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Hedychium spicatum Improves Cognitive Function and Reduces Anxiety in PTZ-Kindled Rats through Non-Cholinergic Mechanisms

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Abstract

Aim: *Hedychium spicatum* (HS) is known to possess antioxidant and neuroprotective properties. The present study aimed to evaluate the protective effects of HS against pentylenetetrazole (PTZ) induced cognitive deficits in rats. **Materials and Methods:** Male Wistar rats (n = 48) were randomly divided into six groups (n = 8): normal control (saline), PTZ control (35 mg/kg, i.p.), PTZ + vehicle (1% CMC, p.o.), PTZ + escitalopram (5 mg/kg, p.o.), PTZ + HS (200 mg/kg, p.o.), and PTZ + HS (400 mg/kg, p.o.). Kindling was induced by administering PTZ (35 mg/kg, i.p.) every 48 h for 20 days. Treatment with HS and escitalopram was initiated on day 6 and continued for 15 days. Behavioural evaluations included the T-maze and novel object recognition tests, the elevated plus maze, and the open field test for exploratory and locomotor activity. After behavioural assessments, brain tissues were collected for biochemical estimation of acetylcholinesterase activity. **Results:** Rats treated with HS showed significant improvements in memory performance in the T-maze and novel object recognition tests, with the higher dose (400 mg/kg) producing effects comparable to those of escitalopram. HS also significantly reduced anxiety-like behaviour in the elevated plus maze. Exploratory and locomotor activities in the open field test remained largely unaffected, indicating the absence of nonspecific stimulatory or sedative effects. However, acetylcholinesterase activity in brain tissue did not change significantly following HS treatment. **Conclusion:** HS attenuated PTZ-induced cognitive deficits and anxiety-like behaviour in rats, suggesting that its beneficial effects may involve mechanisms other than cholinergic modulation.

Major Findings: *Hedychium spicatum* markedly attenuated PTZ-induced cognitive deficits and anxiety-like behaviour in rats, particularly at the 400 mg/kg dose, which demonstrated efficacy comparable to escitalopram. The absence of significant changes in brain acetylcholinesterase activity suggests that the beneficial effects of HS may be mediated through mechanisms other than cholinergic modulation.

Keywords: Anxiety, Cognitive Impairment, *Hedychium spicatum*, Memory Enhancement, PTZ Kindling

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1. Introduction

Epilepsy is a chronic neurological disorder characterised by recurrent and unprovoked seizures resulting from abnormal electrical activity in the brain. It affects approximately 65 million people worldwide and represents one of the most prevalent neurological disorders globally[1]. In addition to seizures, epilepsy is frequently associated with a variety of neuropsychiatric comorbidities, including cognitive impairment, anxiety, and depression[2]. Recent epidemiological studies indicate that individuals with epilepsy have significantly higher rates of psychiatric disorders compared with the general population, highlighting the substantial burden of mental health complications in this condition[3]. These cognitive and emotional disturbances significantly reduce patients' quality of life and complicate clinical management[4]. Experimental and clinical studies have demonstrated that

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recurrent seizures can induce neuronal damage, synaptic dysfunction, and alterations in neurotransmitter systems, particularly in brain regions involved in cognition and emotional regulation, such as the hippocampus, amygdala, and prefrontal cortex[5]. Such alterations may ultimately contribute to deficits in learning, memory, and behavioural regulation.

Among the experimental models used to investigate epilepsy and its associated neurobehavioural consequences, the pentylenetetrazole (PTZ)-induced kindling model is one of the most widely used. PTZ is a chemoconvulsant that induces seizures primarily by antagonising gamma-aminobutyric acid (GABA_A) receptors, thereby causing neuronal hyperexcitability[6]. Repeated administration of subconvulsive doses of PTZ leads to the development of kindling; a phenomenon characterised by progressive intensification of seizure activity and increased seizure susceptibility[7]. In addition to epileptic seizures, PTZ kindling has been reported to produce cognitive deficits, anxiety-like behaviour, and depressive-like symptoms in rodents, making it a valuable experimental model for studying epilepsy-related behavioural dysfunction[8]. These behavioural abnormalities are largely attributed to mechanisms such as oxidative stress, neuroinflammation, mitochondrial dysfunction, and neurotransmitter imbalance, which is known to contribute to neuronal damage during recurrent seizures[9]. Consequently, the PTZ-kindling model has been extensively used for evaluating the anticonvulsant and neuroprotective effects of pharmacological agents and natural compounds.

In recent years, increasing attention has been directed toward the therapeutic potential of medicinal plants in neurological disorders. Plant-derived compounds often possess diverse pharmacological properties, including antioxidant, anti-inflammatory, and neuroprotective activities, which may help counteract the complex pathophysiological mechanisms underlying epilepsy-related brain dysfunction[10]. Natural products rich in polyphenols, flavonoids, and terpenoids have been reported to attenuate oxidative stress and neuroinflammation, thereby protecting neuronal cells from seizure-induced damage[11]. Among such medicinal plants, *Hedychium spicatum* (HS), commonly known as spiked ginger lily, has gained attention due to its broad spectrum of biological activities. Traditionally used in Ayurvedic and folk medicine, *Hedychium spicatum* has been reported to exhibit antioxidant, anti-inflammatory, antimicrobial, and neuroprotective properties[12]. Phytochemical investigations have identified several bioactive constituents in this plant, including essential oils, monoterpenoids, sesquiterpenoids, flavonoids, and phenolic compounds, which are believed to contribute to its pharmacological effects[13,14].

