# Protection of prefrontal cortex neurons and improvement of memory in epileptic rats by 1-triacontanol cerotate

Snehunsu ADHIKARI <sup>1</sup> (b), Sayam SUBHASH <sup>2</sup> (b), Satheesha NAYAK <sup>3</sup> (b), Christofer THOMAS <sup>4</sup> (b), Chinmay SURYAVANSHI <sup>5</sup>\* (b)

- <sup>1</sup> Department of Physiology, Netaji Subhash National Institute of Sports, Sports Authority of India, Ministry of Youth Affairs and Sports, Kolkata, India.
- <sup>2</sup> Department of Physiology, Government Medical College, Kollam, India.
- <sup>3</sup> Department of Basic Medical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka 576104, India.
- <sup>4</sup> Department of Physiology, All India Institute of Medical Sciences, Kalyani, West Bengal, India.
- <sup>5</sup> Department of Physiology, Kasturba Medical College Manipal, Manipal Academy of Higher Education, Manipal, Karnataka 576104, India.
- \* Corresponding Author. E-mail: chinmay.as@manipal.edu (C.S.); Tel. +091-990-294 36 13.

Received: 9 November 2023 / Revised: 27 January 2024 / Accepted: 29 January 2024

**ABSTRACT**: Epilepsy treatment continues to face significant challenges, including drug resistance and cognitive impairment associated with antiepileptic drugs. 1-Triacontanol cerotate (1TAC), an active component isolated from *Marsilea quadrifolia* Linn., has emerged as a potential therapeutic avenue for epilepsy. This study investigated the effects of 1TAC on prefrontal cortical neurons and memory retention in chronically epileptic rats, comparing its efficacy to sodium valproate. Our experiment utilized two-month-old adult male Wistar rats, randomly assigned to one of five groups: I - Vehicle Control, II - Rats receiving Pentylenetetrazol (PTZ) at a dosage of 35 mg/kg body weight intraperitoneally every 48 hours, III - Rats given 200 mg/kg body weight of sodium valproate 30 minutes prior to PTZ administration, IV and V - Rats administered 40 and 80 mg/kg body weight of 1-TAC orally 30 minutes before PTZ challenge, respectively. To assess memory performance, we conducted a passive avoidance test. Subsequently, brain specimens were processed for cresyl violet staining to assess cell densities. Results demonstrated that epileptic rats treated with sodium valproate before PTZ administration, and those given 80 mg of 1-Triacontanol cerotate exhibited significantly enhanced memory retention compared to the untreated epileptic group at both 24- and 48-hours post-challenge. Furthermore, 80 mg of 1-Triacontanol cerotate administration showed a protective effect by significantly reducing the loss of pyramidal cells in the medial prefrontal cortex. This intervention effectively minimized the loss of pyramidal neurons in the medial prefrontal cortex and mitigated memory deficits in chronically epileptic rats.

KEYWORDS: 1-Triacontanol cerotate; epilepsy; Marsilea quadrifolia Linn; memory; prefrontal cortex.

### 1. INTRODUCTION

Epilepsy, a chronic neurological disorder, transcends age and geographical boundaries. According to recent estimates, approximately 49.9 million individuals worldwide are actively experiencing epilepsy, making it a substantial contributor to disability and mortality [1,2]. However, it is imperative to recognize that the impact of epilepsy extends far beyond seizure episodes. The interictal period, the interval between successive seizures, plays a pivotal role in neuronal survival. During the ictal phase, aberrant high-frequency activity in the brain culminates in an excessive release of glutamate, detrimentally affecting the neuronal microenvironment [3,4]. The alteration of the balance between excitatory and inhibitory systems, shifting towards increased excitatory or decreased inhibitory neurotransmission, has been postulated as a potential mechanism for epileptic discharges [5]. This interictal shift towards neuronal hyper-excitability can lead to significant consequences, including neuropsychological and cognitive impairments that affect learning, memory, attention, and related functions [6]. Additionally, during interictal periods, neurons encounter oxidative stress, undergo apoptosis, experience dendritic atrophy in pyramidal neurons, and suffer the loss of neuronal interconnectivity [7–9].

