

Original Research Article

EFFECTS OF ETHANOLIC ROOT EXTRACT OF *CLITORIA* *TERNATA* AGAINST EXPERIMENTALLY INDUCED CONVULSIONS AND ANXIETY IN RODENTS

ABSTRACT

Medicinal plants used in traditional medicine for the treatment of epilepsy have been scientifically shown to possess promising anticonvulsant activities in animal models for screening for anticonvulsant activity and can be a source of newer anticonvulsants. The aim of this study was to investigate the preliminary phytochemical properties, anticonvulsant and anxiolytic activities of ethanolic root extract of *Clitoria ternatea* Linn. Anticonvulsant activity was evaluated against Maximum electroshock (MES) induced convulsion and pentylenetetrazole (PTZ)-induced convulsion model in rats. The effect of the extract at oral dose levels of 200 and 400 mg/kg was evaluated in an experimental rat model, using Phenytoin (25 mg/kg) as positive control. Anxiolytic activity was performed in mice using marble bury test and Lorazepam was used as 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. Triterpenes and steroids are reported to possess anticonvulsant activity in some experimental seizure models such as MES and PTZ. **EECT** contains alkaloids and triterpenes as phytoconstituents, which might be responsible for its anxiolytic action. The results obtained in this study suggest that the ethanolic root extract of *Clitoria ternatea* possess anticonvulsant and anxiolytic activities in rodents.

Keywords: *Clitoria ternatea* Linn, Pentylenetetrazole, Marble bury test, anticonvulsant, anxiolytic

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1. INTRODUCTION

Epilepsy is one of the most common and widespread neurological disorders in the human population. In modern medicine, epilepsy is considered to be a chronic brain syndrome of various aetiology characterized by recurrent seizures and usually associated with loss or disturbance of consciousness. There may be a characteristic body contraction (convulsion). The seizure is due to excessive electrical discharge in the brain and the seizure pattern depends not only on the cause but the origin, extent, intensity and type of epileptic discharge in the brain. Epilepsy is usually controlled, but not cured, with medication. Currently available anticonvulsant drugs are able to efficiently control epileptic seizures in about 50% of the patients; another 25% may show improvement whereas the remainder does not benefit significantly. Furthermore, undesirable side effects of the drugs used clinically often render treatment difficult so that a demand for new types of anticonvulsants exists[1].

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Anxiety is a sensation of unease, discomfort, apprehension, or worried concern that is accompanied by a variety of autonomic and somatic manifestations. Anxiety is a common, emotional, rational, and predictable reaction to real or perceived threat. Anxiety affects most of the population nearly one-eighth of the total population worldwide. Benzodiazepines, being a major class of compounds used for treatment of anxiety present a narrow margin of safety between the anxiolytic effect and unwanted side effects[2].

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Clitoria ternatea Linn belongs to the family *Fabaceae* (*Papilionaceae*) and commonly known as Asian pigeonwings, blue pea, Aparajita. It is a good looking twig herb and very common garden flower plant found all over India especially in southern India. It can also be found in inhabited regions at low and medium altitudes. In various Ayurvedic preparations different parts of this plant have been used as an active ingredient which is used for treatment of several disorders. It is scientifically evaluated for anti-inflammatory, antipyretic, analgesic, larvicidal, insecticidal, antimicrobial, anti-ulcer, anxiolytic, antidepressant, hepatoprotective, tranquilizing and sedative property[3].

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Despite the fact that *Clitoria ternatea* is well known to possess interesting properties in traditional medicine it has not been studied for its anxiolytic and anticonvulsant activities. This study was aimed at providing experimental support for the traditional medicinal use of the ethanolic root extract of *Clitoria ternatea* in the management of epilepsy as well as anxiety.

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2. MATERIALS AND METHODS

2.1 Plant Collection and Drying

Roots of *Clitoria ternatea* were distinguished, gathered, confirmed by a 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 creatures 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 convulsimeter. 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

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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].

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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 200mg/kg, bd.wt., p.o + Electric shock of 150mA for 0.2 sec.
Group – III	EECT 400mg/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.p + Electric shock of 150mA for 0.2 sec.

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.

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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.p.
Group –II	EECT 200mg/kg, bd.wt. p.o.+ PTZ 85 mg/kg, bd.wt., i.p.

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 Bury 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 down 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 replaced 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].

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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., <i>p.o</i>)

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.

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 KH₂PO₄-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 H₂O₂ (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].

$$\text{Hydroxyl radical scavenging activity (\%)} = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}}{\text{Abs}_{\text{sample}}} \times 100$$

where Abs_{sample}= Absorbance of test sample

Abs_{control}= Absorbance of control

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2.7 Histopathology Studies

Histopathological studies are performed in a Pentylene-tetrazole Induced Convulsion model. Brain tissue samples were fixed with neutral buffered formalin for 24 hours, to examine the anticonvulsant activity of *Clitoriaternatea*, tissue sections of the Brain were 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].

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2.8. Statistical analysis

All the values were expressed as arithmetic mean ± 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.

