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Research Paper

Neuroprotective Effect of *Celastrus Paniculatus* Seed Extract on Epilepsy and Epilepsy-associated Cognitive Deficits

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ABSTRACT

Introduction: Cognitive deficit is one of the common comorbidity accompanying epilepsy. The present study evaluated the effect of *Celastrus paniculatus* seed extract on seizure severity and cognitive deficit following the pentylenetetrazole (PTZ)-induced chemical kindling model.

Methods: PTZ kindling model was developed by daily administration of the sub-convulsive dose of PTZ 30 mg/kg for four weeks. After four weeks of induction, the following treatment, namely sodium valproic acid (SVA) 200 mg/kg, *C. paniculatus* 500 mg\kg, pergolide 2 mg/kg, C. paniculatus (250 mg\kg)+ Pergolide (1 mg/kg), and *C. paniculatus* (250 mg\kg)+ SVA (100 mg/kg) were administered 30 minutes prior to PTZ (30 mg/kg) injection for a period of next 14 days. Neurobehavioral parameters, including superoxide dismutase (SOD), Catalase (CAT), glutathione (GSH), and dopamine levels were assessed and the Morris water maze test (MWM) and Grip strength test (GPS) were performed. Hematoxylin & Eosin (H&E) staining of hippocampal cornu ammonis (CA1), CA2, CA3, dentate gyrus (DG), and frontal cortex was performed.

Results: *C. paniculatus* (500 mg/kg) alone and in combination (*C. paniculatus* (250 mg\kg)+ pergolide (1 mg/kg) and *C. paniculatus* (250 mg\kg)+ SVA (100 mg/kg)) significantly ($P<0.05$) reduced the seizure score, mean latency time, and distance traveled in the MWM. However, no significant effect was seen in GPS. Biochemical analysis showed elevated antioxidant markers, namely GSH, CAT, and SOD, and also elevated dopamine levels. *C. paniculatus* and its combination also significantly ($P<0.05$) protected against neuronal loss in the hippocampus and frontal cortex evidenced by H&E staining

Conclusion: *C. paniculatus* alone and in combination with other agents may have the potential to treat epilepsy and associated cognitive deficits

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Highlights

- *Celastrus paniculatus* ameliorate the seizure severity and associated cognitive deficits
- *C. paniculatus* possesses neuronal protection in the hippocampus and frontal cortex and restores the biochemical changes in PTZ kindling model
- *C. paniculatus* is an adjuvant therapy in the treatment of epilepsy

Plain Language Summary

Despite the availability of antiepileptic drugs, patients are poorly responding to them and they are also associated with cognitive deficits. Our study used the herbal treatment (*Celastrus paniculatus*) for the safety and efficacious purpose, which may treat epilepsy and associated cognitive deficits in experimental-induced epilepsy.

1. Introduction

Epilepsy is a chronic neurological disorder that affects 50 million people worldwide, and approximately 5.5 million people in India (Sridharan & Murthy, 1999) age-specific rates, and patterns of epilepsy were chosen for meta-analysis. Both crude values and age-standardized prevalence rates were calculated after accounting for heterogeneity. RESULTS Twenty studies were found involving a sample population of 598,910, among whom 3,207 had epilepsy. This resulted in a crude prevalence of 5.35/1,000. After a correction for heterogeneity due to interstudy variation, the overall prevalence per 1,000 (and its 95% CI. Cognitive deficit is the most common comorbidity with epilepsy (Holmes, 2015). About 20-50% of epileptic patients are associated with memory impairment in early childhood, including learning disability, low intelligence quotient levels, lack of mental intellect, attention deficit, and poor academic outcomes (Holmes, 1995; Lee et al., 2015; Merkena, 2016). Moreover, recurrent neuronal firing disrupts the biochemical cascade and neurochemical and histopathological processes in the brain. The uncontrolled neuronal firing also results in the formation of reactive oxygen species, responsible for damage to antioxidant homeostasis and further damage to the brain (Geronzi et al., 2018; Pearson-Smith & Patel, 2017; Prada Jardim et al., 2017). Despite the availability of efficacious antiepileptic drugs (AEDs), they are associated with severe adverse drug reactions and are devoid of cognitive benefits (Eddy et al., 2011; Ijff & Aldenkamp, 2013; Jost et al., 2016; Wijnen et al., 2017) as is the treatment gap (estimated at 92%. Therefore, a safe and efficacious drug with a low adverse effect profile and possessing cognitive benefits is an unmet medical need.