Standard therapies for epilepsy primarily target the reduction of seizure occurrences by diminishing nerve cell excitability, while not directly interrupting the epileptogenic process [10,11]. Given that epilepsy is a chronic disorder, extended use of antiepileptic treatments is associated with cognitive blunting, as these

How to cite this article: Adhikari S, Subhash S, Nayak S, Thomas C, Suryavanshi C. Protection of prefrontal cortex neurons and improvement of memory in epileptic rats by 1-triacontanol cerotate. J Res Pharm. 2024; 28(5): 1572-1580.

treatments suppress membrane excitability, induce sedation, and augment postsynaptic inhibition, ultimately reducing excessive neuronal excitability linked to seizure development [12–14]. Despite the utilization of three generations of antiepileptic drugs (AEDs), substantial progress in seizure prevention has not been achieved, as the 'one-drug-one-disease' treatment approach has proven unsuccessful [15]. Furthermore, even with the adoption of multiple drug approaches like polytherapy, approximately 40% of individuals with epilepsy exhibit treatment resistance [16]. Compounding these challenges is the observation that in low-income countries, where 80-90% of patients lack access to AEDs due to financial constraints, epilepsy-related mortality is 2.6 times higher than in the general population [17]. Considering these factors, the management of chronic epilepsy remains a formidable challenge, and herbal medicine emerges as a potential solution due to its cost-effectiveness and reduced side effects.

*Marsilea quadrifolia* Linn (MQ), commonly known as European water clover, is an aquatic fern species with a widespread distribution in regions such as Asia, Africa, and Australia. This plant has a rich history in traditional medicine, where it has been used to address various health issues, including fever, asthma, and inflammation. In a previous study, we reported the antiepileptic potential of MQ methanolic extract in preventing seizures and the resulting neuronal damage in Pentylenetetrazol (PTZ)-induced epileptic rats [18]. Additionally, our investigation unveiled the significant impact of 1-Triacontanol cerotate (1TAC), an isolated compound from MQ, in reducing reactive oxidative stress in the brain and alleviating memory deficits [19]. Building on these prior findings, this study aims to evaluate the effects of 1TAC on pyramidal neurons in the prefrontal cortex and memory retention in chronically epileptic rats, comparing its efficacy with sodium valproate (SV), a standard antiepileptic drug.

# 2. RESULTS

# 2.1. Effect of 1TAC on the Two-Compartment Passive Avoidance Test

Memory retrieval was assessed using the step-through passive avoidance task to evaluate the impact of 1TAC treatment prior to chronic PTZ kindling. During the exploratory trial, rats with PTZ kindling exhibited a slightly longer latency ( $11.5 \pm 1.84$  seconds) to enter the dark compartment in the first trial. However, this difference was not statistically significant (F (4, 45) = 2.477, p = 0.57) (Table 1). Subsequently, in later trials, all groups demonstrated a decreased latency to enter the dark chamber, which aligns with rats' natural preference for dimly lit environments. Additionally, there was no significant difference in the average latencies across the three trials between the groups upon entering the dark compartment (F (4, 45) = 0.8409, p = 0.51) (Figure 1A).

Entrance latency	VC	PTZ+DW	SV+ PTZ	1TAC40+PTZ	1TAC80+PTZ
(seconds)					
Trial 01	$7.00 \pm 0.36$	$11.50 \pm 1.84$	$7.50 \pm 0.99$	$10.67 \pm 1.50$	8.00 ± 1.21
Trial 02	$6.33 \pm 1.12$	$7.50 \pm 2.33$	$6.50 \pm 1.18$	$10.00 \pm 1.37$	$6.17\pm0.87$
Trial 03	$5.33 \pm 0.21$	$7.83 \pm 0.48$	$6.50 \pm 1.06$	$6.83 \pm 0.83$	$4.67\pm0.76$

**Table 1.** Effect of 1-Triacontanol Cerotate (1TAC) on entrance latency to the dark compartment.

Data expressed as mean ± SEM; VC-Vehicle Control; PTZ+DW - Pentylenetetrazol + Distilled Water; SV+PTZ - Sodium Valproate + Pentylenetetrazol; 1TAC40+PTZ - 1-Triacontanol cerotate 40mg + Pentylenetetrazol; 1TAC80+PTZ - 1-

Triacontanol cerotate 80mg + Pentylenetetrazol.