3. RESULTS

3.1 Preliminary Phytochemical Analysis

The preliminary phytochemical investigation for ethanolic root extract of *Clitoriaternatea* showed presence of alkaloids, flavonoids, proteins, phenols, triterpenoids, carbohydrates and steroids.

3.2 Acute Toxicity Studies

Ethanolic root extract of *Clitoriaternatea* 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 as shown in table 4. Phenytoin at a dose of 25mg/kg protected the rats against Maximal Electroshock induced convulsion.

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	Recovery/Death
Control	10±0.51	10.53±0.40	11.93±0.46	169.6±0.88	Recovery
EECT 200mg/kg	7.9± 0.54 ^{**A}	7.43±0.34 ^{*A}	8.9±0.41 ^{*A}	136.5±0.88 ^{*A}	Recovery
EECT 400mg/kg	6.6±0.33 ^{*A}	5±0.25 ^{*A}	6.9±0.33 ^{*B}	80.3±0.98 ^{*A}	Recovery
Phenytoin 25mg/kg	2.4±0.28 [*]	2.3±0.23 [*]	4.5±0.31 [*]	49.3±0.88 [*]	Recovery

The values are expressed as mean±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= $p < 0.001$).

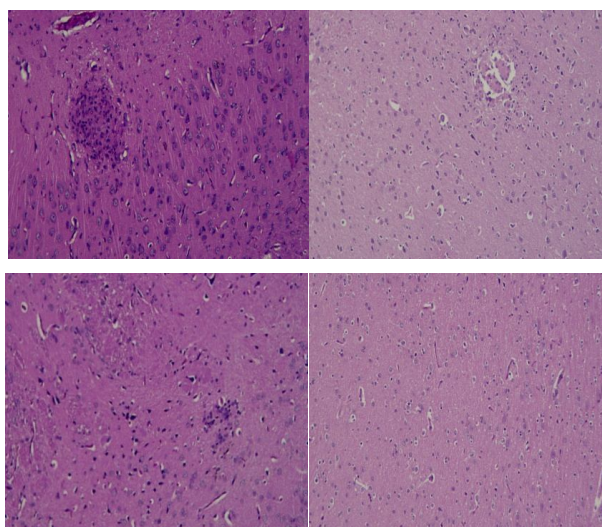
3.5 Pentylene tetrazole Induced Convulsions (PTZ)

The Ethanolic root extract of *Clitoria ternatea* Linn significantly ($p < 0.05$) increased the latency of convulsion's but decreased the duration of convulsion in the treated group (EECT 200 & 400mg/kg) compared to control group as depicted in table 5. Phenytoin showed a significant anticonvulsant activity at a dose of 25mg/kg.

Table 5: Effect of Ethanolicroot extract of *Clitoriaternateon* Pentylenetetrazole Induced Convulsions (PTZ)

Groups	Latency of convulsion's (sec)	Duration of convulsions (sec)	Recovery/Death
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

The values are expressed as mean ± 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).



= pyknosis= inflammatory cells

Figure 1: Histopathology of rat's brain in Pentylenetetrazole Induced Convulsions

- A. Chronic administration of PTZ induces histological aberration in the brain hippocampus shows elevated inflammatory infiltration, necrosis, pyknosis, and congestion in PTZ control rat.
- B. Administration of EECT 200mg/kg shows moderate inflammatory infiltration, necrosis, pyknosis, and congestion in the hippocampal region when compared with PTZ control rat.

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- C. Administration of EECT 400mg/kg shows mild inflammatory infiltration, necrosis, pyknosis, and congestion in the hippocampal region when compared with PTZ control rat.
- D. Phenytoin (25mg/kg) treatment significantly ($p < 0.05$) inhibited PTZ-induced histological aberrations in the hippocampal region, evident by decreased inflammatory infiltration, necrosis pyknosis and congestion as compared to PTZ control rat [10].

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The histological results should be presented before micrographs (A, B, C and D).

3.6 Marble Bury 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.

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., p.o)	5.6±0.33 ^{**A}
3	EECT(400mg/kg, bd.wt., p.o)	4.1±0.30 ^{*ns}
4	Lorazepam (0.05mg/kg, bd.wt., p.o)	3.3±0.21 [*]

The values are expressed as mean ± 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:

Table 7: Effect of EECT on Hydroxyl radical Scavenging assay

S.No	Compound	Concentration (µg/mL)	% Inhibition (Mean±SEM)	IC ₅₀ value (µg/mL)
1	Ascorbic acid	10	28.7±0.25	19.76
		20	50.6±0.32	
		30	64.4±0.43	
		40	72.6±0.58	
		50	74.8±0.75	

2	EECT	10	26.5±0.27	24.0
		20	47.2±0.38	
		30	62.3±0.54	
		40	70.2±0.63	
		50	74±0.81	

Figure 2: Effect of EECT on *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 IC₅₀ 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 IC₅₀ value was found to be 19.76 µg/ml.