Celastrus Paniculatus (CP) is an herb well known for its medicinal properties and belongs to the Celastreceae family. It is found in tropical and subtropical regions. The plant contains sesquiterpene alkaloids, like celestine, malkanguniol, paniculatin, and celapanin as major active constituents and ample flavonoids and tannins, triterpenoids, and steroids (Bhanumathy et al., 2010; Shashank & Mistry, 2017). In folk medicine, CP has shown a beneficial effect on various pathological conditions, such as muscle cramps, backache, sciatica, osteoarthritis, facial paralysis, and various neurological disorders (Kulkarni et al., 2015; Saroya & Singh, 2018). Experimental data have suggested the beneficial role of CP oil in neuronal protection against glutamate-induced toxicity by modulating glutamate receptors (Godkar et al., 2004). It has also been shown to possess a cognitive benefit as evidenced in stress-induced cognitive dysfunction and possesses dose-dependent anti-cholinesterase activity in the rat brain (Bhagya et al., 2016). However, the effect of CP seed extract on epileptogenesis and associated cognitive deficits has not been studied. Therefore, the present study evaluated the neuroprotective of CP seed extract alone and combination in with A pentylenetetrazole (PTZ)-induced kindling model.

2. Materials and Methods

Animals

Adult male Wistar rats (200-250 g) were taken from the Advance small animal facility center of the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India. Rats were accommodated each three in polypropylene cages crumpled with husk and kept at a controlled temperature (25±2°C) with relative humidity (RH) (60-70%) under a normal 12 h light and dark

cycle. Animals were housed with free access to food and water and allowed to acclimatize for one week before the experiments. The experimental study was initiated after approval of the Institutional Animal Ethics Committee (IAEC) (88/IAEC/598) and Institutional Biosafety Committee (IBC) (613/IBC). The experiments were performed according to the guidelines of CPCSEA (Committee for Control and Supervision of Experiments on Animals).

Drugs and chemicals

Pentylenetetrazole (PTZ), pergolide, and sodium valproic acid (SVA) were purchased from Sigma-Aldrich, USA. CP seeds extract (Batch no. SHPL/SAMPLE/JTMSIDE) was purchased from Vaidya Hokum Chand Agrawal (VHCA) Ayurveda, Pvt. Ltd India. Enzyme-Linked immunosorbent assay (ELISA) kit (Lot no. 05/2018 (96T)) was used for quantitative analysis of dopamine and purchased from Bio-Rad Laboratories, USA. All the chemicals and reagents used in the study were of analytical grade and accompanied by a certificate of analysis. PTZ was diluted in the saline and administered by intraperitoneal (i.p.) route. Among the interventions, SVA was diluted in distilled water, and pergolide and CP were diluted in 0.5% carboxymethylcellulose (CMC). The oral route was used for administering all the interventions.

Experimental design

A total of 50 six male Wistar rats were approved for the study. We used 42 rats for the experiment and kept the remaining for replacement (mortality during the model induction or any other deficit hindering experimental assessment).

A training period of four days was given to the rats for the Morris water maze (MWM). A probe trial was conducted at the end of the training period to assess memory retention. Baseline readings were obtained for both the MWM and Grip strength test (GPS). After that, PTZ (30 mg/kg, i.p.) was given daily for the next 28 days to all rats except the vehicle control group. Weekly data were recorded for the assessment of successful kindling. After kindling induction, rats were randomized into seven groups (n=6 in each group), namely: (1) Vehicle control (NC): 0.5% CMC (1 mL/kg/per oral), (2) PTZ: PTZ (30 mg/kg/i.p.), (3) SVA: Sodium valproic acid (200 mg/kg; oral), (4) Pergolide: Pergolide (2 mg/kg; oral), (5) CP: *C. paniculatus* (500 mg/kg/per oral), (6) CP+SVA: *C. paniculatus* (250 mg/kg; oral)+sodium valproic acid (100 mg/kg; oral), and (7) CP+Pergolide: *C. paniculatus* (250 mg/kg; oral)+pergolide (1 mg/kg; oral). The treatments were administered 30 minutes before the PTZ injection for 14 days. The doses of CP and SVA were selected based on the previous literature (Kulkarni et al., 2015; Löscher et al., 1993). Neurobehavioural, biochemical, and histological assessments were made as described below (Figure 1).

PTZ-induced kindling model

PTZ kindling model was developed as per lab standard protocol (Dhir, 2012). The sub convulsive dose of PTZ (30 mg/kg, i.p.) was given daily for 28 days or till kindling. The seizure scoring was calculated based on the Racine scale. The rats were placed in a transparent plexiglass chamber for score assessment. The scaling is as follows: "0: No response, 1: Ears and facial twitching, 2: Myoclonic jerks without rearing, 3: Myoclonic jerks and rearing, 4: Turn over into side position, tonic-clonic seizures, and 5: Turn over onto back position, generalized tonic-clonic convulsions". The confirmation of model induction was the occurrence of stage 2 of seizure for five consecutive days or stage 4 for three consecutive days (De Sarro et al., 1999; Yazdi, et al., 2020). The severity of the seizure score was recorded at baseline.

Neurobehavioral assessment

Morris water maze

The cognitive deficits were evaluated by the MWM test using Ethovision Noldus XT 11.5 (EV115-06266) tracking system. The test protocol was followed as per the literature (Prakash et al, 2013a; Prakash et al, 2013b; Vorhees & Williams, 2006). The endpoints assessed were mean latency time and distance traveled to reach the platform at baseline, and days 28, 35, and 42. The cut-off period of all trials was kept at 120 sec.

