for this activity. EECA can be a safe and effective approach/alternative for the treatment of number of CNS depressant disorders. More extensive study is necessary to determine the exact mechanism of action of the ethanolic extract *Clitoria annua* and its active ingredients.

Acknowledgement

The authors are very thankful to the management of P. RAMI REDDY MEMORIAL COLLEGE OF PHARMACY, KADAPA, for providing the facilities for working, technical support and discussions.

Conflict of Interest

The authors have declared no Conflict of Interest.

Ethical Statement

Institutional Animal Ethical Committee (IAEC) of P.Rami Reddy Memorial College of Pharmacy (1423/PO/Re/S/11/CPCSEA) approved the study.

Author Contribution

All authors contributed equally.

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