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Attenuation of Cognitive Dysfunction in Pediatric Epilepsy with the Co-Administration of Piperine

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Abstract: *Epilepsy is a common neurological disorder with spontaneous recurrent seizures triggered by abnormal electric impulse in brain cortex. It results in cognitive and learning problems along with seizures in children. Present investigation is aimed to decrease the cognitive problems in pediatric epilepsy with adjunct administration of Piperine along with Topiramate. Pentylenetetrazole induced seizures in mice was used as the experimental model. Seizure assessment was done according to the scale given by modified Racine. Cognitive function assessment was done by Morris water maze test and passive avoidance test. Results indicated the maximum protection from seizures both in topiramatetreated and in topiramate co administered piperine groups with maximum latency time to seizures than other groups. In Morris water maze test, there was a significant($p<0.0001$) increase in cognition (31.33%) when piperine co administered with topiramate. It can be concluded that the co administration of piperine in pediatric epilepsy along with topiramate therapy will support in overcoming the cognitive dysfunction.*

Keywords: *Epilepsy, Topiramate, Piperine and Pentalynetetrazole*.

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I. Introduction

Epilepsy is one of the most common neurological disorders with an incidence rate of 0.3 to 0.5%. It affects all age groups, but in children it not only involves seizures but also the problems like, memory deficits, learning disabilities and behavioral problems[1]. These psychological problems may worsen in about 70% of children and lead the adolescents with mental retardation2]. It was also reported that, antiepileptic treatment in pregnant women causes the cognitive impairment in new born [3]. Topiramate (TPM) belongs to the group of novel antiepileptic drugs. TPM was reported as effective and well tolerated treatment in children of age under 12[4]. TPM has an advantage of considerable separation from effective to neurotoxic dose[3] and also found to be nontoxic to the developing brain [5]. TPM has a drawback of high incidence of cognitive problems and adverse effects on some components of cognition that appear to be long lasting even at low doses [6].Recent pharmacological studies show the herbal therapies and functional foods with increased attention as an alternate strategies to prevent various diseases. Among the available herbal remedies, Piperine (PIP) was found to possess both cognitive enhancing and anti-epilepticeffects [7]. The aim of present investigation is to prevent the cognitive problems of TPM therapy with the co-administration of PIP.

II. Materials and Methods

PentyleneTetrazole and Piperine were purchased from Sigma Aldrich, Mumbai. 0.5% Carboxy Methyl Cellulose (CMC) was purchased from SD fine chemicals, Mumbai, Topiramatewas a gift sample from Dr. Reddy's Labs, Bachupally. All the other reagents used were of analytical grade.

2.1. Animal Experiments

Male young albino mice weighing ≈30 g (2-3 weeks) were obtained from the central animal house of Swami RamanandaTirtha Institute of Pharmaceutical Sciences, Nalgondaand were group housed in poly-acrylic cages with not more than four animals per cage. They were maintained under standard laboratory conditions with a natural 12H light and dark cycle. Standard pellet diet and tap water were provided. 12H before investigations, the mice were deprived of food. The study protocol was approved by the Institutional Animal Ethics Committee of SwamiRamanandaTirtha Institutes of Pharmaceutical Sciences

(1468/PO/a/11/CPCSEA/107/2013) and conducted according to the CPCSEA guidelines. The animals were randomly divided into five groups.
Control: Mice were not given with any treatment but administered 0.5% CMC through PO and named as control.
Epilepsy was induced by the administration of PTZ (60mg/kg/week) to all the remaining groups.
Group 1: This group was served as disease control group.
Group 2: Mice were administered TPM (20mg/kg/week) in normal saline through IP route.
Group 3: Mice were administered PIP (5mg/kg/week) in 0.5% CMC through PO.
Group 4: Mice were treated with TPM (20mg/kg/week) with co-administration of PIP (5mg/kg/week).
The mice were treated with TPM and PIP 30 min before PTZ administration.

2.2. Seizure assessment

The animals were placed in cage and observed for 30 min for latency of seizure onset and mortality. The animals that survived after that period of time were considered to be protected. Furthermore, each seizure was classified according to a modified Racine scale. This scale consists of six stages that correspond to the successive developmental stages of motor seizures[8].

