



18(1): 1-10, 2022; Article no.INDJ.90785 ISSN: 2321-7235, NLM ID: 101632319

Effects of Ethanolic Root Extract of *Clitoria ternatea* against Experimentally Induced Convulsions and Anxiety in Rodents

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/INDJ/2022/v18i130212

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/90785

Original Research Article

Received 18 June 2022 Accepted 20 August 2022 Published 24 August 2022

ABSTRACT

In animal models for screening for anticonvulsant activity, it has been scientifically established that medicinal herbs used in traditional medicine for the treatment of epilepsy possess promising anticonvulsant properties and can be a source of newer anticonvulsants. This study's objective was to evaluate the ethanolic root extract of Clitoria ternatea Linn for its preliminary phytochemical components, anticonvulsant, and anxiolytic effects. Anticonvulsant activity was evaluated against Maximum electroshock (MES) induced convulsion and pentylenetetrazole (PTZ)-induced convulsion model in rats. Using phenytoin (25 mg/kg) as a standard drug, the efficacy of the extract at oral dose levels of 200 and 400 mg/kg were evaluated in an experimental rat model. The marble bury test was used to assess the mice for anxiolytic activity, and lorazepam served as the standard drug at a dose of 0.05 mg/kg. Phytochemical screening revealed that C. ternatea extract contain carbohydrates, flavonoids, alkaloids, proteins, triterpenoids, phenols and steroids. The ethanolic extract significantly decreased the duration of tonic flexion and tonic extension in MES induced model (p<0.05). The ethanolic extract significantly increased the latency of convulsion and decreased the duration of convulsion in PTZ induced model (p<0.05). The ethanolic root extract were found to be significantly decrease the number of marbles buried in the treated groups as compared to control group, indicating anxiolytic activity. According to specific investigations, terpenes and steroids exhibited anticonvulsant effects in some experimental seizure models, including MES and PTZ. Alkaloids and triterpenes, which are phytoconstituents in ethanolic extract of *Clitoria ternatea* (EECT), might be the basis of its anxiolytic actions. Based on the findings of the study, *Clitoria ternatea*'s ethanolic root extract has anticonvulsant and anxiolytic effects on animals.

Keywords: Clitoria ternatea Linn; pentylenetetrazole; marble burying test; anticonvulsant; anxiolytic activity.

1. INTRODUCTION

One of the most prevalent and common neurological conditions affecting people epilepsy. Today's medical community views epilepsy as a chronic brain condition with multiple aetiologies that is marked by repeated seizures and frequently accompanied by loss or alteration of consciousness. There could be noticeable body rigidity (convulsion). The origin, extent, intensity, and type of the epileptic discharge in the brain all play a role in the seizure pattern, which is caused by an excessive amount of electrical discharge in the brain. Medication is typically used to manage epilepsy but not to cure it. About 50% of patients can effectively control their epileptic seizures using anticonvulsant medications already on the market; another 25% may improve; and the remaining patients do not see any appreciable benefits. In addition, problematic side effects of the medications used in clinical settings frequently make therapy challenging, creating a desire for novel anticonvulsants [1].

Anxiety is a sensation of unease, discomfort, apprehension, or worried concern that is accompanied by a variety of autonomic and somatic manifestations. The majority of people nearly one-eighth of the world's population experience anxiety. A significant family of drugs used to treat anxiety, benzodiazepines, have a small window of safety between their anxiolytic effects and undesirable side effects [2].