A GPS was used to assess the muscle function of the rats. Rats were acclimated to the testing room an hour before testing. The grip strength apparatus consists of a thread fastened to two vertical wooden boards and is kept in position by a horizontal wooden board. The individual rats were made to hang vertically using the two forelimbs. The length of time to hold the thread before falling was assessed at baseline and on days 28, 35, and 42. The maximum trial length was kept at 150 s. Three trials were taken, and the inter-trial duration was kept at 15-20 minutes (Deacon, 2013).

Tissue preparation for biochemical and histopathological parameters

On day 42, animals were euthanized using a high dose of pentobarbital sodium (100 mg/kg; i.p.) and transcardially perfused with 0.9% normal saline. The brain was extracted and stored in Phosphate Buffer Saline (PBS) at -20 for the estimation of glutathione (GSH), catalase, superoxide dismutase (SOD), and dopamine. A part of the brain was stored in 10% formaldehyde for H&E staining. For quantitative analysis, the samples were homogenized in ice-cold PBS at pH 7.4 and then transferred to different aliquots as per test requirements. The supernatant of all seven experimental groups was used for quantitative analysis by UV-spectrophotometer.

Biochemical analysis

Oxidative stress markers, including GSH, Catalase (CAT), and SOD were estimated in the whole brain. GSH was estimated by Jollow 1974 method (Kumar, et al., 2012) and expressed as nmol/mg protein. CAT was estimated by Claiborne's 1985 method (Kumar et al., 2012) and expressed as unit/gram tissue. SOD level was measured by the pyrogallol autoxidation method and expressed as Units/mL (Marklund & Marklund, 1974; Nandi & Chatterjee, 1988). All three methods have been standardized earlier in our laboratory.

Dopamine estimation

The dopamine level in brain tissue was estimated by the ELISA kit as per manufacturer instructions. The level of dopamine was expressed as µg/ml.

Histopathological analysis

The formalin-embedded brain was dissected into coronal sections. The hippocampus and frontal cortex were preserved and stained in H&E dye to assess the neurodegenerative changes in the frontal cortex, DG, CA1, CA2, and CA3 of the hippocampal layers. Overall hippocampal neuronal damage was scored by a semi-quantitative scoring system as follows: "Score 0: Normal (no injury or rare isolated apoptotic neuron); Score 1: Rare neuronal injury (<5 clusters); Score 2: Occasional neuronal injury; Score 3: Frequent neuronal injury (<15 clusters); and Score 4: Diffuse neuronal injury" (Myung et al., 2004).

Statistical analysis

Data were expressed as Mean±SEM. Quantitative data, such as behavioral parameters, latency time, and biochemical estimations were assessed by one-way ANOVA followed by post hoc Bonferroni test. The R software, version 3.5.2 was used for statistical analysis. The $P<0.05$ was considered statistically significant.

3. Results

PTZ kindling model and effect of the CP alone and in combination on seizure score

During model induction, there was a progressive significantly ($P<0.001$) increase in seizure score compared to baseline, and days 7, day 14, day 21, day 28, indicating successful PTZ kindling (Figure 2 A). On day 28, myoclonic jerks with rearing and tonic-clonic seizures were observed in all PTZ (30 mg/kg)-treated rats. On days 35 and 42, we found a significant decrease in the seizure score severity in treatment groups, namely SVA, CP, pergolide, CP+pergolide, and CP+SVA compared to the PTZ-treated group ($P<0.05$) (Figure 2 B). The given data revealed the protective effect of CP alone and in combination on reducing seizure score starting from day 7 to day 14 of the treatment.

Effect of the CP alone and in on behavioral parameters (MWM and GPS)

On day 28 of kindling, escape latency increased in all groups: PTZ, SVA, CP, pergolide, CP+pergolide, and CP+SVA compared to the vehicle group indicating an impairment in spatial learning and memory. On day 35, SVA, CP, pergolide, CP+pergolide, and CP+SVA groups showed no significant effect on spatial memory compared to the PTZ-treated group ($P>0.05$). However, on day 42, treatment with SVA, CP alone, and CP+SVA decreased the escape latency and distance traveled in kindled rats, which was statistically significant in contrast to the PTZ-treated group ($P<0.05$). This indicates a progressive improvement in learning and memory following 14 days of treatment. The treatment with pergolide alone and in combination increased the escape latency and distance traveled, thereby negatively affecting memory (Figure 3).

On day 28 of the kindling, the latency to fall decreased in all groups compared to the vehicle-treated group but it was not significant ($P>0.05$) (Figure 3). On days 35 and 42, no significant difference was found between treatment groups than the PTZ group ($P>0.05$).