2.3. Cognitive function assessment

First, the initial transfer latency for the Morris water maze and then the initial latency for passive avoidance were noted down for mice. This was followed by drug and PTZ administration. 24H after administration the animals were observed for retention latency. Passive avoidance test was performed on 34 and 35days. Morris water maze test was conducted from 14^{th} to 21^{st} days.

2.3.1. Morris water maze test

Briefly, the apparatus consisted of a circular tub (180X50 cm) filled with water and a depth of 24 cm[9, 10] A transparent escape platform (diameter =8 cm) was placed 1 cm below the water surface. The mice were placed in water at the midpoint. The mice were allowed to swim freely until they found and climbed onto the platform. If a mouse failed to locate the platform within 60s, it was placed on the platform for 5s. During training each mouse was submitted to 6 trials per day, and the starting position was changed at each trial. After 5 training days, there was a gap of 7 days. During data analysis the latency to platform on day 7 with visible platform was measured and this indicates the adaptive memory and learning.

2.3.2. Passive avoidance test

During training, the mice were placed in an illuminated box (40×40×30 cm) connected to a dark box (40×40×30 cm), which was equipped with a metal grid floor. Entrance to the dark box was punished by administration of an electric foot shock (0.7 mA for 2 seconds). Animals that did not enter the dark compartment were excluded from the experiment. On the next day (24 hours later), the same animals were placed in the illuminated box and observed up to 180 seconds.Latency to entry into the dark box was recorded. Cognitive function was tested with a passive avoidance task at baseline and 24 hours later[8].

2.4. Statistics

Statistical analysis was done by two way ANOVA followed by Dunnet's multiple comparison and value of $P < 0.05$ was used as statistical significance. Data are expressed as mean ± SEM. The treated group animal variables compared with normal control group variables.

III. Results and Discussion

3.1. Seizure assessment

In the seizure score paradigm vehicle control group did not show any seizure score. PTZ control group showed significant seizure score. While TPM with PIP treated group showed the seizure score less than other treated groups. Over the treatment period of 5 weeks animals almost in all groups except in PTZ group indicated slow recovery and gradual increased response to the drug treatment from week 3.

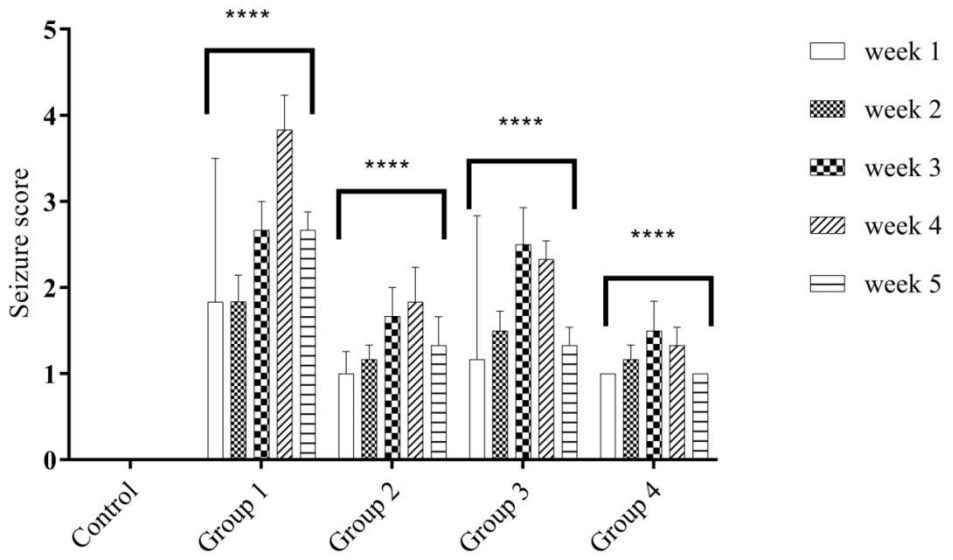

Fig No 1: Seizure score of the animals. Values are expressed as mean±SEM. Data marked with **** are p<0.0001 when compared to control group by two way ANOVA followed by Dunnets multiple comparison test. F (16, 125) = 2.909 with an overall interaction significant at p<0.001