Clitoria ternatea Linn belongs to the family Fabaceae (Papilionaceae) and commonly known as Asian pigeonwings, blue pea, Aparajita. It is a pretty garden flower plant with twigs that can be found all over India, but it is most abundant in southern India. It can also be found in inhabited regions at low and medium altitudes. Different parts of this plant have been employed as active constituents in numerous Avurvedic medicines that are used to cure a variety of illnesses. Its anti-inflammatory, antipyretic, analgesic, larvicidal, insecticidal, antimicrobial, anti-ulcer, anxiolytic, antidepressant, hepatoprotective,

tranquillizing, and sedative properties have been scientifically studied [3]. Although *Clitoria ternatea* is well known for having intriguing qualities in traditional medicine, its anxiolytic and anticonvulsant effects have not been investigated. This study aims to provide experimental support for the traditional usage of *Clitoria ternatea's* ethanolic root extract in the treatment of anxiety and epilepsy.

2. MATERIALS AND METHODS

2.1 Plant Collection and Drying

Roots of *Clitoria ternatea* were distinguished, gathered, confirmed by botanist, Government degree school, Kukatpally, Medchal locale. *Clitoria ternatea* roots were cleaned and dried under conceal for around 14 days and powdered. The powdered material was stored.

2.2 Preparation of Ethanolic Root Extract of *Clitoria ternatea* (Soxhlet)

The powdered material of *Clitoria ternatea* root were dried and separated with ethanol by soxhlation strategy.

2.3 Preliminary Phytochemical Analysis of the Extract

The concentrate was exposed to fundamental phytochemical examinations to recognize different phytoconstituents present in the ethanolic concentrate of *Clitoria ternatea* root [4].

2.4 Acute Toxicity Testing

The intense poisonousness studies were completed utilizing OECD 425 rules. Present review was done in CPCSEA endorsed creature place of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India. (Reg. No. 1175/PO/ERe/S/08/CPCSEA).

2.5 Animal Housing

The creatures (rodents and mice) were housed in poly acrylic confines with not in excess of six

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animals for each enclosure; with 12 h light/12 h dim cycle. Creatures have free admittance to standard eating routine and drinking water not indispensable. The creatures were permitted to adapt the lab climate for seven days before the beginning of the examination. The consideration and support of the creatures were completed according to the endorsed rules of the advisory group with the end goal of control and oversight of tests on creatures (CPCSEA).

2.6 *In vivo* Methods for Evaluation of Anticonvulsant and Anxiolytic Activity

In vivo evaluation of Anticonvulsant activity of the ethanolic root extract of *Clitoria ternatea* was carried out using the following models.

2.6.1 *In-vivo* Methods for Evaluation Anticonvulsant Activity

2.6.1.1 Maximal Electro Shock Induced Convulsions (MES)

In this method 24 healthy Wistar Albino rats of either sex weighing 150-200 gm are selected and divided into 4 groups, containing 6 in each group. Group I (Control) is administered with saline (0.9% NaCl). Group II is administered with Ethanolic extract of *Clitoria ternatea* (EECT) 200 mg/kg, bd.wt., *p.o.* Group III is administered with EECT 400 mg/kg, bd.wt., *p.o.* Group IV is administered with standard drug Phenytoin 25 mg/kg, bd.wt, *i.p.* All these drugs are administered 30 minutes prior to induction of seizures to all the groups using electro convulsiometer. A 50Hz alternating current of 150mA for 0.2 seconds was delivered through the ear electrodes. The different phases of convulsions are noted down along with the duration of each phase for recording various parameters. Various phases of convulsions like tonic flexion, extension, clonus, stupor and mortality are observed. The decreased duration of tonic flexion and extension was considered as a protective measure against MES induced seizures [5].

2.6.1.2 Pentylenetetrazole Induced Convulsions

24 healthy Wistar Albino rats of either sex weighing 150-200 gm are selected for this study.

They are divided into 4 groups, containing 6 in each group. Group I (Disease control) is administered with saline (0.9% NaCl). Group II is administered with EECT 200 mg/kg, bd.wt., p.o. Group III is administered with EECT 400 mg/kg, bd.wt., p.o. Group IV is administered with standard drug Phenytoin 25 mg/kg, bd.wt, i.p. After 30 minutes, rats in all groups received Pentylenetetrazole (PTZ) at a dose of 85 mg/kg, bd.wt., *i.p.* and animals are observed for 1 hour. The parameters like latency of convulsion, duration of convulsion, and recovery/ death were observed as measures of anticonvulsive property. Decreased duration of convulsion indicates that the extract has an ability to abolish the effect of PTZ induced convulsions [6]. The animals after the treatment were subjected to histopathological studies.

