Title: Neuroprotective Effect of Celastrus Paniculatus Seed Extract in Epilepsy and Epilepsy-Associated Cognitive Deficits

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Abstract

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

Methods: PTZ kindling model was developed by daily administration of sub-convulsive dose of PTZ 30 mg/kg for 4 weeks. After 4 weeks of induction, the following treatment namely Sodium valproic acid (SVA) 200 mg/kg, C- paniculatus 500 mg/kg, Pergolide 2 mg/kg alone and combination groups C- paniculatus 250 mg/kg+ Pergolide 1 mg/kg and C- paniculatus 250 mg/kg+ SVA 100 mg/kg were administered 30 minute prior to PTZ 30 mg/kg injection for a period of next 14 days. Neurobehavioral parameters Morris water maze test (MWM), Grip strength test (GPS) brain superoxide dismutase (SOD), Catalase, reduced glutathione (GSH) and dopamine level. Hematoxylin & Eosin (H&E) staining of hippocampus pyramidal layers Cornu ammonis (CA1), CA2, CA3, dentate gyrus (DG), and frontal cortex were assessed.

Results: C- paniculatus 500 mg/kg alone and combination groups C- paniculatus 250 mg/kg+ Pergolide 1 mg/kg and Celastrus paniculatus 250 mg/kg+ SVA 100 mg/kg significantly (p<0.05) reduced the seizure score, mean latency time, and distance traveled in MWM. However, no significant effect was seen in grip strength test. Biochemical analysis showed elevated antioxidant markers namely GSH, catalase, and SOD & elevated dopamine levels. C- paniculatus and its combination were also found significantly (p<0.05) protected against neuronal loss in hippocampus and frontal cortex as evidenced by H&E staining.

Conclusion: C- paniculatus alone and its combination may have the potential to treat epilepsy and associated cognitive deficits.

Keywords: C- paniculatus, Hippocampus, Kindling, Oxidative stress, Pentylenetetrazole
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). 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 intelligent 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, neurochemical and histopathological processes in the brain. The uncontrolled neuronal firing also results in formation of reactive oxygen species which is responsible for damage of antioxidant homeostasis and further damage to the brain (Geronzi, Lotti, & Grosso, 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, Rickards, & Cavanna, 2011; Ijff & Aldenkamp, 2013; Jost et al., 2016; Kutt & Jensen, 1986; Wijnen et al., 2017). Therefore, a safe and efficacious drug with a low adverse effect profile and possessing cognitive benefit is an unmet medical need.

*C. paniculatus* (*C. paniculatus*) is a herb well known for its medicinal properties and belongs to Celastreaceae family. It is found in tropical and sub-tropical regions. The plant contains sesquiterpine alkaloids like celastrine, malkanguniol, paniculatin, and celapanin as major active constituents and ample flavonoids and tannins, triterpenoids, and steroids (Bhanumathy, Harish, Shivaprasad, & Sushma, 2010; Shashank, Sv, & Mistry, 2017). In folk medicine, *C. paniculatus* has shown a beneficial effect in various pathological conditions such as muscle cramps, backache, sciatica, osteoarthritis, facial paralysis, and various neurological disorders (Kulkarni, Agarwal, & Garud, 2015; Saroya & Singh, 2018). Experimental data have suggested the beneficial role of CP oil in neuronal protection against glutamate-induced toxicity by modulating glutamate receptor. (Godkar, Gordon, Ravindran, & Doctor, 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, Christofer, & Shankaranarayana Rao, 2016). However, the effect of CP seed extract in epileptogenesis and associated cognitive deficits has not been studied. Therefore, present study evaluated the neuroprotective of *C. paniculatus* seed extract alone and combination in PTZ-induced kindling model.
2. Material & Methods

2.1 Animals

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

2.2 Drug and chemicals

Pentylenetetrazole (PTZ), Pergolide, Sodium valproic acid (SVA) were purchased from (Sigma-Aldrich), USA. C. paniculatus 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 was purchased from Bio-Rad Laboratories, USA. All the chemicals and reagents used in the study were of analytical grade and were 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 & CP was diluted in 0.5% carboxymethylcellulose (CMC). The oral route was used for administering all the interventions.

2.3 Experimental design

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

2.4 PTZ- induced kindling model

PTZ kindling model was developed as per lab standard protocol(Dhir, 2012). The sub convulsive dose of PTZ 30mg/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, 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, Naccari, & De Sarro, 1999; Yazdi, Doostmohammadi, Pourhossein Majarshin, & Beheshti, 2020). The severity of the seizure score was recorded at baseline.
2.5 Neurobehavioral assessment

2.5.1 Morris water maze

The cognitive deficits were evaluated in the Morris water maze test under *Ethovision Noldus XT* 11.5 (EV115-06266) software tracking system. The test protocol was followed as per the literature (Prakash, Chopra, & Medhi, 2013; Prakash, Medhi, & Chopra, 2013; Vorhees & Williams, 2006). The endpoints assessed were mean latency time and distance traveled to reach the platform at baseline, day 28, 35, and 42. The cut off period of all trials was kept at 120 sec.