Table 1. Effect of *C. paniculatus* alone and in combination on antioxidant markers

Groups	Mean±SD		
	GSH (nmol/mg Protein)	Catalase (Unit/g Tissue)	SOD (Unit/mg Protein)
Vehicle	28.9±6.2	2.82±0.24	41.93±5.81
PTZ	16.65±4.44	0.37±0.12	13.10±3.00$^{###}$
SVA	20.09±1.33	2.30±0.12	32.80±3.39*
CP	34.39 ±2.55	2.74±0.31	31.41±4.07
Pergolide	29.33±5.44	4.64 ±0.54	39.70±2.94**
CP+Pergolide	38.33±5.92	5.43±1.19**	40.48±2.30***
CP+SVA	29.33±8.71	5.17±1.55*	37.74±3.62**

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Abbreviation: CAT: Catalase; SOD: Superoxide dismutase; PTZ: Pentylenetetrazole (30 mg/kg); SVA: Sodium valproate (200 mg/kg); CP: *C. paniculatus* (500 mg/kg); Pergolide: Pergolide (2 mg/kg); CP+pergolide: *C. paniculatus* (250 mg/kg)+pergolide (1 mg/kg); CP+SVA: *C. paniculatus* (250 mg)+sodium valproate (100 mg/kg).

Statistical significance was determined by one-way ANOVA followed by Bonferroni post hoc test; $^{##}$P<0.01 and $^{###}$P<0.001 in comparison to the vehicle control, *P<0.05 and **P<0.01 and ***P<0.001 in comparison to the PTZ group.

Effect of CP alone and in combination on oxidative stress markers (GSH, CAT, and SOD)

The brain GSH level decreased in the PTZ group compared to the vehicle-treated group. In the intergroup analysis, the brain GSH level increased in the CP and CP+pergolide, and CP+SVA groups compared to the PTZ group, but it was not significant (P>0.05) (Table 1).

The brain CAT level reduced in the PTZ group compared to the vehicle group but it was not significant (P>0.05). In the intergroup analysis, a statistically significant difference was found in CAT level in the combined groups compared to the PTZ group (P<0.01 and P<0.05). However, CAT level in the SVA, CP, and pergolide groups significantly changed compared to the PTZ-treated group (Table 1).

The brain SOD level decreased in the PTZ group and was statistically significant compared to the vehicle group (P<0.001). The intergroup analysis showed that SOD level significantly increased in the SVA, pergolide, CP+pergolide, and CP+SVA groups (P<0.05, P<0.01, P <0.001, and P<0.01, respectively) compared to the PTZ group (Table 1).

Effect of CP alone and in combination on dopamine level

The brain dopamine level reduced in the PTZ group compared to the vehicle group. In the intergroup analysis, brain dopamine level significantly increased in the SVA, CP, and CP+pergolide groups compared to the PTZ group (P<0.05). However, pergolide alone and in combination (CP+SVA) showed markedly elevated dopamine levels compared to the PTZ group (P<0.01) (Figure 4).

Effect of the CP alone and in combination on histopathological neuronal scoring of the hippocampus and frontal cortex

The overall hippocampal neuronal damage expressed by nuclear chromatin clumping, hypereosinophilia, and condensation of cytoplasm (Figure 5 A) significantly increased in the PTZ group compared to the vehicle group (P<0.01). Treatment with SVA, CP, pergolide CP+pergolide, and CP+SVA caused a statistically significant decrease in the histopathological score compared to the PTZ group (P<0.01) (Figure 5 B). The decrease in neuronal injury score in groups receiving CP alone and on combination indicate its neuronal protection in PTZ kindled rats.