Previous studies have demonstrated that plant extracts with strong antioxidant activity can protect neuronal cells against oxidative damage and improve cognitive function in various neurological disorders[15,16]. Since oxidative stress and neuronal degeneration are key factors involved in seizure-induced cognitive impairment, natural compounds possessing antioxidant and neuroprotective properties may provide therapeutic benefits in epilepsy-associated behavioural deficits. However, despite the traditional medicinal use and promising pharmacological profile of *Hedychium spicatum*, limited experimental evidence is available regarding its potential role in improving cognitive and emotional disturbances associated with epileptic conditions.

Therefore, the present study was designed to investigate the protective effects of *Hedychium spicatum* against cognitive impairment, anxiety-like behaviour, and depressive-like symptoms induced by PTZ kindling in rats. Behavioural assessments were performed using established experimental paradigms, including the T-maze and novel object recognition tests for evaluating memory function, the elevated plus maze for assessing anxiety-related behaviour, and the open field test for measuring locomotor and exploratory activity. In addition, biochemical analysis of acetylcholinesterase activity in brain tissue was carried out to explore potential mechanisms underlying the observed behavioural effects. Through this integrated approach, the study aimed to provide experimental evidence supporting the neuroprotective potential of *Hedychium spicatum* in seizure-induced cognitive and behavioural dysfunction.

2. Materials and Methods

2.1 Animals

Male Wistar rats (180-200 g; 4-6 weeks old) were obtained from *in vivo* Biosciences (CCSEA No. 1165/PO/RcBiBt-S/NRc-L/08/CPCSEA). Animals were housed under controlled laboratory conditions with a 12-h light/dark cycle, temperature maintained at 24 ± 2°C, and relative humidity of 40–60%, with free access to standard food and water except during specific experimental food-restriction

schedules. Upon arrival, the animals were quarantined and allowed to acclimatise to the laboratory conditions for seven days before the initiation of the experiment, during which they were maintained under standard housing conditions with free access to food and water. Allbehavioural assessments were performed during the light phase (10:00–18:00hrs (24 HR time cycle). Animal care followed the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), India, and the study protocol was approved by the Institutional Animal Ethics Committee of KLE College of Pharmacy, Bengaluru (IAEC No. 03/BVR/2021).

2.2 Drugs

Pentylenetetrazole (PTZ) was procured from Yarrow ChemPvt. Ltd, Mumbai, India. Escitalopram oxalate, a standard drug, was received as a gift sample from Micro Labs Pvt. Ltd, Bengaluru, India. Escitalopram was used as the standard drug because it is a selective serotonin reuptake inhibitor (SSRI) widely reported to exhibit antidepressant and anxiolytic effects and has been shown to improve cognitive and behavioural deficits associated with neurological disorders. Therefore, it was used as a reference compound to compare the neuroprotective and behavioural effects of *Hedychium spicatum* in PTZ-kindled rats. The test drugs, *Hedychium spicatum* rhizomes, were collected from Mao, Manipur, India, and the ethanolic extract was obtained from Green Chem. The authentication of the rhizomes was done by Dr N. Dhatchanamoorthy, Assistant Professor, Plant Systematics and Nomenclature, Foundation for Revitalisation of Local Health Traditions, Bengaluru 560064.

2.3 Experimental Groups

A total of 48 animals were used in the study. Naive Wistar rats were assigned to six groups, each consisting of a sample size of n=8 (Table 1).

Total number of animals used in the study = 48.

All standard and HS treatments were for consecutive 15 days (from 6-20), after which animals were subjected to behavioural tests to assess spatial navigation and decision making, anxiety-like behaviour, exploratory and locomotor activity, and the Recognition and Discrimination index.

2.4 PTZ-induced Kindling Procedure

PTZ-induced kindling, a well-established animal model[17]. PTZ (35 mg/kg, i.p.) was administered on alternate days (every 48 h) for a period of 20 days to induce kindling in rats. The intensity of convulsions was recorded on a six-stage modified Racine scale[17,18].

2.5 Modified Racine Scale

- Scale 0: No response
- Scale 1: Facial and ear twitching
- Scale 2: Myoclonic jerks without rearing
- Scale 3: Rearing, myoclonic jerks
- Scale 4: Clonic-tonic seizures, turning over into side position
- Scale 5: Generalised clonic-tonic seizures, turning over into the back position
- Scale 6: Death

The Modified Racine scale was used to grade the animal's seizure severity after 30 minutes of observation following intraperitoneal administration.

Seizure intensity score

Group II–VI's convulsion intensity was graded by a skilled observer. Animals given PTZ exhibited behaviour based on the six-unit Racine scale.

2.6 Treatment

The pharmacological treatment paradigm began on the sixth day of the kindling induction and continued for 15 days. The vehicle and normal control groups received intraperitoneal injections of saline and 1% CMC, respectively.