A grip strength test 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 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, day 28, 35, and 42. The maximum trial length was kept at 150s. Three trials were taken, and inter trial duration was kept at 15-20 minutes (Deacon, 2013).

2.6 Tissue preparation for biochemical and histopathological parameters

On day 42, animals were euthanized using a high dose of pentobarbital sodium i.p. (100mg/kg) and were transcardially perfused with 0.9% normal saline. The brain was extracted and stored in phosphate buffer saline (PBS) at -20°C for the estimation of reduced glutathione (GSH), catalase, superoxide dismutase (SOD), 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 requirement. The supernatant of all seven experimental groups was used for quantitative analysis by UV-Spectrophotometer.

2.6.1 Biochemical analysis

Oxidative stress markers GSH, catalase, and SOD were estimated in the whole brain. Reduced glutathione was estimated by Jollow 1974 method (B. Kumar, Arora, Kuhad, & Chopra, 2012) and expressed as nmol /mg protein. Catalase was estimated by Claiborne 1985 method (B. Kumar et
al., 2012) was expressed in unit/gram tissue. SOD level was measured by pyrogallol autoxidation method and expressed as Unit/ml (MARKLUND & MARKLUND, 1974; Nandi & Chatterjee, 1988). All three methods have been standardized earlier in our laboratory.

2.6.2 Dopamine estimation

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

2.6.3 Histopathological analysis

The formalin embedded brain was dissected into the coronal section. The hippocampus and frontal cortex were preserved and stained in hematoxylin and eosin (H&E) dye to assess the neurodegenerative changes in frontal cortex, DG, CA1, CA2 and CA3 of hippocampal layers. Overall hippocampal neuronal damage was scored by semi quantitative scoring system as: "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); Score 4: Diffuse neuronal injury" (Myung et al., 2004).

2.7 Statistical analysis

Data were expressed as mean ± SEM (standard error of mean). 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 version 3.5.2 was used for statistical analysis. The p-value < 0.05 was considered statistically significant.

3. Results

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

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

3.2 Effect of the CP and combination on Behavioral parameters (MWM, GPS)

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

On day 28 of the kindling, the latency to fall was decreased in all groups compared to the vehicle-treated group and were found non-significant (p>0.05) (Fig. 3 GPS). On day 35 and 42, no significant difference was found between treatment groups than the PTZ group (p>0.05).

3.4 Effect of CP and combination on oxidative stress (GSH, catalase, SOD)

The brain GSH level was decreased in the PTZ group as compared to the vehicle-treated group. In the intergroup analysis, the brain GSH level was increased in CP alone and combination group (CP+pergolide and CP+SVA) as compared to the PTZ group but was found non-significant (p>0.05). (Table 1).
The brain catalase level was reduced in the PTZ group as compared to the vehicle group but was found non-significant (p>0.05). In the intergroup analysis, a statistically significant difference was found in combination groups as compared to the PTZ group (p<0.01) (p<0.05), thereby signifying that the combination groups possess an effect on catalase. Whereas SVA, CP, Pergolide alone groups were found to affect comparable to the PTZ treated group (Table 1).

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

3.5. Effect of CP and combination on dopamine level

The brain dopamine level was reduced in the PTZ group as compared to the vehicle group. In the intergroup analysis, brain dopamine level was significantly increased in SVA, CP, and CP+pergolide groups compared to the PTZ group (p<0.005). Whereas the pergolide alone and combination of CP+SVA showed markedly elevated dopamine levels compared to the PTZ group (p<0.01). (Fig. 4).

3.6. Effect of the CP and combination on histopathological neuronal scoring of the hippocampus and frontal cortex

The overall hippocampal neuronal damage expressed by nuclear chromatin clumping, hyper eosinophilia, condensation of cytoplasm (Fig. 5A) was significantly increased in the PTZ group as compared to the vehicle group (p<0.01). The treatment with SVA, CP, pergolide CP+pergolide, and CP+SVA has shown to cause statistically significant decrease in the histopathological score compared to the PTZ group (p<0.01). (Fig. 5B). The decreased in neuronal injury score in CP alone, and combinations indicate its neuronal protection in PTZ kindled rats.