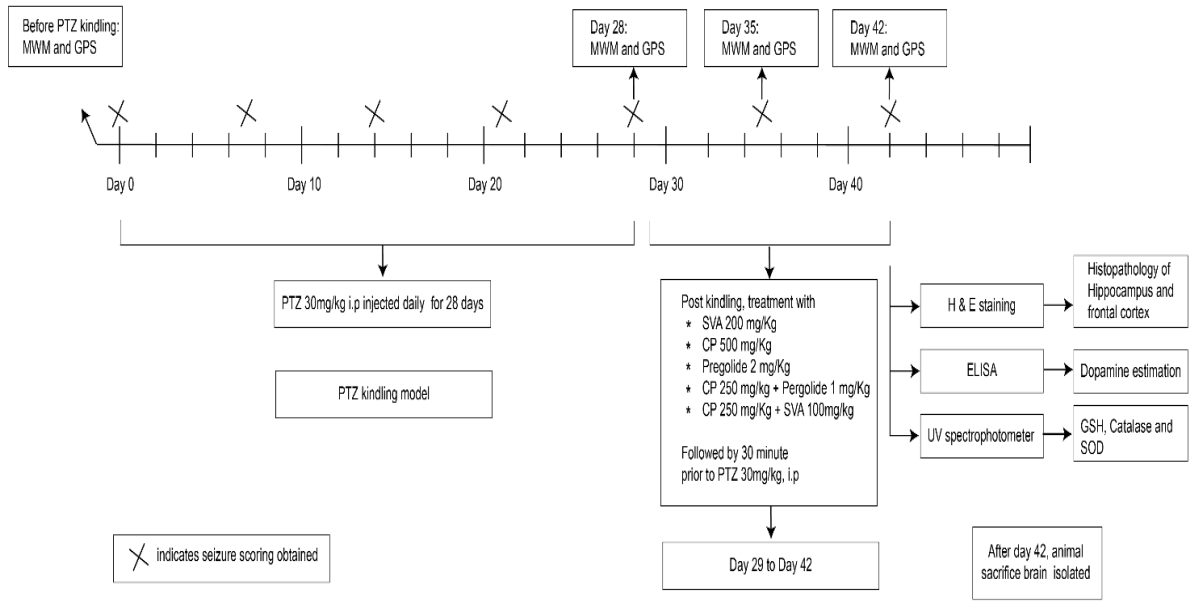

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Figure 1. Experimental design: Morris water maze (MWM) and grip strength test (GPS) were done at baseline without allocating any treatment to rats

After baseline reading, PTZ (30 mg/kg, i.p.) was given to all rats daily except the vehicle group from day 1 to day 28 or until kindling to induce the model, and then treatments were allocated from day 29 to day 42 (for 14 days). After day 42, the rats were sacrificed and their brains were isolated for histopathology assessment and evaluation of antioxidant markers, and dopamine levels.

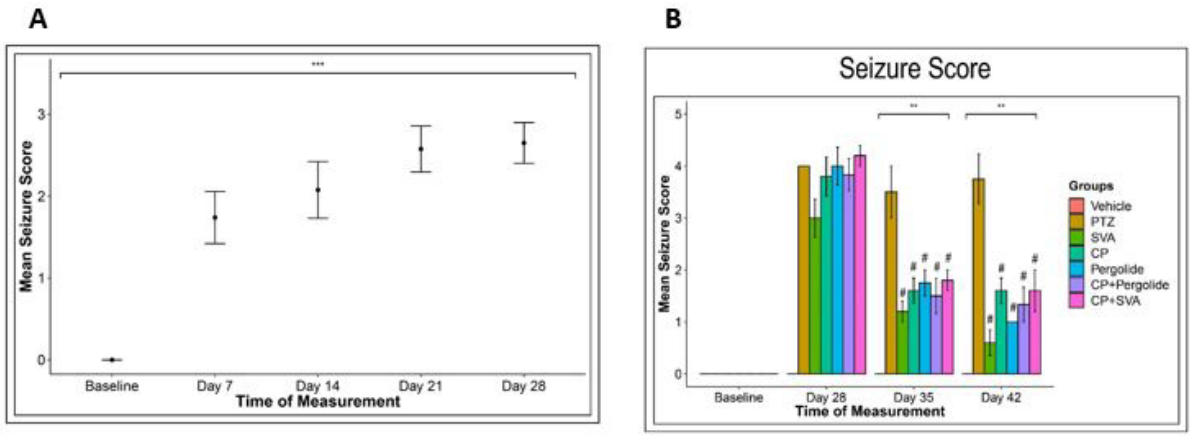

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Figure 2. PTZ kindling model and the effect of the *C. paniculatu*s alone and in combination on seizure score

The vehicle control group did not receive PTZ (30 mg/kg) showing a score 0.

A) Seizure scoring was recorded on days 0, 7, 14, and 28 to induce the PTZ kindling model.

B) Seizure scoring was recorded with treatment on days 0, 28, 35, and 42. Data were expressed as Mean±SEM (n=6). One-way ANOVA followed by Bonferroni post hoc test analyzed the data. #P<0.05 compared to the PTZ group. **P<0.01.

Abbreviation: PTZ: Pentylenetetrazole (30 mg/kg); SVA: Sodium valproate (200 mg/kg); CP: *C. paniculatus* (500 mg/kg); Pergolide: Pergolide (2 mg/kg); CP+pergolide: *C. paniculatus* (250 mg/kg)+pergolide (1 mg/kg); CP+SVA: *C. paniculatus* (250 mg)+sodium valproate (100 mg/kg).