Retention memory tests conducted at 24 hours (F (4, 45) = 4.122, p = 0.006) and 48 hours (F (4, 45) = 4.416, p = 0.004) after the shock revealed significant differences among the groups. As depicted in the graph, PTZ-kindled epileptic rats exhibited retention memory impairment in both the 24-hour (48  $\pm$  9.71 seconds) and 48-hour (M = 51.33, SD = 13.06) post-shock tests compared to the control group (131  $\pm$  31.02 seconds) (Fig. 1A, B). These findings suggest impaired memory consolidation in the PTZ kindling group. Epileptic rats treated with sodium valproate before PTZ demonstrated significantly better memory compared to the epileptic group of animals after 24 hours (126.33  $\pm$  11.08 seconds) and 48 hours (104.83  $\pm$  17.21 seconds). Interestingly, epileptic animals that received 1TAC at 80 mg/kg body weight (136.83  $\pm$  18.62 seconds) showed results similar to rats that received sodium valproate (p < 0.05). The latency was significantly higher

compared to the PTZ group. Furthermore, rats receiving 40 mg/kg body weight ( $82.67 \pm 16.15$  seconds) exhibited better memory retention than the epileptic animals (Figure 1B).



**Figure 1.** Effect of chronic epilepsy and treatment with SV and 1TAC on latency to enter the dark compartment. Data expressed as mean  $\pm$  SEM; (**A**) - exploratory trials, (**B**) 24 hours and (**C**) 48 hours after the shock trial. One-way ANOVA followed by Tukey's post hoc test, \*p<0.05 vs. VC. ##p<0.01; # p<0.05 vs. PTZ+DW.

VC-Vehicle Control; PTZ+DW - Pentylenetetrazol + Distilled Water; SV+PTZ - Sodium Valproate + Pentylenetetrazol;1TAC40+PTZ - 1-Triacontanol cerotate 40mg + Pentylenetetrazol; 1TAC80+PTZ - 1-Triacontanol cerotate 80mg + Pentylenetetrazol

The retention test after 48 hours displayed a similar trend to the results at 24 hours, with one notable exception. In all groups, there was a decrease in the time taken to enter the dark compartment, except in the PTZ kindling group, which experienced an increase in latency from 24 to 48 hours. Animals administered with 80 mg/kg body weight of 1TAC ( $113.67 \pm 8.39$  seconds) stayed in the light compartment for a longer duration compared to the PTZ kindling group ( $51.33 \pm 13.06$  seconds). The 1TAC group exhibited a more significant change compared to the sodium valproate group ( $104.83 \pm 17.21$  seconds), indicating better memory consolidation. (Figure 1C)

# 2.2. Effect of 1TAC on neuronal cell count in the medial prefrontal cortex (mPFC)

Cell density in prefrontal cortex sections stained with cresyl violet (CV) was examined to assess the effect of ITAC. Photomicrographs of the studied groups, illustrating the cell densities, are presented in Fig. 2. It's evident that epilepsy led to a reduction in cells in the prefrontal cortex. The quantitative estimation of neuronal cell counts in the mPFC revealed significant differences among the various treatment groups (F (4, 45) = 12.88, p < 0.001). Cell counts of epileptic rats (M = 206.67, SD = 11.04) showed a significant reduction compared to the vehicle group (M = 311.5, SD = 5.65) in the square mm area, with an estimated reduction of around 34%. Figure 3 demonstrates that treatment with valproate (M = 272.17, SD = 4.83) significantly prevented neuronal loss in the frontal cortex compared to the epileptic rats. Animals pre-treated with 40 mg/kg body weight of 1TAC (M = 246.33, SD = 17.14) were unable to protect against neuronal loss due to epilepsy in the medial prefrontal cortex. However, a higher dose of 80 mg/kg body weight (M = 296.83, SD = 14.32) was found to be protective, as the treatment significantly prevented epilepsy-induced neuronal loss, with the difference in cell density of animals treated with 80 mg/kg body weight being only 5% less than the vehicle control group. (Figure 3)