Table 1. Experimental design

Group	Treatment	Dose	Route	Number of Animals (n)
Group I	Normal Control	Saline	i.p.	8
Group II	PTZ Control	PTZ (35 mg/kg)	i.p.	8
Group III	PTZ + Vehicle	1% CMC	p.o.	8
Group IV	PTZ + Standard	Escitalopram (5 mg/kg)	p.o.	8
Group V	PTZ + HS (Low dose)	*Hedychium spicatum* (200 mg/kg)	p.o.	8
Group VI	PTZ + HS (High dose)	*Hedychium spicatum* (400 mg/kg)	p.o.	8

2.6.1 Escitalopram Oxalate

For 15 days, escitalopram, a selective serotonin reuptake inhibitor (SSRI), was given orally at a dose of 5 mg/kg in freshly made saline[19].

2.6.2 Ethanolic Extract of Hedychium spicatum

Two distinct doses of 200 and 400 mg/kg of the ethanolic extract were diluted in 1% CMC and given orally to the corresponding groups[13].

After completion of the kindling and treatment protocols, the animals were subjected to various behavioural assessments, following which they were sacrificed, and specific brain regions were collected for subsequent biochemical analyses.

2.7 Evaluation of Behavioural Parameters

The animals were subjected to the T-Maze alteration task, Elevated PlusMaze (EPM), Open Field Test (OFT) and Novel Object Recognition Test (NORT).

2.8 Test for Spatial Navigation and Decision Making

T-Maze, a 'T' shaped apparatus having three arms, was used to assess the spatial working memory in rodents by a spontaneous reward alteration task[20-24]. The apparatus comprises a start arm, two goal arms, and a manually operated door positioned 10 cm from the start to form a small holding area. Each goal arm contains a circular well at the end for the placement of food pellets. The experimental procedure involves three stages: habituation, acquisition, and retention.

2.8.1 Habituation

After a partial fast, the animals were free to roam the maze and eat the pellets that were positioned in the goal arms. To ensure adequate acclimatisation, this phase lasted for two days.

2.8.2 Acquisition

During the acquisition phase, animals completed six trials per day for six days. Food was alternatively put in the goal arms, requiring the animals to make different choices to collect the prize. The number of accurate entries, defined as those into the baited arm, was tallied for analysis. To eliminate olfactory cues, the maze was cleaned with 70% ethanol after every two trials, with a 30-second gap between trials.

2.8.3 Retention Test

Retention test has been conducted 48h after the last training day. Six test trials were conducted to examine retention memory, with both arms kept open and one arm containing a reward (food pellet). The rats were permitted to walk about and had to make the correct decision. The bait was changed spontaneously after each correct entry. The time taken and the correct/incorrect choice made were recorded during the memory retention testing.

2.9 Test for Anxiety-like Behaviour

One of the most widely used tests for assessing anxiety behaviours in rodents is the Elevated plus maze (EPM). The test was conducted in a cross-shaped maze that was 50 cm above the floor and had two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm × 40 cm). A mounted camera was used to capture each rat's movements for five minutes while it was positioned in the centre, facing an open arm, for offline analysis. After the test, the rats were returned to their cages. The time spent in both open and closed arms, the number of entrances into each arm, the number of head dips in the open arms, and the number of rearing in the closed arms were among the factors that were evaluated[20-24].

2.10 Test for Exploratory, Locomotor and Anxiety-like Behaviour

The open field was used to analyse anxiolytic-like behaviour. The Open Field Test (OFT) was performed in a square timber arena with black-painted floors and inner walls. Rats were placed at the corner of the arena facing the centre. Their behaviour was recorded for 5 minutes. The arena was cleaned with 70% ethanol to remove any olfactory cues. During the test, data were gathered on numerous variables, including the total number of squares crossed on both sides of the periphery and centre, the time spent in each area, and the number of rearing responses[20-24].

2.11 Test for Recognition and Discrimination Index

The Novel Object Recognition Test (NORT) serves as a non-aversive and non-spatial behavioural assay designed to assess recognition memory by evaluating an animal's preference for a novel object over a familiar one. The experiment was performed in an open-field arena and comprised three stages: habituation, training, and testing. In the habituation

phase, each rat was placed at the centre of the field and allowed a ten-minute period to freely explore the apparatus. During the training session conducted the following day, two identical objects were positioned in opposite corners, and the animals were permitted to investigate them for ten minutes. After a retention interval of 24 hours, one of the familiar objects was substituted with a novel one, and rats were again given ten minutes to explore during the testing phase. All behavioural sessions were video-recorded and later analysed offline. The measured variables included the exploration time for the novel object (Tn), exploration time for the familiar object (Tf), along with calculated discrimination and recognition indices[20-24].

$$Discrimination\ Index = \frac{T_n - T_f}{Total\ time\ with\ both\ objects}$$

$$Recognition\ index = \frac{T_n}{Total\ time\ with\ both\ objects}$$

2.12 Biochemical Studies

24 hours after the behavioural experiments, the animals were sacrificed using isoflurane anaesthesia, the brain was removed, and the prefrontal cortex (PFC), Hippocampus (HPC), and Septum (SP) were isolated for additional biochemical analysis. Acetylcholinesterase (AChE) activity estimation was carried out with the harvested brain regions.