 Table 1. Experimental study design for scheduled drug treatment in Maximal Electro Shock

 Induced Convulsions (MES)

Groups	Treatment
Group – I	Control (Normal saline) + Electric shock of 150mA for 0.2 sec.
Group – II	EECT 200 mg/kg, bd.wt., p.o + Electric shock of 150mA for 0.2 sec.
Group – III	EECT 400 mg/kg, bd.wt., p.o + Electric shock of 150 mA for 0.2 sec.
Group – IV	Standard drug Phenytoin 25 mg/kg, bd.wt., <i>i.p</i> + Electric shock of 150mA for 0.2 sec.

Table 2. Experimental study design for scheduled drug treatment in Pentylenetetrazole
Induced Convulsions

Groups	Treatment
Group –I	Disease control (Normal saline) + PTZ 85 mg/kg, bd.wt., <i>i.p</i> .
Group –II	EECT 200 mg/kg, bd.wt. <i>p.o</i> . + PTZ 85 mg/kg, bd.wt., <i>i.p.</i>
Group –III	EECT 400 mg/kg, bd.wt. <i>p.o.</i> + PTZ 85 mg/kg, bd.wt., <i>i.p</i>
Group –IV	Standard drug Phenytoin (25 mg/kg, bd.wt, <i>i.p)</i> + PTZ 85 mg/kg, bd.wt., <i>i.p</i> .

2.6.2 *In vivo* Methods for Evaluation of Anxiolytic Activity

2.6.2.1 Marble Burying Test (MBT)

In this method Swiss Albino mice of either sex weighing about 25-30 gm are selected and divided into 4 groups containing 6 in each group. Standard cages are filled with husk about 5 cm deep. Bedding material was lightly tamped town to create even and flat surface. In each cage, 10 glass marbles is placed on the bedding material by grid pattern, maintaining approximately 4 cm distance between the marble. Group I (control) is administered with saline (0.9% NaCl). Group II is administered with EECT 200 mg/kg, bd.wt., p.o. Group III is administered with EECT 400 mg/kg. bd.wt., p.o. Group IV is administered with standard drug Lorazepam 0.05 mg/kg, bd.wt., p.o. After 1 hour of oral drug administration, mice are placed in respected cages. Mice are allowed to explore the cages for 30 minutes. During the experimental period, experimental room is kept silent and undisturbed environment was maintained. After 30 minutes, mice were removed carefully from the cages and placed in their original cages. Numbers of marbles buried by the mice are measured. Marble was considered buried if 2/3 area of the marble is covered with the bedding material [7].

2.6.3 *In-vitro* Determination of hydroxyl radical scavenging activity

Hydroxyl radical is one of the potent reactive oxygen species in the biological system that reacts with poly unsaturated fatty acid moieties of the cell membrane phospholipids and causes damage to the cell.

2.6.3.1 Procedure

In hydroxyl radical scavenging assay, the reaction mixture was prepared by adding 100 mL of 2- deoxy- D ribose (28 mM in 20 mM KH_2PO_4 -KOH buffer, pH 7.4), 500 mL of EECT at different

concentrations (10, 20, 30, 40, 50 mg/mL), 200 mL EDTA (1.04 mM) and 200 mM FeCl₃, 100 mL of H_2O_2 (1 mM) and 100 mL ascorbic acid (1mM), and incubated at 37°C for 1 h. 1mL thiobarbituric acid (1%) and 1mL of trichloroacetic acid (2.8%) was added to resultant mixture and again incubated at 100°C for 20 min. After cooling, absorbance of resultant solution was measured at 532 nm, against a blank sample [8].