4. Discussion

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

In the present study, we found that daily administration of PTZ 30mg/kg reduced the threshold for seizure-induced tonic-clonic seizure 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 500mg/kg alone and in combination with pergolide and SVA has shown to reduce the seizure score, decreasing the latency time and distance traveled to reach the platform in Morris water maze, suggesting its protective role in seizure and cognitive deficits. The treatment, however, did not show significant benefit on the grip strength test. Previously, CP has been shown to improve memory in chronic stress-induced cognitive impairment and nitro-propionic induced Huntington disease-like symptoms (Bhagya et al., 2016; Malik, Karan, & Dogra, 2017). Therefore, the current finding of improvement of 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 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, neurodegenerative disorders like Alzheimer's disease, etc. (Balmus, Ciobica, Antioch, Dobrin, & Timofte, 2016; Salim, 2016). Concordance to previous results: In the present study, the sub-convulsive dose of PTZ causes oxidative damage in response to altered antioxidant enzymes' levels. Simultaneously, the antioxidant defense mechanism responds poorly due to prolonged seizure, thus enhancing the occurrence of neurodegeneration in the epileptic brain (Geronzi et al., 2018). The treatment with CP 500mg/kg and its combination elevated the levels of GSH, catalase, and SOD, suggesting its antioxidant property. The present results can be correlated with previous studies that various herbal drugs, including CP, have shown antioxidant and neuroprotective effects in neurodegenerative disorders (da Rocha et al., 2011;
Godkar et al., 2004; M. H. V. Kumar & Gupta, 2002; Malik et al., 2017). The antioxidant property of CP might control the recurrent seizure episodes in this study.

Furthermore, to correlate the dopamine with seizure and associated cognitive deficits, we assessed the level of dopamine in brain tissue. The study result reveals PTZ 30mg/kg lowers the levels of brain dopamine. The treatment of CP500mg/kg alone and in combination with pergolide and SVA significantly increased the levels of dopamine. It was comparable to positive control pergolide (D1 receptor agonist), thereby suggesting that increased dopamine levels may show protective roles in epilepsy and associated cognitive impairment by acting through D2 receptors. The 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 via 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 was shown to exacerbate 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 and inferior blade of the dentate (Dhir, 2012; Kotloski, Lynch, Lauersdorf, & Sutula, 2002). The treatment groups CP500mg/kg and combination with SVA100mg/kg and pergolide 1 mg/kg ameliorate these neuronal losses, thereby suggesting its neuroprotective effect in the epileptic brain. The maximum neuroprotection seen with the CP group might be due to activation of dopaminergic and GABAergic action in the hippocampus.

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

_C- paniculatus_ seed extract alone and its combination possesses anticonvulsant, memory enhancing, and antioxidant properties. Further experiments are required in identifying active ingredient responsible for the beneficial effect.

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Declaration of interest

Authors declared no conflict of interest
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Table 1: Effect of *C- paniculatus* and combination on antioxidant markers; GSH, Catalase, SOD. Data were presented as mean ± SEM. Statistical significance was determined by one way ANOVA followed by post hoc test bonferroni; ## p<0.01 and ###p<0.001 in comparison to vehicle control, *p<0.05 and **p<0.01 and ***p<0.001 in comparison to PTZ group. PTZ: Pentylenetetrazole 30mg/kg, SVA: Sodium valproate 200mg/kg, CP: *C- paniculatus* 500 mg/kg, Pergolide: pergolide 2mg/kg, CP+pergolide: *C- paniculatus* 250 mg/kg+ pergolide 1mg/kg, CP+SVA: *C- paniculatus* 250 mg+ Sodium valproate 100mg/kg.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (nmol/mg protein)</th>
<th>Catalase (Unit/g tissue)</th>
<th>SOD (Unit/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>28.9 ± 6.2</td>
<td>2.82 ± 0.24</td>
<td>41.93 ± 5.81</td>
</tr>
<tr>
<td>PTZ</td>
<td>16.65 ± 4.44</td>
<td>0.37 ± 0.12</td>
<td>13.10 ± 3.00***</td>
</tr>
<tr>
<td>SVA</td>
<td>20.09 ± 1.33</td>
<td>2.30 ± 0.12</td>
<td>32.80 ± 3.39 *</td>
</tr>
<tr>
<td>CP</td>
<td>34.39 ± 2.55</td>
<td>2.74 ± 0.31</td>
<td>31.41 ± 4.07</td>
</tr>
<tr>
<td>Pergolide</td>
<td>29.33 ± 5.44</td>
<td>4.64 ± 0.54</td>
<td>39.70 ± 2.94**</td>
</tr>
<tr>
<td>CP+ Pergolide</td>
<td>38.33 ± 5.92</td>
<td>5.43 ± 1.19 **</td>
<td>40.48 ± 2.30***</td>
</tr>
<tr>
<td>CP+SVA</td>
<td>29.33 ± 8.71</td>
<td>5.17 ± 1.55*</td>
<td>37.74 ± 3.62**</td>
</tr>
</tbody>
</table>
Fig. 1. Experimental design: Behavioral parameters, Morris water maze (MWM) and grip strength test (GPS) was done at day0 without allocating any treatment to rats. After baseline reading, PTZ 30mg/kg, i.p was given to all rats daily except vehicle control from day 1 to day28 or until kindling to induce the model, and then treatments as per the groups were allocated from day 29 to day 42 (for 14 days). After day 42 rats were sacrificed and brain isolated for histopathology, antioxidant markers and dopamine estimation.
Fig. 2: PTZ kindling model and effect of the *C. paniculatus* and combination on seizure score. Vehicle control did not received PTZ30mg/kg showing score 0. A: Seizure scoring was recorded at day 0, 7, 14, 28 to induce the PTZ kindling model. B: Seizure scoring was recorded with treatment at day 0, 28, 35 and 42. Data were expressed in Mean ± SEM (n=6). One way ANOVA followed by Bonferroni post hoc analysis. # represents P<0.05 compared to PTZ group. ** represents overall significant (P<0.01). PTZ: Pentylenetetrazole 30mg/kg, SVA: Sodium valproate 200mg/kg, CP: *C. paniculatus*500mg/kg, Pergolide: pergolide 2mg/kg, CP+pergolide: *C. paniculatus*250mg/kg+ pergolide 1mg/kg, CP+SVA: *C. paniculatus*250mg+ Sodium valproate 100mg/kg.
**Figure 3**: Effect of the *C. paniculatus* and combination on neurobehavioral analysis (MWM and GPS). The outcomes assessed were latency to reach platform (sec) and distance travelled (cm) in case of MWM and latency to fall (sec) in case of GPS.