2.13 Estimation of Acetylcholinesterase Activity

Acetylcholinesterase (AChE) activity in brain homogenates was measured using a modified Ellman-based spectrophotometric assay that assesses the rate of thiocholine production from acetylthiocholine in the presence of DTNB[25]. The reaction between DTNB and freed thiocholine creates a yellow chromophore, and the absorbance change was measured at 412 nm with a microplate reader. Homogenates were prepared in cold phosphate buffer and centrifuged to yield clear supernatants for analysis[26]. Substrate and DTNB concentrations were modified in accordance with previous guidelines that described enhanced sensitivity and decreased background absorbance to reduce interference from tissue pigments and guarantee the best possible signal response. The test was done at room temperature, and the reaction kinetics were recorded immediately after adding the substrate. Micromoles of thiocholine produced per minute per milligram of protein were used to measure AChE activity[27].

2.13.1 Procedure

The dissected tissues were weighed, placed in phosphate buffer (0.1 M, pH 8.0), and homogenate was made. After aliquoting 40 µl of the homogenate, add 200 µl of phosphate buffer and 80 µl of 0.01 M DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)) and incubate for 2 minutes at 27°C. To start the reaction, 10 µl of acetylthiocholine iodide (ATC, final concentration 1 mM) was added. The absorbance was measured at 412 nm every 60 seconds for 10 minutes using an ELISA/microplate reader. All samples were run in duplicate. The protein concentration of each homogenate was measured using the Bradford technique on an aliquot of the same homogenate. AChE activity was measured as µmol ATC hydrolyzed/h/mg of protein[25,26].

The following equation was used to calculate enzyme activity:

Enzyme activity (µmol ATC/h/mg protein)

$$= 5.74 \times 10^{-4} \times \frac{Absorbance}{Protein\ concentration}$$

2.14 Statistical Analysis

The data were examined with GraphPad Prism 7.0 software. Any significant changes between groups were examined using one-way and two-way ANOVAs. A post-hoc test was used to compare groups, and data were presented as mean ± SEM.

3. Results

The ethanolic extract of *Hedychium spicatum* did not cause any alterations such as muscle spasms, sedation, weight loss, lacrimation, diarrhoea, or urine. The survival rate was 100%, indicating that the herbal extract did not cause any toxic effects. The data obtained from the animals' behavioural and biochemical estimations were compared to the normal and disease control groups.

3.1 Effect of *Hedychium spicatum* on Pentylenetetrazole-Induced Memory Impairment in the T-Maze Alteration Task

Kindling caused by successive doses of PTZ impairs learning in the T-maze alteration task. After 6 days of training (36 trials), the kindled rats made fewer correct choices as shown in the figure than the control group

(p<0.001; Figure 1A). Treatment with the test drug *Hedychium spicatum* at a low dose of (200 mg/kg) and high dose of (400 mg/kg) for 15 days, improved working memory on the 5th day (p<0.01, Figure 1B) and on the 6th day (Figure 1B, p<0.001), indicating a significant increase in the number of correct choices possessing memory enhancing abilities when compared to the disease control group. Escitalopram treatment completely restored PTZ-induced memory impairment in the T-maze task.

In the T-maze memory retention test, performance was quantified by recording the number of correct arm choices made by each rat. Compared to the normal control group, the PTZ and vehicle-treated groups showed a significant reduction in the number of correct choices, indicating impaired working memory. Treatment with *Hedychium spicatum* significantly improved performance, with the low dose (200 mg/kg; p < 0.01, Figure 1C) and high dose (400 mg/kg; p < 0.001) and escitalopram (10 mg/kg; p < 0.01) groups showing a significant increase in the number of correct choices compared to the PTZ control group, indicating improved working memory ability.

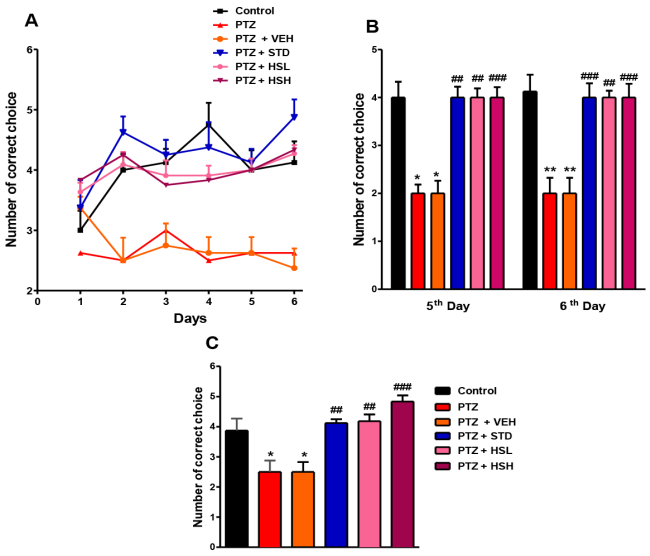


Figure 1. Effect of PTZ and *Hedychium spicatum* treatment on correct choices (**A**) Learning behaviour during training; (**B**) Correct choices assessment on 5th and 6th days; (**C**) Correct choices in retention assessment. Control: Untreated rats (n=8); PTZ: Kindled animals (8): pentylenetetrazole 35 mg/kg i.p.; PTZ + Veh (8): CMC 1% v/v; PTZ + STD (8): Escitalopram oxalate 5 mg/kg *p.o.*, PTZ + HS200 (8), PTZ + HS400 (8) mg/kg/day for 15 days orally. Data were expressed as Mean ± SEM. Statistical analysis was done using Two-way ANOVA followed by Bonferroni's post hoc test. *p<0.05, **p<0.01 as compared with normal control; ##p<0.01, ###p<0.001 compared with PTZ group.