 $Hydroxyl radical scavenging activity (\%) = \frac{AAbs \ sample \ - \ Abs \ control}{Abs \ sample} x \ 100$

where Abs _{sample} = Absorbance of test sample Abs _{control} = Absorbance of control

2.7 Histopathology Studies

Histopathological studies are performed in a Pentylenetetrazole Induced Convulsion model. Brain tissue samples were fixed with neutral buffered formalin for 24 hours, to examine the anticonvulsant activity of Clitoria ternatea, tissue Brain were sections of the examined histopathological. Tissues were fixed with 10% buffered formalin and treated using a tissue processor. The treated tissue was embedded in a paraffin block and a rotary microtome was used to cut sections approximately 5 µm thick. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for morphological changes such as elevated inflammatory infiltration, necrosis, pyknosis, and congestion [9].

2.8 Statistical Analysis

All the values were expressed as arithmetic mean \pm SEM & were analysed by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. p< 0.05 was the criterion for statistical significance.

Table 3. Experimental study design for scheduled drug treatment in Marble Bury Test

Groups	Treatment
Group –I	Control (Normal saline)
Group –II	EECT 200 mg/kg, bd.wt., <i>p.o.</i>
Group –III	EECT 400 mg/kg, bd.wt., <i>p.o.</i>
Group –IV	Standard drug (Lorazepam 0.05 mg/kg, bd.wt., p.o)

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3. RESULTS

3.1 Preliminary Phytochemical Analysis

Clitoria ternatea's ethanolic root extract underwent a preliminary phytochemical screening that revealed the presence of alkaloids, flavonoids, proteins, phenols, triterpenoids, carbohydrates, and steroids.

3.2 Acute Toxicity Studies

Ethanolic root extract of *Clitoria ternatea* was tried on Swiss Albino mice up to a portion of 2000 mg/kg bd. wt. The creatures didn't show any indications of poisonousness or mortality up to 2000 mg/kg bd. wt. different morphological and social characters were seen during the review. Thus, the concentrate was viewed as protected up to 2000 mg/kg bd. wt.

3.3 Dose Selection

From poisonousness studies, a portion of 2000 mg/kg bd. wt. was distinguished to be protected, and the functioning portion was considered as 1/10th i.e., 200 mg/kg, bd. wt. In the current review pharmacological assessments were finished utilizing 200 mg/kg. bd. wt. furthermore 400 mg/kg, bd. wt.

3.4 Maximal Electro Shock Induced Convulsions (MES)

There was significant (p<0.05) decrease in the duration of tonic Flexion and tonic Extension after administration of EECT (200 & 400 mg/kg) compared to control group was shown in Table 4. Phenytoin at a dose of 25mg/kg protected the rats against Maximal Electroshock induced convulsion.

The values are expressed as mean \pm SEM (n=6) analysis was performed with one way ANOVA followed by Dunnett's multiple comparison test against Control (*p<0.0001, **p<0.01) and

Standard (A=p<0.0001, B=p<0.001). R = Recovery D = Death.

3.5 Pentylenetetrazole Induced Convulsions (PTZ)

Table 5 illustrates that *Clitoria ternatea* Linn's ethanolic root extract significantly (p<0.05) increased convulsion latency while reducing convulsion latency in the treated group (EECT 200 & 400 mg/kg) compared to control group. At a dose of 25 mg/kg, phenytoin demonstrated a considerable anticonvulsant efficacy.

The values are expressed as mean \pm SEM (n=6) analysis was performed with one way ANOVA followed by Dunnett's multiple comparison test against Disease control (*p<0.0001) and against standard (A=p<0.0001). R = Recovery D = Death.