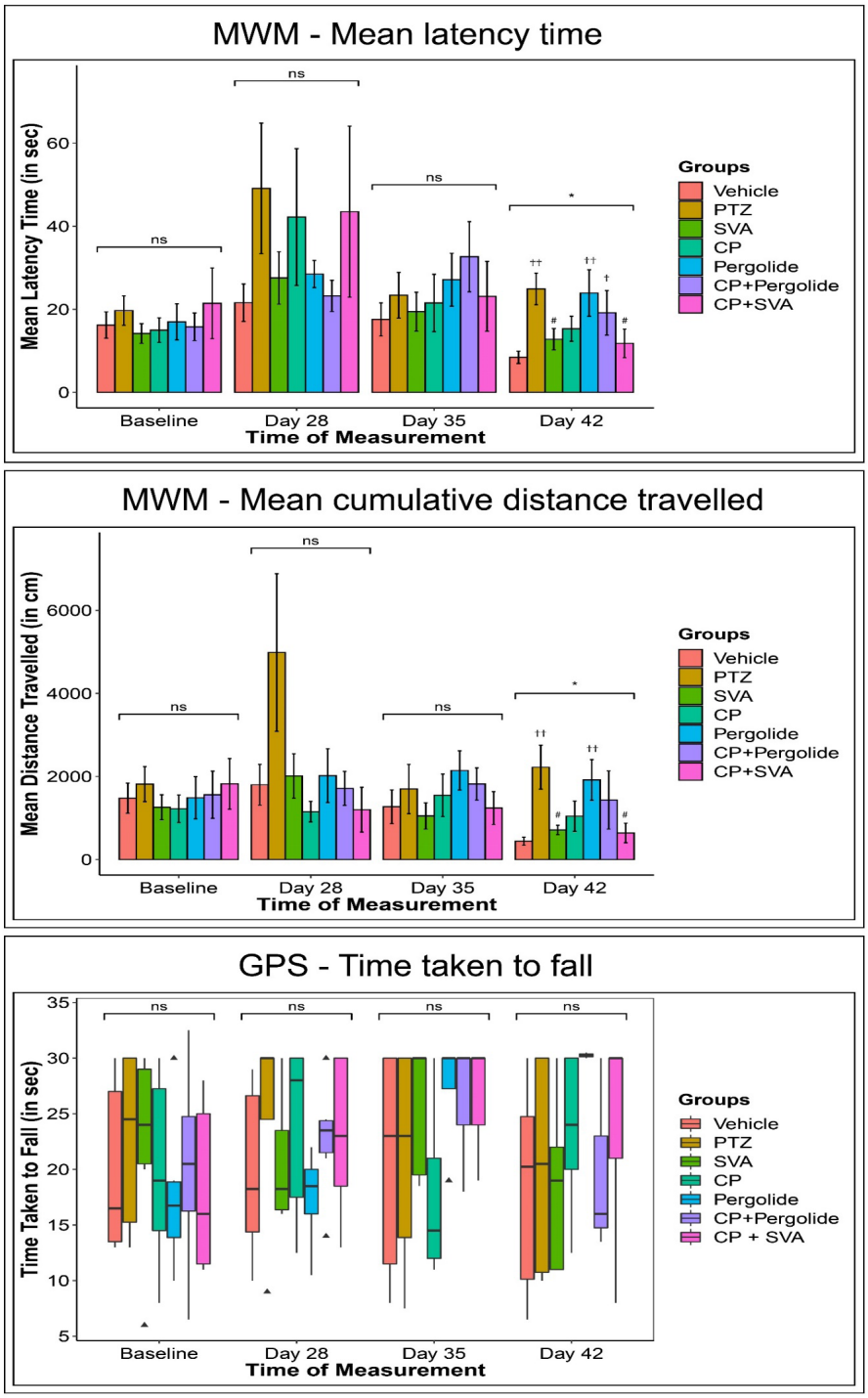

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Figure 3. Effect of *C. paniculatus* alone and in combination on neurobehavioral parameters using Morris water maze (MWM) and Grip strength test (GPS). The outcomes assessed were latency to reach the platform (sec) and distance traveled (cm) in the MWM and latency to fall (sec) in GPS

Data were expressed as Mean±SEM (n=6). One-way ANOVA followed by Bonferroni post hoc analysis analyzed the data. † and ††P<0.05 and P<0.01 compared to the vehicle group. #P<0.05 compared to the PTZ group. *P<0.05.

Abbreviation: PTZ: Pentylenetetrazole (30 mg/kg); SVA: Sodium valproate (200 mg/kg); CP: *C- paniculatus* (500 mg/kg); Pergolide: Pergolide (2 mg/kg); CP+pergolide: *C. paniculatus* (250 mg/kg)+pergolide (1 mg/kg); CP+SVA: *C. paniculatus* (250 mg)+sodium valproate (100 mg/kg).

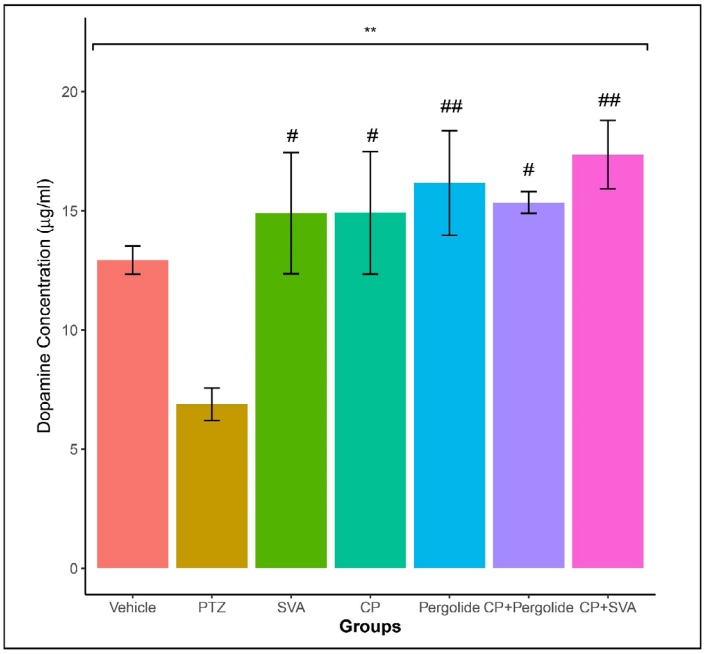

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Figure 4. Effect of *C. paniculatus* alone and in combination on dopamine level

Data are expressed as Mean±SEM (n=6). One-way ANOVA followed by Bonferroni post hoc test analyzed the data. # and ##P<0.05 and P<0.01 compared to the PTZ group. **P<0.01.