3.2 Effect of *Hedychium spicatum* on Pentylenetetrazole-induced Anxiety-like Behaviourin EPM

A 15-day treatment with a high dose of HS (400 mg/kg) resulted in a substantial increase in open arm entries (p<0.01, Figure 2A); however, treatment with a low dose of HS (200 mg/kg) resulted in no statistical difference. Treatment with an ethanolic extract of *Hedychium spicatum* at a low dose of (200 mg/kg) and a high dose of (400 mg/kg) for 15 days enhanced the duration spent in the open arms, but the difference was not statistically significant when compared to the disease control group (p>0.05, Figure 2B). Standard drug escitalopram demonstrated anxiolytic activity in rats administered PTZ.

The total number of entries in the PTZ and vehicle administered groups decreased consistently when compared to the normal control group. Although the HS low dosage (200 mg/kg) did not increase total arm entries, the high dose (400 mg/kg) and escitalopram treated animals did, but the difference was not statistically significant when compared to the disease control group (p>0.05, Figure 2C). PTZ and the vehicle group showed fewer head dips than the normal control group, but when the test drug *Hedychium spicatum* was administered for 15 days at a low dose of 200 mg/kg and a high dose of 400 mg/kg, the number of head dips increased. However, the increased head dips were not statistically significant (p>0.05, Figure 2D).

3.3 Effect of *Hedychium spicatum* on Pentylenetetrazole-induced Exploratory Behaviourin the Open Field Test

PTZ injections did not change exploratory behaviour and locomotion. Over 15 days, rats treated with escitalopram oxalate (5 mg/kg) demonstrated increased exploratory activity. The administration of low-dose HS (200 mg/kg) and high-dose HS (400 mg/kg) increased exploration and locomotion, although there was no significant change when compared to the disease group (p>0.05, Figure 3A and 3B).

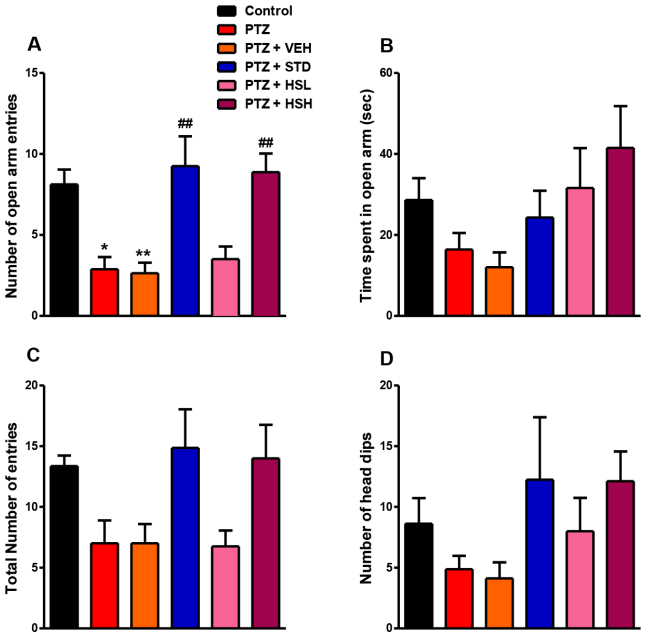


Figure 2. Effect of PTZ and *Hedychium spicatum* treatment on anxiety-like behaviour (**A**) Number of open arm entries; (**B**) Time spent in open arm (sec); (**C**) Total number of entries; (**D**) Number of head dips. Control: Untreated rats (n=8); PTZ: Kindled animals (8): pentylenetetrazole 35 mg/kg i.p.; PTZ + Veh (8): CMC 1% v/v; PTZ + STD (8): Escitalopram oxalate 5 mg/kg, PTZ + HS200 (8), PTZ + HS400 (8) mg/kg/day for 15 days orally. Data were expressed as Mean ± SEM. Statistical analysis was done using One-way ANOVA followed by Tukey's post hoc test. $^{*}p<0.05$, $^{**}p<0.01$ as compared with normal control; $^{\#\#}p<0.01$ compared with PTZ group.

3.4 Effect of *Hedychium spicatum* on Pentylenetetrazole-induced Altered Novel Object Recognition Memory

The 15-day treatment with low dosage HS 200 mg/kg and high dose HS 400 mg/kg resulted in a significant ($p<0.001$) decrease in exploration time with familiar objects (Figure 4A). The low dose of HS 200 mg/kg resulted in an increase in exploration time with novel objects, but this was not significant. However, the high dose of HS 400 mg/kg resulted in a statistically significant ($p<0.01$) increase in exploration time with novel objects, indicating restorative memory abilities when compared to the disease control group (Figure 4B). The standard treatment group spent much more time ($p< 0.001$) with the novel object.

Both low-dose HS 200 mg/kg and high-dose HS 400 mg/kg treated groups showed an increase in exploration time with the novel object compared to the time spent with the familiar object, indicating restored recognition memory (Figure 4C). Similar results were seen with escitalopram treated group.