- A. Chronic PTZ administration produces histological alterations in the brain's hippocampus, which in PTZ control rats exhibits increased inflammatory infiltration, necrosis, pyknosis, and congestion.
- B. When compared to PTZ control rats, the hippocampus area after administration of EECT 200 mg/kg exhibits considerable inflammatory infiltration, necrosis, pyknosis, and congestion.
- C. When compared to PTZ control rats, administration of EECT 400 mg/kg causes minor inflammatory infiltration, necrosis, pyknosis, and congestion in the hippocampus region.
- D. When compared to PTZ control rats, phenytoin (25 mg/kg) treatment significantly (p<0.05) prevented PTZinduced histological aberrations in the hippocampus region. This was shown bv а reduction in inflammatorv infiltration, necrosis pyknosis, and congestion [10].

Table 4. Effect of Ethanolic root extract of Clitoria ternatea on Maximal Electro Shock Induced Convulsions (MES)

Groups	Time in seconds of various phase of convulsion				
	Flexion	Extension	Clonus	Stupor	R/D
Control	10±0.51	10.53±0.40	11.93±0.46	169.6±0.88	R
EECT 200 mg/kg	7.9± 0.54 ^{**A}	7.43±0.34 ^{*A}	8.9±0.41 ^{*A}	136.5±0.88 ^{*A}	R
EECT 400 mg/kg	6.6±0.33 ^{*A}	5±0.25 ^{*A}	6.9±0.33 ^{*B}	80.3±0.98 ^{*A}	R
Phenytoin 25 mg/kg	2.4±0.28 [*]	2.3±0.23 [*]	4.5±0.31 [*]	49.3±0.88 [*]	R

Table 5	. Effect o	of Ethanolic	root extract o	f Clitoria	ternatea oi	n Pentylene	tetrazole l	Induced
			Conv	ulsions (F	PTZ)	-		

Groups	Latency of convulsion's (sec)	Duration of convulsions (sec)	R/D
Disease control	120.1±0.64	70.8±0.59	R
EECT 200mg/kg (bd., wt.)	170.1±0.70 ^{*A}	48.8±0.68 ^{*A}	R
EECT 400mg/kg (bd., wt.)	218.8±0.63 ^{*A}	29.85±0.72 ^{*A}	R
Phenytoin 25mg/kg	387.9±0.60 [*]	16.0±0.50 [*]	R



Fig. 1. Histopathology of rat's brain in Pentylenetetrazole Induced Convulsions

Table 6. Effect of Ethanolic root extract of Clitoria ternatea on Marble bury test

S. No	Groups	No of marbles buried Mean±SEM
1	Control	7±0.36
2	EECT(200mg/kg, bd.wt., <i>p.o</i>)	5.6±0.33 ^{**A}
3	EECT(400mg/kg, bd.wt., <i>p.o</i>)	4.1±0.30 ^{*ns}
4	Lorazepam (0.05mg/kg, bd.wt., p.o)	3.3±0.21 [*]



Fig. 2. Effect of EECT on hydroxyl radical scavenging assay

S. No	Compound	Concentration (µg/mL)	% Inhibition (Mean±SEM)	IC ₅₀ value (ug/ml.)
1		10	28.7±0.25	
		20	50.6±0.32	
		30	64.4±0.43	
	Ascorbic acid	40	72.6±0.58	
		50	74.8±0.75	
2		10	26.5±0.27	24.0
		20	47.2±0.38	
		30	62.3±0.54	
	EECT	40	70.2±0.63	
		50	74±0.81	

 Table 7. Effect of EECT on Hydroxyl radical Scavenging assay

3.6 Marble Burying Test (MBT)

In marble bury test the number of marbles buried were recorded to determine its anxiolytic effect. The Ethanolic root extract of *Clitoria ternatea* Linn significantly (p<0.05) decreased the no of marbles buried in the treated group (EECT 200 & 400mg/kg) compared to control group was shown in Table 6. Lorazepam showed a significant anxiolytic activity at a dose of 0.05mg/kg.