Abbreviation: PTZ: Pentylenetetrazole (30 mg/kg); SVA: Sodium valproate (200mg/kg); CP: *C- paniculatus* (500 mg/kg); Pergolide: Pergolide (2 mg/kg); CP+pergolide: *C. paniculatus* (250 mg/kg)+pergolide (1 mg/kg); CP+SVA: *C. paniculatus* (250 mg)+Sodium valproate (100 mg/kg).

4. Discussion

The present study evaluated the effect of CP seed extract on seizure severity and seizure-associated neurobehavioral changes, oxidative stress, dopamine levels, and changes in hippocampal CA1, CA2, CA3, DG areas, and frontal cortex in the PTZ kindling model. PTZ kindling model is a gold standard tool in the screening of novel drugs for epilepsy (Dhir, 2012; Prakash et al., 2013b). PTZ is a chemoconvulsant, an antagonist of GABAA receptor. It is used to induce absence–like seizures in rats (Dhir, 2012). CP alone and in combination has a beneficial effect against seizure and associated cognitive deficits.

In the present study, we found that daily administration of PTZ (30 mg/kg) reduced the threshold for seizure-induced tonic-clonic seizures along with impairment in learning and memory. It has been reported that repeated stimulus of PTZ promotes neuronal loss in the CA1 and CA3 layers of the hippocampus and prefrontal cortex. These areas are generally responsible for the formation of spatial memory and cognitive function (Dhir, 2012; Kälviäinen et al., 1998; Wang et al., 2019). Treatment with CP (500 mg/kg) alone and in combination with pergolide and SVA has been shown to reduce the seizure score and decrease the latency time and distance traveled to reach the platform in MWM, suggesting its protective role in seizure and cognitive deficits. The treatment, however, did not show significant benefit on the GPS. Previously, CP has been shown to improve memory in chronic stress-induced cognitive impairment and nitropropionic-induced Huntington disease-like symptoms (Bhagya et al., 2016; Malik et al., 2017). Therefore, the current finding of improvement in seizure-induced cognitive impairment further strengthens CP's use as a potential antiepileptic treatment.

Consistent with the previously reported studies, the repeated seizure stimulus alters the brain's oxidative stress and antioxidant enzyme homeostasis (Geronzi et al., 2018; Xie et al., 2012; Zhu et al., 2017). This phenomenon is further noticed in multiple neuropsychiatric disorders, such as schizophrenia, depression, bipolar disorder, and neurodegenerative disorders, like Alzheimer's disease, etc. (Balmus et al., 2016; Salim, 2016). In concordance with previous results, in the present study, the sub-convulsive dose of PTZ caused oxidative damage in response to altered antioxidant enzyme levels. Simultaneously, the antioxidant defense mechanism responds