During the test phase, the PTZ and vehicle-treated groups exhibited significantly lower discrimination (Figure 5A) and recognition (Figure 5B) indices for both familiar and novel objects compared with the normal control group ($p<0.001$). In contrast, rats treated with low and high doses of HS, a standard drug, showed a significant increase in the discrimination index (Figure 5A) toward familiar objects and the recognition index

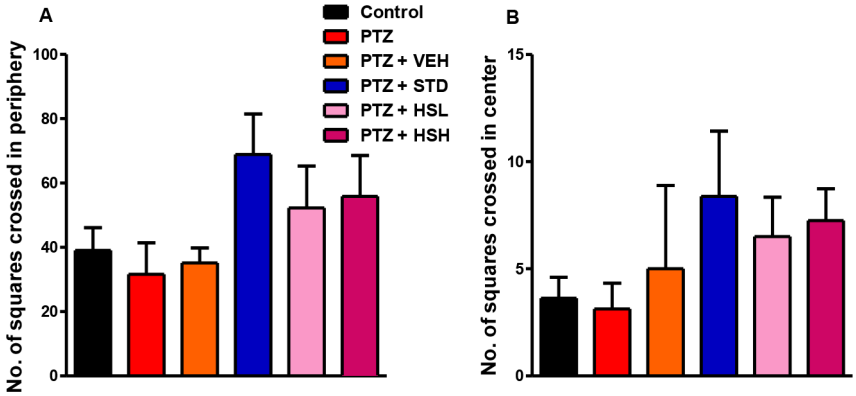


Figure 3. Effect of PTZ and *Hedychium spicatum* treatment on exploratory behaviour and locomotion (**A**) Number of squares crossed in periphery; (**B**) Number of squares crossed in centre. Control: Untreated rats (n=8); PTZ: Kindled animals (8): pentylenetetrazole 35 mg/kg i.p.; PTZ + Veh (8): CMC 1% v/v; PTZ + STD (8): Escitalopram oxalate 5 mg/kg, PTZ + HS200 (8), PTZ + HS400 (8) mg/kg/day for 15 days orally. Data were expressed as Mean ± SEM. Statistical analysis was done using One-way ANOVA followed by Tukey's post hoc test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared with normal control; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared with PTZ group.

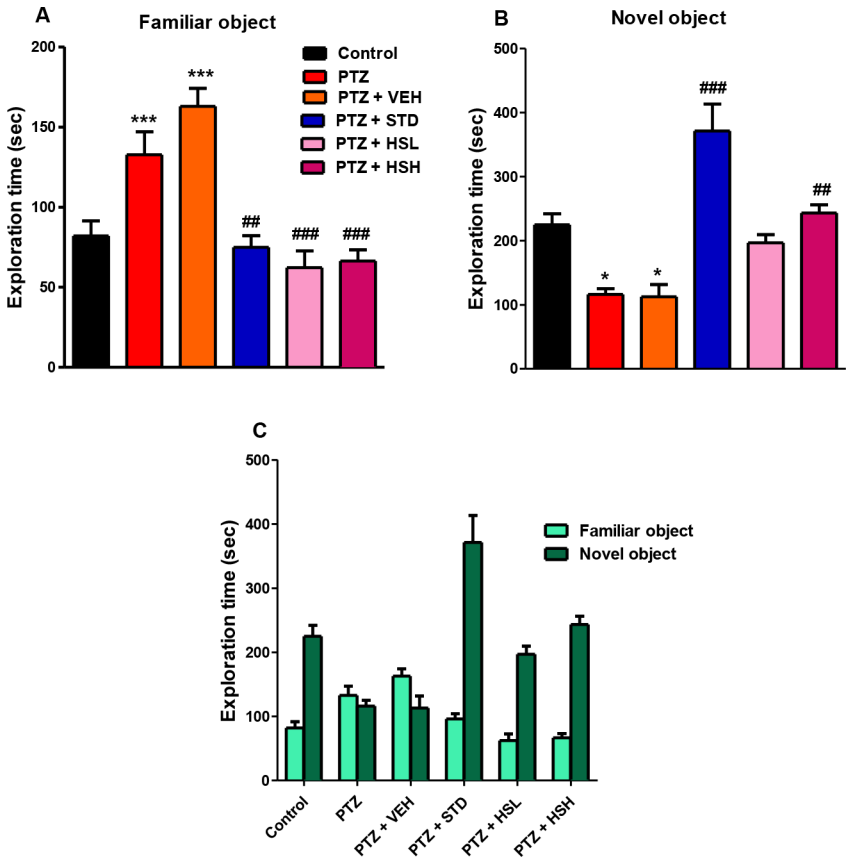


Figure 4. Effect of PTZ and *Hedychium spicatum* treatment on altered novel object (**A**) Exploration time with familiar object; (**B**) Exploration time with novel object; (**C**) Comparison of exploration time between familiar and novel object. Control: Untreated rats (n=8); PTZ: Kindled animals (8): pentylenetetrazole 35 mg/kg i.p.; PTZ + Veh (8): CMC 1% v/v; PTZ + STD (8): Escitalopram oxalate 5 mg/kg, PTZ + HS200 (8), PTZ + HS400 (8) mg/kg/day for 15 days orally. Data were expressed as Mean ± SEM. Statistical analysis was done using One-way ANOVA followed by Tukey's post hoc test; $^{*}p<0.05$, $^{***}p<0.001$ as compared with the normal control; $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared with the PTZ group.