The values are expressed as mean \pm SEM (n=6) analysis was performed with one way ANOVA followed by Dunnett's multiple comparison test against Disease control (p<0.0001, p<0.05) and against standard (A=p<0.0001, ns=non-significant).

3.7 *In vitro* Hydroxyl Radical Scavenging Assay

The anti-oxidant activity of ethanolic root extract of *Clitoria ternatea* was carried out by hydroxyl radical scavenging assay. EECT has shown increase in percentage inhibition of hydroxyl radicals with increase in dose and its IC_{50} value was found to be 24 µg/ml which is represented in Table 7. The potential of the extract was comparable to that standard Ascorbic acid and IC_{50} value was found to be 19.76 µg/ml.

4. DISCUSSION

In the present research ethanolic root extract of *Clitoria ternatea* was studied for anticonvulsant activity by using MES induced convulsions and pentylenetetrazole induced convulsions and anxiolytic activity by experimental models namely marble bury test. Both MES and PTZ-induced seizures were reduced when *Clitoria ternatea*'s ethanolic root extract was used. The two assays

that are most frequently used to measure anticonvulsant activity are these two. EECT showed anticonvulsant effect by shortening the length of tonic flexion and tonic extension in MES-induced seizures. Using the MES test, substances that are active against generalised tonic-clonic seizures are identified. The maximal electroshock test is also said to forecast the effectiveness of anticonvulsants in preventing partial seizures [11].

According to the findings of the current study, Clitoria ternatea's ethanolic root extract has anticonvulsant properties in rats when used to prevent pentylenetetrazole-induced seizures, which may be brought on by blocking GABAergic pathways. In contrast to glutamic acid, which is an excitatory neurotransmitter in the brain, GABA is the main inhibitory neurotransmitter. It has been established that the underlying causes of epilepsy are the suppression of the GABA neurotransmitter and the increase of the action of glutamic acid. Therefore, it is conceivable that the anticonvulsant effects of EECT against seizures caused by PTZ in this study may be attributable to the stimulation of GABA neurotransmission [12]. The results show that the EECT protected significant number of animals against seizures induced by pentylenetetrazole and also increased the latency period but decreased duration of convulsion and in a dose dependent manner. histopathological In examination of the rat's brain tissue in Pentylenetetrazole Induced Convulsions model Ethanolic root extract of Clitoria ternatea at a dose of 200 and 400mg/kg shows decreased inflammatory infiltration, necrosis, pyknosis, and congestion in the hippocampal region when compared with PTZ control rat.

One of the most prevalent categories of psychiatric diseases is anxiety disorders. Each

day activities are affected by anxiety disorders. Common signs of anxiety disorders include difficulty sleeping, loss of appetite, inability to concentrate at work, sluggish coordination, and excessive concern or fear about anything. The immunological and cardiovascular systems of a person might be impacted by anxiety disorders. Treatment for anxiety disorders involves using drugs including benzodiazepines, azapirones, monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), and serotonin-norepinephrine inhibitors (SNRIs). Anxiolytic reuptake medication evaluation is done using the marble bury test. Since rodents exhibit neophobia (fear of unfamiliar or strange objects), they bury marbles out of worry or terror. Reduced marble burying by mice is a sign of anxiolytic action.

In the marble bury test, lorazepam 0.05 mg/kg (group 4), EECT 400 mg/kg (group 3), and EECT 200 mg/kg all significantly reduced the number of marbles bury (group 2). Mice given lorazepam 0.05 mg/kg (group 4) had the fewest marble buries, with a p<0.05 compared to the control group. The group with the most significant anxiolytic effect was as a consequence [13].