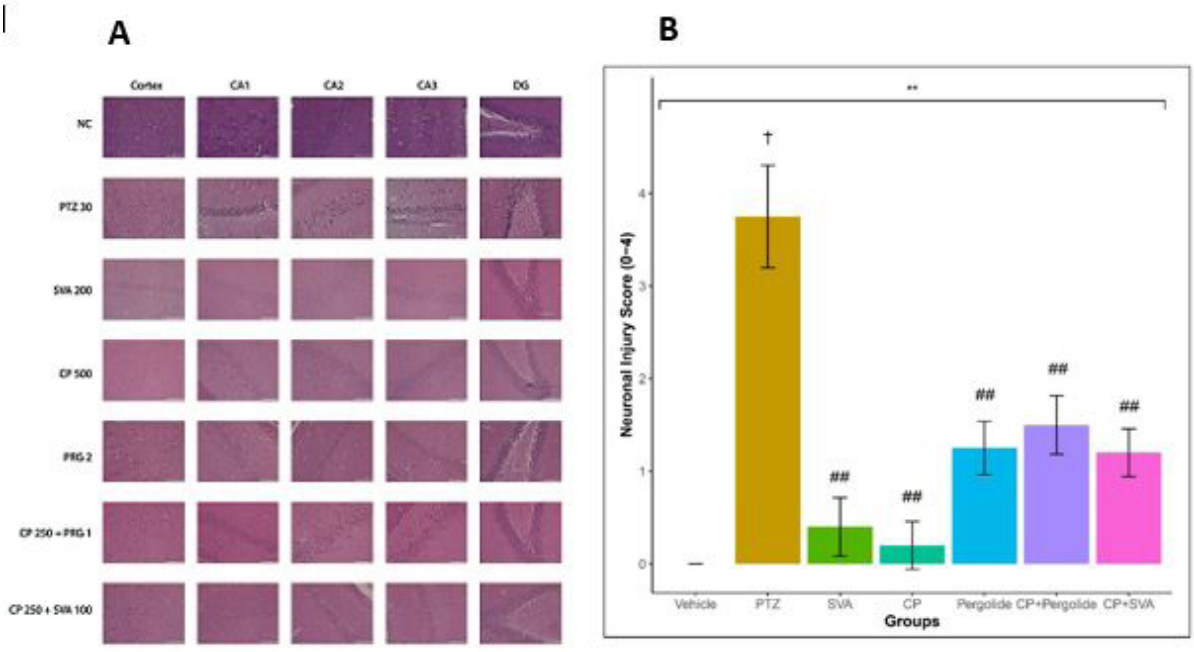

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Figure 5. Effect of the *C. paniculatus* alone and in combination on histopathological neuronal scoring of the hippocampus and frontal cortex

A) H&E staining was used to indicate the changes in the hippocampal CA1, CA2, CA3 areas, DG, and frontal cortex after treatments. Shrunken and darkly pigmented nuclei of neurons showed chromatin clumping (arrows) and cytoplasmic vacuolation in the form of neuronal damage in contrast to the treatment groups.

B) Graphical representation of neuronal injury of the hippocampus in different groups. Data were expressed as Mean±SEM (n=3) one-way ANOVA followed by Bonferroni post hoc test analyzed the data. compared to the vehicle group, ##P<0.01 compared to the PTZ group. **P<0.001.

Abbreviation: PTZ: Pentylenetetrazole (30 mg/kg); SVA: Sodium valproate (200 mg/kg); CP: *C. paniculatus* (500 mg/kg); Pergolide: Pergolide (2 mg/kg); CP+pergolide: *C. paniculatus* (250 mg/kg)+pergolide (1 mg/kg); CP+SVA: *C- paniculatus* (250 mg)+sodium valproate (100 mg/kg).

poorly due to prolonged seizures, thus enhancing the occurrence of neurodegeneration in the epileptic brain (Geronzi et al., 2018). Treatment with CP (500 mg/kg) alone and in combination elevated the levels of GSH, CAT, and SOD, suggesting its antioxidant property. The present results can be correlated with previous studies, in which various herbal drugs, including CP, have shown antioxidant and neuroprotective effects on neurodegenerative disorders (da Rocha et al., 2011; Godkar et al., 2004; Kumar & Gupta, 2002; Malik et al., 2017). The antioxidant property of CP possibly controlled the recurrent seizure episodes in this study.

Furthermore, to correlate dopamine with seizure and associated cognitive deficits, we assessed the level of dopamine in brain tissue. PTZ (30 mg/kg) lowered the levels of brain dopamine. Treatment with CP (500 mg/kg) alone and in combination with pergolide and SVA significantly increased the levels of dopamine. It was comparable to the positive control group (D1 receptor agonist), suggesting that increased dopamine levels may show protective roles in epilepsy and associated cognitive impairment by acting through D2 receptors. Previous studies also have shown D2 agonistic CP seed oil activity in an animal model of depression (Valecha & Dhingra, 2016). In epilepsy, dopamine modulates the seizure by acting through D1 and D2-like receptors. D1 receptor maintains the prefrontal cortex's cognitive functions (Starr, 1996; Wang et al., 2019), and stimulating D2- like receptors induces an antiepileptic effect (Bozzi & Borrelli, 2013).

In the histopathological analysis, PTZ (30 mg/kg) exacerbated the neuronal loss by changes in the neurons' normal morphology in the hippocampus. Similarly, previous studies have reported that the repeated induction of seizure leads to prominent axonal sprouting in the CA3 and CA1 areas and the inferior blade of the dentate (Dhir,

2012; Kotloski et al., 2002). Treatment with CP (500 mg/kg) alone and in combination with SVA (100 mg/kg) and pergolide (1 mg/kg) ameliorated these neuronal losses, thereby suggesting its neuroprotective effect on the epileptic brain. The maximum neuroprotection in the CP group might be due to the activation of dopaminergic and GABAergic neurons in the hippocampus.

Our study has limitations. We used a minimum number of animals for experimentation. The active ingredient in the CP was not identified. Despite these limitations, the study has its strengths. The study addresses a critical unmet medical need, identifying a drug with both antiepileptic and cognitive benefits. We evaluated both neurobehavioral and biochemical parameters and used a gold standard model for antiepileptic evaluation.

5. Conclusion

CP seed extract alone and in combination possesses anticonvulsant, memory-enhancing, and antioxidant properties. Further experiments are required for identifying active ingredients responsible for CP beneficial effect.

Ethical Considerations

Compliance with ethical guidelines

The study was approved by Institutional Animal Ethics Committee (IAEC) (Code: 88/IAEC/598) and Institutional Biosafety Committee (IBC) (Code: 613/IBC).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflicts of interests.

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