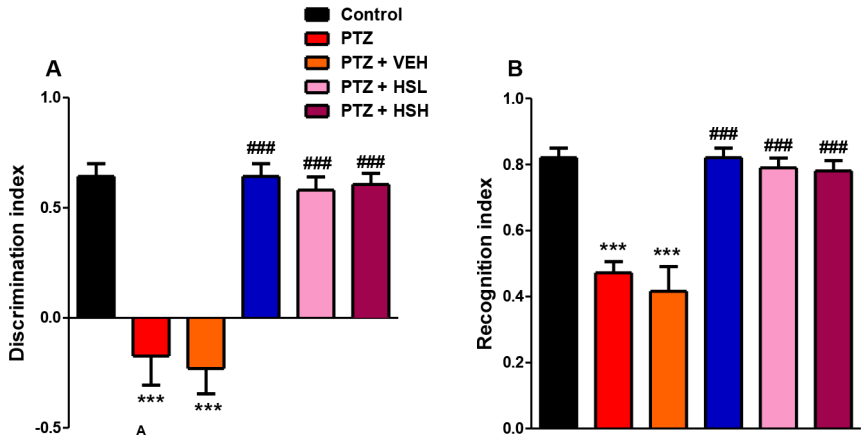


Figure 5. Effect of PTZ and *Hedychium spicatum* treatment on altered novel object recognition test (**A**) Discrimination index; (**B**) Recognition index. Control: Untreated rats (n=8); PTZ: Kindled animals (8): pentylenetetrazole 35 mg/kg i.p.; PTZ + Veh (8): CMC 1% v/v; PTZ + STD (8): Escitalopram oxalate 5 mg/kg, PTZ + HS200 (8), PTZ + HS400 (8) mg/kg/day for 15 days orally. Data were expressed as Mean ± SEM. Statistical analysis was done using One-way ANOVA followed by Tukey's post hoc test; $^{***}p<0.001$ as compared with the normal control; $^{\#\#\#}p<0.001$ compared with the PTZ group.

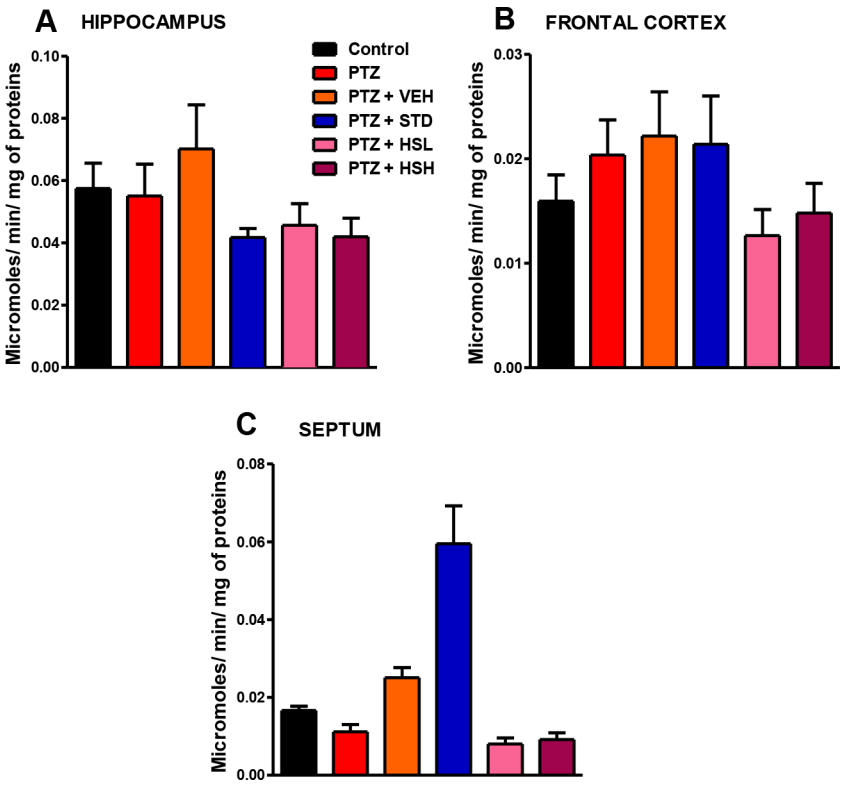


Figure 6. Effect of PTZ and *Hedychium spicatum* treatment on AChE Activity in (**A**) Hippocampus; (**B**) Frontal Cortex; (**C**) Septum. Control: Untreated rats (n=4); PTZ: Kindled animals (4): pentylenetetrazole 35 mg/kg i.p.; PTZ + Veh: CMC 1% v/v (4); PTZ + STD (4): Escitalopram oxalate 5 mg/kg, PTZ + HS200 (4), PTZ + HS400 (4) mg/kg/day for 15 days orally. Data were expressed as Mean ± SEM. Statistical analysis was done using One-way ANOVA followed by Tukey's post hoc test.

(Figure 5B) toward novel objects compared with the disease control group ($p<0.001$).

3.5 Effect of *Hedychium spicatum* Treatment on AChE Activity

To get an insight into the role of cholinergic neurotransmission in PTZ-induced kindling and the effects of test drugs on it, we performed AChE assay in the hippocampus, prefrontal cortex and septum tissues. The PTZ group did not elicit any significant changes in the levels of AChE in all three brain regions as compared to the normal control. The low dose (200 mg/kg) and high dose of *Hedychium spicatum* (400 mg/kg), and escitalopram showed no beneficial effects on the levels of AChE, as shown in Figure 6.