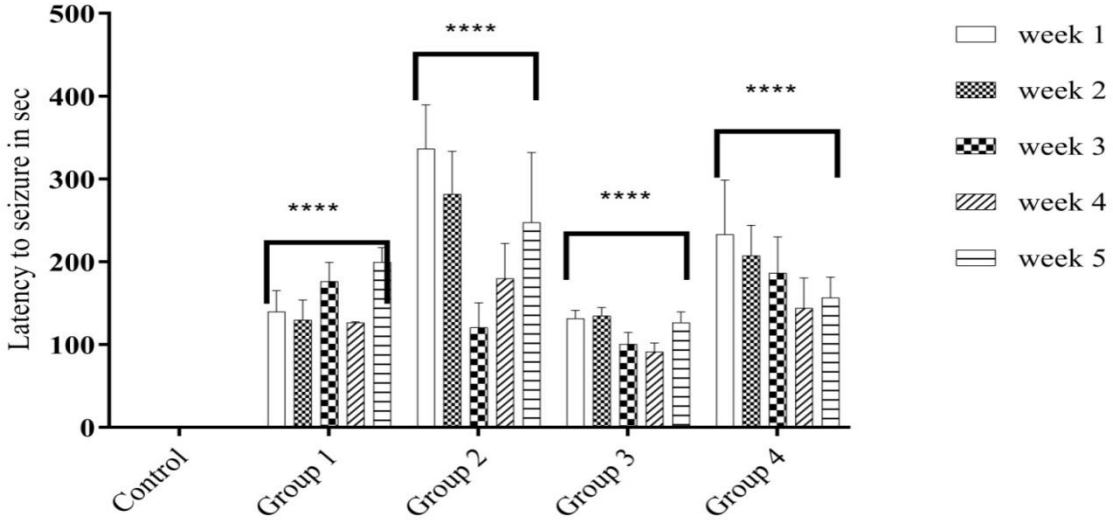

Fig No 2: Latency to seizures. Values are expressed as mean±SEM. Data marked with **** are p<0.0001 when compared to control group by two way ANOVA followed by Dunnet's multiple comparison test. F (16, 125) = 9.345 with an overall interaction significant at p<0.0001

The latency to seizure was found to be more in TPM treated group indicating the maximum protection offered by the drug against PTZ treatment. PIP treated group showed the considerable latency to seizure but was less than TPM treated group. A clear evidence of drug effect in TPM treated animals from week 3 is visible with the increased latency time to seizure in the next period. Even the similar effect is visible but less with group 3 and 4. PIP alone could not show much increase the latency to seizure.

3.2. Cognitive assessment

3.2.1. Morris water Maze Test

With respect to Morris water maze test, the latency time to reach the platform indicates the memory/cognitive function of the animal. Less is the time taken to reach the platform; more will be the cognitive function. From Fig3, it is observed that the group treated with both PIP and TPM significantly (p<0.05) decreased the latency time to reach the platform. This effect was even found to be consistent

throughout the testing period of 7 days. Similar effects were observed with the group treated with PIP alone. The group treated with TPM alone did not show significant decrease in latency time to reach the platform.