The pharmacological activity of anxiolytics is known to increase the amount of GABA in the cerebral hemisphere. The direct stimulation of GABA receptors, which are involved in anxiety, would have an anxiolytic effect. The major secondary metabolites in many plants that are thought to be responsible for their sedative and anxiolytic effects are known as alkaloids. Alkaloids and triterpenes. which are phytoconstituents in EECT, may be the cause of its anxiolytic effects. Due to the involvement of numerous CNS chemical mediators, there are numerous theories to explain how anxiolytic drugs work. The anxiolytic effect of EECT may result from interactions between the extract and neural substrates, chemicals such as noradrenaline, serotonin, GABA, BZD, hormones (testosterones), or natural endogenous mediators of the body that are thought to be responsible for aggressive and anxiety-like conditions.

According to certain reports, triterpenes and steroids have anticonvulsant properties in some experimental seizure models, including MES and PTZ [14].

The pathophysiology of various disease, include atherosclerosis, diabetes, cancer, arthritis, and ageing, is related to reactive oxygen species Reddy V et al.; INDJ, 18(1): 1-10, 2022; Article no.INDJ.90785

produced either endogenously (ROS) or exogenously. Normally, a cell is shielded from the damaging effects of ROS by cellular antioxidant enzymes and radical free scavengers. However, oxidative stress, which can be extremely harmful, is caused when the production of ROS overwhelms the antioxidant defense and leads to oxidative destruction of cellular macromolecules. Inducing oxidations that harm membranes, such as membrane lipid peroxidation and a decrease in membrane fluidity, ROS can damage the lipids in cell membranes. Free radicals easily combine and damage biomolecules including proteins, lipids, and carbohydrates, rendering them inert and causing harm to cells, tissues, and organs in the process. Additionally, they have deleterious effects that affect intracellular oxidation-reduction states and inactivate enzymes [15]. However, the biologically most significant target of oxidative attack is DNA, and it is commonly believed that ongoing oxidative damage to DNA plays a key role in the aging-related development of serious malignancies. Free radicals like superoxide and hydroxyl radicals are known to play a role in the development of cancer.

The hydroxyl radical is the most reactive ROS and targets practically all body molecules. It starts the peroxidation of lipids in cell membranes, which produces malondialdehyde, a mutagenic and carcinogenic compound. Cell damage results from its reaction with the polyunsaturated fatty acid moiety of cell membrane phospholipids. The ascorbic acidiron-EDTA model of the •OH producing system was employed in this investigation. Ascorbic acid, iron, and EDTA work together in this entirely aqueous system to produce the hydroxyl radical. The ethanolic root extract of Clitoria dose-dependent ternatea demonstrated а reduction of oxidation in this investigation as well. Therefore, it can also be assumed that *Clitoria* ternatea's ethanolic root extract offers scavenging ability against oxidizing agents like hydroxyl ions. In this context, Clitoria ternatea can prove to be effective as a potent antioxidant agent since it is found to exhibit considerable in vitro antioxidant activity [16].

5. CONCLUSION

According to the results of the current investigation, *Clitoria ternatea's* ethanolic root extract has significant anticonvulsant and anxiolytic efficacy. For the isolation, identification, and confirmation of the exact mechanism, further research is required.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The Institutional Animal Ethics Committee of GRCP approved the research entitled "Effects of Ethanolic Extract of *Clitoria ternate* against experimentally induced convulsions and anxiety in rodents" with Regd number. 1175/PO/Re/S/08/CPCSEA. All animal experiments were carried out in accordance with CPCSEA guidelines.