Data were expressed in Mean ± SEM (n=6). One way ANOVA followed by Bonferroni post hoc analysis. †, †† represents p<0.05, p<0.01 compared to vehicle group. # represents P<0.05 compared to PTZ group. * represents P<0.05 overall significant. PTZ: Pentylenetetrazole 30mg/kg. SVA: Sodium valproate 200mg/kg, CP: *C. paniculatus* 500mg/kg, Pergolide: pergolide 2mg/kg, CP+pergolide: *C. paniculatus* 250mg/kg+ pergolide 1mg/kg, CP+SVA: *C. paniculatus* 250mg+ Sodium valproate 100mg/kg.
Fig. 4. Effect of the *C. paniculatus* and combination on dopamine level. Data expressed as Mean ± SEM (n=6). One way ANOVA followed by Bonferroni post hoc analysis. #, ## represents p<0.05, p<0.01 compared to PTZ group. ** represents P<0.01 overall significant. PTZ: Pentylenetetrazole 30mg/kg, SVA: Sodium valproate 200mg/kg, CP: *C. paniculatus* 500mg/kg, Pergolide: pergolide 2mg/kg, CP+pergolide: *C. paniculatus* 250mg/kg + pergolide 1mg/kg, CP+SVA: *C. paniculatus* 250mg + Sodium valproate 100mg/kg.
Fig. 5. Effect of the *C. paniculatus* and combination on histopathological neuronal scoring of hippocampus and frontal cortex. A: H&E staining results showed the microphotograph of hippocampus CA1, CA2, CA3, DG and frontal cortex region with PTZ and treatments groups. Shrunken and dark pigmented nuclei of neurons showing chromatin clumping (arrows) and cytoplasmic vacuolation in the form of neuronal damage in contrast to treatment groups. B: Graphical representation of neuronal injury of hippocampus in different groups. Data were expressed as Mean ± SEM (n=3) one way ANOVA followed by Bonferroni post hoc test. † represents compared to vehicle group, ## represents p<0.01 compared to PTZ group. ** represents p<0.001 overall significant. PTZ: Pentylenetetrazole 30mg/kg, SVA: Sodium valproate 200mg/kg, CP: *C. paniculatus* 500mg/kg, Pergolide: pergolide 2mg/kg, CP+pergolide: *C. paniculatus* 250mg/kg+ pergolide 1mg/kg, CP+SVA: *C. paniculatus* 250mg+ Sodium valproate 100mg/kg.
Highlights:

1. *C-paniculatus* ameliorate the seizure severity and associated cognitive deficits
2. *C-paniculatus* possesses neuronal protection in hippocampus and frontal cortex and restore the biochemical changes in PTZ kindling model
3. *C-paniculatus* as an adjuvant therapy in the treatment of epilepsy

Plain language summary:

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