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4. DISCUSSION

In present study the 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. Ethanolic root extract of *Clitoria ternatea* inhibited both MES and PTZ induced seizures. These two tests are most widely used for testing of anticonvulsant activity. EECT attenuated the duration of tonic flexion and tonic extension in MES induced seizures indicating anticonvulsant activity. The MES test identifies agents with activity against generalized tonic clonic seizures. It is also proposed that maximal electroshock test predicts anticonvulsant effects against partial seizure [11].

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The results of the present study indicate that ethanolic root extract of *Clitoria ternatea* possesses anticonvulsant activity in rats in pentylenetetrazole induced seizures which may elicit seizures by inhibiting GABAergic mechanisms. GABA is the major inhibitory

neurotransmitter, while glutamic acid is an excitatory neurotransmitter in the brain. The inhibition of GABA neurotransmitter and the enhancement of the action of glutamic acid have been shown to be the underlying factors in epilepsy. It is therefore, possible that the anticonvulsant effects shown in this study by EECT against seizures produced by PTZ may be due to the activation 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. In histopathological 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.

Anxiety disorders are known to be one of the most common types of psychiatric disorders. Anxiety disorders affect person's daily activities. Person suffering from anxiety disorder experiences common symptoms like disturbance in sleep, loss of appetite, unable focus on work, poor coordination, excess worry or fear about anything. Anxiety disorders can also affect person's immune and cardiovascular system. Medicines like benzodiazepines, Azapirones, monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs) and serotonin- norepinephrine reuptake inhibitors (SNRIs) are used for the treatment of anxiety disorders. Marble bury test is used for evaluation anxiolytic drugs. As neophobia (fear of strange or novel objects) is seen in rodents, they bury marbles due to anxiety or fear responses. Decrease in the number of marble buried by mice indicates anxiolytic activity.

In marble bury test, Significant decrease in number of marble bury was observed with the treatment of Lorazepam 0.05 mg/kg (group 4), EECT 400 mg/kg (group 3), EECT 200 mg/kg (group 2). Lowest number of marble bury was observed by mice treated with Lorazepam 0.05mg/kg (group 4), $P \leq 0.05$ when compared with control. Hence highest significant anxiolytic activity was observed with this group [13].

Anxiolytics are known to exert pharmacological action by causing an increase in GABA content in the cerebral hemisphere. GABA receptors are involved in anxiety and their direct activation would have an anxiolytic effect. Alkaloids are the most important secondary metabolites in many plants that are held responsible for their sedative and anxiolytic actions. EECT contains alkaloids and triterpenes as phytoconstituents, which might be responsible for its anxiolytic action. There are various ways of explaining the mechanisms of action of anxiolytic agents because of involvement of many CNS chemical mediators. The anxiolytic

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effect of EECT may be due to interaction of the extract with the neural substrates or chemical mediators like nor-adrenaline, serotonin, GABA, BZD, hormones (testosterones) and magnesium or natural endogenous mediators of the body which are implicated to be responsible for aggressive and anxiety-like condition.

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Triterpenes and steroids are reported to possess anticonvulsant activity in some experimental seizure models such as MES and PTZ [14].

Reactive Oxygen species (ROS) generated endogenously or exogenously are associated with the pathogenesis of various diseases such as atherosclerosis, diabetes, cancer, arthritis and ageing process. Normally the cellular antioxidant enzymes and free radical scavengers protect a cell from toxic effects of ROS. However, oxidative damage of cellular macromolecules occurs when generation of ROS overtakes the antioxidant defense and results in oxidative stress, which may be very damaging. ROS can attack lipids in cell membranes inducing oxidations that cause membrane damage such as membrane lipid peroxidation and a decrease in membrane fluidity. Free radicals readily combine and oxidize biomolecules such as carbohydrates, proteins and lipids making them inactive with subsequent damage to cells, tissues and organs. They also exert toxic effects including inactivation of enzymes and alteration of intracellular oxidation-reduction states [15]. But DNA is probably the most biologically significant target of oxidative attack, and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age related development of major cancers. The involvement of free radicals like superoxide and hydroxyl radicals in carcinogenesis is well established.

The most reactive ROS that attacks almost every molecule in the body is the hydroxyl radical. It initiates the peroxidation of cell membrane lipids yielding malondialdehyde, which is mutagenic and carcinogenic. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. The model used in this study was ascorbic acid-iron-EDTA model of $\bullet\text{OH}$ generating system. This is a totally aqueous system in which ascorbic acid, iron and EDTA conspire with each other to generate the hydroxyl radical. In this study also, the ethanolic root extract of *Clitoria ternatea* exhibited a dose dependent inhibition of oxidation. Hence it may also be inferred that the ethanolic root extract of *Clitoria ternatea* possess scavenging activity 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

The present study concludes that the Ethanolic root extract of *Clitoria ternatea* possess significant anticonvulsant and anxiolytic activity. Further study is required for isolation & identification of active constituents and to confirm exact mechanism.

ETHICAL APPROVAL

The Institutional Animal Ethics Committee of GRCP approved the research entitled “Effects of Ethanolic Extract of *Clitoria ternatea* 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.

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