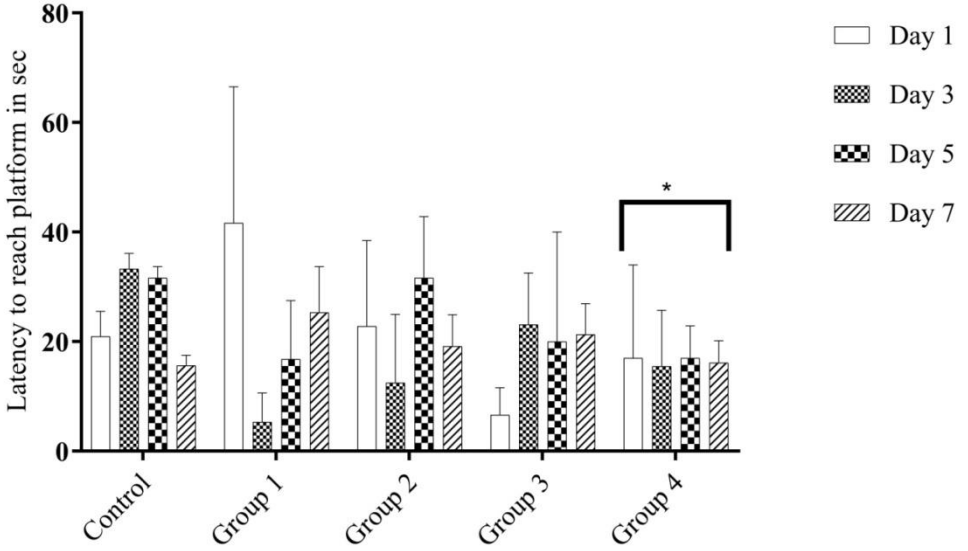

Fig No 3: Latency to reach the platform in Morris water maze test. Values are expressed as mean±SEM. Data marked with * are significant at p<0.05, when compared to control group by two way ANOVA followed by Dunnets multiple comparison test. F (12, 100) = 4.939with an overall interaction significant at p<0.0001

3.2.2. Passive avoidance test

Memory retention was evaluated by passive avoidance test. From Fig 4, it was found that PTZ treatment significantly (p<0.0001) damaged the memory retention. TPM treatment decreased the latency to avoid the shock significantly (p<0.0001) with a 13.78% delay in the retention. In the groups treated with PIP alone and in combination with TPM there is clear memory retention by 7.5% and 31.88% respectively

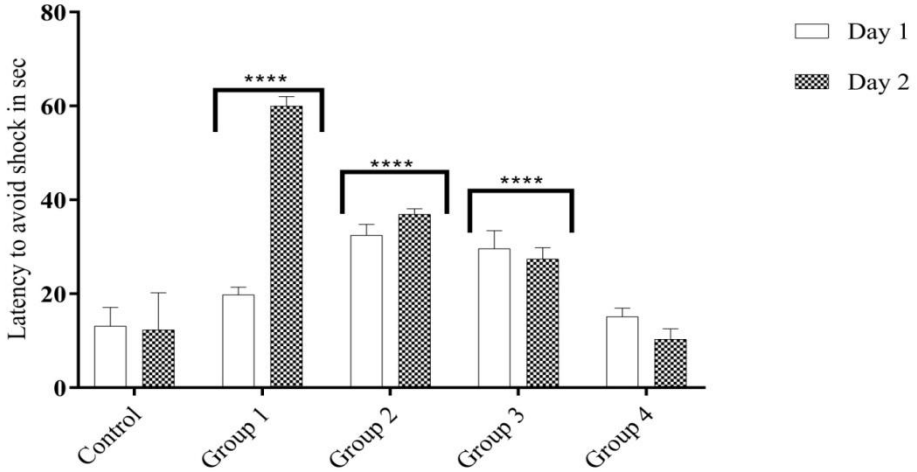

Fig No4: Latency to avoid shock in passive avoidance test. Values are expressed as mean±SEM. Data marked with **** are p<0.0001 when compared to control group by two way ANOVA followed by Dunnets multiple comparison test. F (4, 50) = 87.2with an overall interaction significant at p<0.0001

IV. Discussion

TPM is one among the wide available drug treatments in pediatric epilepsy. Its monotherapy is effective and well tolerated in patients with newly diagnosed epilepsy [11]. It is also effective and well tolerated in children under 12 years in a broad range of epilepsy syndromes, including refractory partial and symptomatic generalized epilepsy [4]. Recent study proved the effectiveness of TPM as safe alternate for prophylaxis of pediatric migraine with a maintenance dose of < 2mg/kg/day [12]. TPM significantly improves quality of life

based on seizure frequency in children with epilepsy, suggesting its potential clinical benefits[13]. The adverse effect of TPM on some components of cognition was reported to be long-lasting, even at low doses, and this may influence further occupational functioning or academic achievement[6].A recent study says, the quality of life in children with epilepsy is affected by cognitive comorbidities, language disorders which may further lead to academic under achievement long term social, professional and psychological problems [14].In present investigation, adjunct PIP therapy was given along with TPM treatment with an aim to solve the cognitive dysfunction.