4. Discussion

The present study investigated the protective effects of *Hedychium spicatum* against pentylenetetrazole (PTZ)-induced cognitive impairment and anxiety-like behaviour in rats. PTZ kindling is a widely used experimental model to study epileptogenesis and its associated neurobehavioural abnormalities, including learning deficits and emotional disturbances[28]. Repeated administration of PTZ produces progressive seizure susceptibility through disruption of the balance between excitatory and inhibitory neurotransmission, particularly through enhanced glutamatergic activity and reduced GABAergic inhibition. These alterations lead to neuronal hyperexcitability, oxidative stress, and hippocampal dysfunction, which ultimately contribute to cognitive impairment and behavioural abnormalities observed in epileptic conditions[29]. Previous studies have reported that PTZ-induced seizures are associated with significant deficits in memory, increased anxiety-like behaviour, and neurochemical alterations in the hippocampus and cortex[30].

In the present investigation, PTZ-treated animals exhibited impaired performance in the T-maze and novel object recognition tasks, indicating deficits in spatial

and recognition memory. These findings are consistent with earlier reports demonstrating that PTZ kindling disrupts hippocampal-dependent learning and memory processes[29,30]. The hippocampus plays a critical role in memory formation, and seizure activity can impair synaptic plasticity and long-term potentiation in this region. Treatment with *Hedychium spicatum* significantly improved memory performance in both behavioural paradigms, suggesting its potential protective effect against seizure-associated cognitive dysfunction. The higher dose of *Hedychium spicatum* demonstrated effects comparable to the standard drug escitalopram, indicating that the plant extract may possess significant neuroprotective properties.

In addition to cognitive deficits, PTZ-kindled animals demonstrated increased anxiety-like behaviour, as evidenced by reduced open-arm exploration in the elevated plus maze. Anxiety and mood disturbances are common comorbidities in epilepsy and are often associated with alterations in neurotransmitter systems and stress-related neuroendocrine pathways[31,32]. The significant reduction in anxiety-like behaviour observed in *Hedychium spicatum*-treated groups suggests that the plant may exert anxiolytic effects[33,34]. Importantly, locomotor and exploratory activity in the open field test remained largely unchanged following treatment, indicating that the behavioural improvements were not due to nonspecific stimulant or sedative effects. These findings further support the behavioural specificity of *Hedychium spicatum* in improving neurobehavioural outcomes.

The neuroprotective effects of *Hedychium spicatum* observed in the present study may be attributed to its diverse phytochemical constituents and pharmacological activities. Previous phytochemical investigations have shown that the rhizomes of *Hedychium spicatum* contain essential oils rich in monoterpenes and sesquiterpenes, along with other bioactive compounds possessing antioxidant and anti-inflammatory properties[16]. Oxidative stress plays a significant role in seizure-induced neuronal damage by promoting lipid peroxidation, mitochondrial dysfunction, and apoptotic pathways[35]. Natural compounds with antioxidant potential are therefore considered promising therapeutic candidates for reducing seizure-induced neurotoxicity[36,37]. Although acetylcholinesterase activity did not show significant alterations following treatment in the present study, the behavioural improvements suggest that other mechanisms, such as antioxidant activity, modulation of neurotransmitter systems, or neuroprotective effects, may contribute to the observed benefits.

Overall, the findings of the present study demonstrate that *Hedychium spicatum* significantly attenuates PTZ-induced cognitive deficits and anxiety-like behaviour in rats. These results highlight the potential of *Hedychium spicatum* as a natural neuroprotective agent for managing epilepsy-associated cognitive and behavioural impairments. However, the precise molecular mechanisms underlying these effects remain to be elucidated. Future studies focusing on oxidative stress markers, inflammatory mediators, and neurotransmitter pathways may provide further insights into the therapeutic potential of *Hedychium spicatum* in epilepsy and related neurological disorders.

5. Conclusion

In conclusion, the present study demonstrates that *Hedychium spicatum* significantly attenuates PTZ-induced cognitive impairment and anxiety-like behaviour in rats. Treatment with *Hedychium spicatum* improved memory performance in behavioural tasks and reduced anxiety-related responses without affecting general locomotor activity, indicating a specific neurobehavioural protective effect. The higher dose of *Hedychium spicatum* produced effects comparable to the standard drug escitalopram. However, no significant changes were observed in brain acetylcholinesterase activity, suggesting that the protective effects may involve mechanisms other than cholinergic modulation, possibly related to its antioxidant or neuroprotective properties. These findings suggest that *Hedychium spicatum* may serve as a promising natural therapeutic candidate for managing epilepsy-associated cognitive and behavioural deficits, although further studies are required to elucidate the precise molecular pathways involved.

6. Competing Interests Disclaimer

Authors have declared that they have no known competing financial interests OR non-financial interests, or personal relationships that could have appeared to influence the work reported in this paper.

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Conceptualisation, G.B. and B.V.; methodology, M.M and G.B.; investigation, M.M and G.B.; datacuration, M.M and G.B.; writing-original draft preparation, M.M and G.B.; writing-review and editing, B.V.; supervision, B.V. All authors have read and agreed to the published version of the manuscript

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