ACKNOWLEDGEMENT

The authors are grateful to the management of the Gokaraju Rangaraju College of pharmacy, for the constant support and encouragement during the course of the work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- 1. Twinomujuni SS, Oloro J, Alele PE. Anticonvulsant and anxiolytic activity of the leaf aqueous and ethanolic extracts of *Melanthera scandens* in a rat model. African Journal of Pharmacy and Pharmacology. 2016;10(12): 216-222.
- Geethu K, Vishnupriya K, Thamodaran G, Suresh V, Senthil K. Evaluation of anticonvulsant and anxiolytic activity of methanolic extracts of leaves of *Syzygium aqueum* (Brum. f.). European Journal of Pharmaceutical and Medical Research. 2017;4(12):224-230.
- Singh NK, Gupta JK, Shah K, Mishra P, Tripathi A, Chauhan NS, Upmanyu N. A Review on *Clitoria ternatea* (Linn.): Chemistry and Pharmacology. 2017; 1-17.
- Mostafavi H, Pezhhanfar S. Qualitative Phytochemical Analysis of Ajwain (*Trachyspermum Ammi*) From North-West Iran. International Research Journal of Pharmacy. 2015;6(9):610-615.
- 5. Ganga Μ, Raju Fernandes J, Vijayanarayana K. Evaluation of antiepileptic activity of the alcoholic extract Adhatoda vasica leaves of in rats. Research Journal of Pharmaceutical.

Biological and Chemical Sciences. 2011;2(3):5-10.

- Reddy VNVLS, Raju GM, Sirisha S, Nikitha K. Neuropsychological Screening on Acalypha indica Whole Plant Extract. Journal of Young Pharmacists. 2020;12(2) Suppl:s82-s6.
- Jagtap PN, Mhetre OS. Study of *Passiflora* incarnata Alone and with Asparagus Racemosus for Anxiolytic Activity in Mice. Journal of Medicinal Plants Studies. 2021;9(4):147-156.
- Sneha Nawale, Priyanka N, Sujit Das, Ganga Raju M. Data of in vivo screening of antiulcer activity for methanolic extract of *Vernonia elaeagnifolia* DC. Data in brief, 2019;23(103753):1-13.
- 9. Raju MG, Manisha R, Reddy VNVLS, Niharika M. Anti-ulcer Activity of Ethanolic Extract of *Alstonia scholaris* Bark: An *in vivo* and *in silico* Approach. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2022;11(1): 14-32.
- Tao Z, Chun-Yan H, Hua P, Bin-Bin Y, Xiaoping T. Phyllathin From *Phyllanthus Amarus* Ameliorates Epileptic Convulsion and Kindling Associated Post-Ictal Depression in Mice via Inhibition of NFkB/TLR-4 Pathway. Dose Response. 2020;18(3):1559325820946914.
- 11. Sudhir G. Ramani and Rajiv Bajracharya. Antiepileptic and Anxiolytic Activity of ethanolic extract of *Brassica nigra* L Koch seeds on Wistar Albino rats. European Journal of Pharmaceutical and Medical Research. 2016;3(4):394-402.
- 12. Swarnamoni Das, Phulen Sarma. A Study on the Anticonvulsant and Antianxiety activity of Ethanolic Extract of *Punica granatum* Linn. International Journal of Pharmacy ad Pharmaceutical Sciences. 2014;6(2):38.
- Njunge K, Handley SL. Evaluation of marble-burying behavior as a model of anxiety. Pharmacology Biochemistry and Behaviour. 1991;38(1):63-67. DOI:10.1016/0091-3057(91)90590-x
- Barua CC, Begum SA, Barua AG, Borah 14. RS. Lahkar Μ. Anxiolytic and anticonvulsant activity methanol of Alternanthera extract of leaves of brasiliana (L.) Kuntze (Amaranthaceae) in laboratory animals. Indian Journal of Experimental Biology. 2013;51:450-457.

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- 15. Suvarchala Reddy NVL, Anarthe SJ, Raghavendra NM. Evaluation of *in-vitro* antioxidant activity of methanol extracts of *Asystasia gangetica* (L).T (Chinese violet). Natural Products: An Indian Journal. 2009;5(2):78-80.
- Jacob L, Latha MS. In vitro antioxidant activity of Clitoria ternatea Linn. International Journal of Research Phytochemical and Pharmacological Sciences. 2013;3(1):35-39.

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Peer-review history: The peer review history for this paper can be accessed here: https://www.sdiarticle5.com/review-history/90785