Pentylenetetrazole (PTZ) is a tetrazole derivative and is used to induce convulsions in animal models by inhibiting GABA activated channels[15]. More over oxidative stress involved in PTZ model at a dose above 40mg/kg leads to an increase in the free radical generation[16]. Another study reported that, PTZ induced seizure activity mimics the oxidative stress in brain by altering membrane phospholipid metabolism and ultimately results in the release of free radicals leading to epilepsy[17].

From the results of seizure score and latency to seizure, it was observed that animals treated with the drug TPM had shown good recovery from PTZ induced seizures. Anticonvulsant effect was found to be better with the time period of treatment. All the investigations about seizures depicted the same up to 5 weeks. The possible reason might be the ability of TPM to reduce the epilepsy by a combination of mechanisms including activation of voltage sensitive sodium channels and increased levels of ɣ amino butyric acid (GABA) levels through GABA receptors [18].

We can observe the protection from seizures even in the groups treated with PIP. It showed control over seizures but not effective as TPM. PIP is an alkaloid from piper genus (Piperaceae family). It is known to have many pharmacological actions. PIP was reported to execute anticonvulsant action by multiple mechanisms including activation of GABA neurotransmission and increased amino acid levels in striatum. It'santi-inflammatory and antioxidant properties also help in controlling convulsions [19]. Literature also supports the anti-oxidant activity of PIP. It can inhibit lipid peroxide formation and can reduce acid phosphatase resulting in the free radical scavenging [20]. These might be the reasons for the additive effect of controlled seizure score with the co administration of TPM and PIP.

Cognitive function was assessed by Morris water maze test and passive avoidance test. More latency to reach the platform indicates cognitive dysfunction or memory impairment. Similarly more latency to avoid shock in passive avoidance test also indicates the same. Observations from the study indicated that the animals in disease control and in TPM treatment showed distinct cognitive dysfunction. Structural alterations, channelopathies, bad synapses and improper dendrites might be the reasons for cognitive impairment in pediatric epilepsy[21]. Seizures increase the accumulation of free radicals in the brain and decrease the natural anti-oxidant defense mechanism[22].Reported clinical proofs also indicate TPM induced cognitive impairment including attention, memory and language difficulties. Possible mechanisms may be apoptosis related neuronal suppression or the drug gets bio activated by free radical species and may bind to DNA, protein or lipids[23].

In our present investigation there is a significant reduction in latency time in groups treated with PIP indicating enhanced cognitive function. PIP could exert memory enhancing effect by increasing serotonin levels in hippocampus region of the brain. It is the region which plays important role in learning and memory [24]. PIP can improve neuron density in the hippocampus and alsoinhibits acetyl cholinesterase enzyme leading to increase in the acetyl choline, a neuro transmitter in the brain [25]. These might be the underlying mechanisms for the cognitive enhancement by PIP.

In PTZ induced epilepsy, there is a possibility of generation of free radicals and they can accumulate excessively in the brain [17, 26]. This will attenuate neuronal function resulting in neuro degeneration with cognitive decline. Glutathione is an important endogenous defense mechanism against oxidative stress. It can either detoxify the oxygen free radicals or directly reduces the lipid peroxidation. PIP being an antioxidant elevates the glutathione levels or prevents its depletion [27]. This will help in controlling the oxidative damage and protecting the cognitive function.

V. Conclusion

TPM is the drug with significant control over seizure and with improved quality of life available for pediatric epilepsy. It is one of the best available options for early pediatric epilepsy. The drug treatment suffers with the problems of cognitive dysfunction and learning disabilities. PIP is the natural alkaloid with clinical evidence of epileptic control, antioxidant and cognitive improvement effects. Co administration of PIP with TPM in adjusted doses is going to improve the quality of life in pediatric epilepsy without leading to long term social, professional and psychological problems.

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