**Figure 2.** Representative image of prefrontal cortex sections stained with cresyl violet for different groups. Scalebar representing 40µm length. VC-Vehicle Control; PTZ+DW - Pentylenetetrazol + Distilled Water; SV+PTZ - Sodium Valproate + Pentylenetetrazol; 1TAC40+PTZ - 1-Triacontanol cerotate 40mg + Pentylenetetrazol; 1TAC80+PTZ - 1-Triacontanol cerotate 80mg + Pentylenetetrazol.



**Figure 3.** Effect of 1TAC on pyramidal cell count in the medial prefrontal cortex. Data expressed as Mean ± SEM. One-way ANOVA followed by Tukey's post hoc test, \*\*\*p<0.001; \*\*p<0.01 vs. VC. ###p<0.001; ## p<0.01 vs. PTZ+DW.

VC-Vehicle Control; PTZ+DW - Pentylenetetrazol + Distilled Water; SV+PTZ - Sodium Valproate + Pentylenetetrazol;1TAC40+PTZ - 1-Triacontanol cerotate 40mg + Pentylenetetrazol; 1TAC80+PTZ - 1-Triacontanol cerotate 80mg + Pentylenetetrazol

# 3. DISCUSSION

In this study, we conducted experiments employing a well-established rat model of chronic epilepsy induced by a sub-convulsive dose of PTZ at 35 mg/kg body weight, administered once every 48 hours. Our findings indicate that 1TAC exhibits promise as an antiepileptic agent, providing protection against brain damage induced by seizures. This investigation focuses on evaluating the impact of 1TAC on memory impairments associated with epilepsy and neuronal loss in the prefrontal cortex. Given the dose-dependent response observed in our prior research involving 1TAC, we administered two doses in this study, specifically 40 and 80 mg/kg body weight. Furthermore, we compared their effects with those of a standard pharmacological drug, sodium valproate, to validate the efficacy of the active component extracted from MQ. Our study demonstrates that 1TAC significantly ameliorates memory impairment, as assessed by passive avoidance tasks, and prevents neuronal loss in the medial prefrontal cortex. Notably, we observed that the 80 mg/kg body weight dose of 1TAC is more effective than the 40 mg/kg body weight dose and more effective than sodium valproate treatment in rats.

Passive avoidance testing serves as a widely employed and standardized behavioural paradigm for assessing memory function in experimental rodent models. Previous studies have demonstrated memory impairment, as assessed through passive avoidance testing, in various chronic epilepsy models [20-22]. The causes of learning and memory impairment in epilepsy are multifaceted and may result from structural and functional loss of neurons and their connectivity. Neuronal loss can be attributed to increased neuronal calcium influx, leading to glutamate excitotoxicity, reduced acetylcholinesterase activity, diminished or aberrant neurogenesis, increased NMDAR activity with decreased GABAergic function, and oxidative stress in brain regions including the hippocampus, amygdala, and frontal cortex [4,20]. Many studies involving herbal remedies have shown therapeutic potential, with a protective mechanism possibly related to their ability to scavenge free radicals and act as antioxidants. These herbal treatments have demonstrated the capacity to prevent neuronal death and, consequently, neuronal loss. In our experimental model, a chronic epileptic model, we administered 1TAC as a protective agent into the brain prior to PTZ injection. Therefore, 1TAC also functions as a protective agent against epilepsy-induced brain damage. Previous research has revealed that 1TAC could reverse the levels of antioxidants (MDA and GSH) in the hippocampus and frontal cortex [19]. These reversals were associated with improvements in hippocampal-dependent learning, a decrease in the frequency of seizures, and increased neuronal densities in the hippocampus. Our present study demonstrates that 1TAC protected frontal cortical neurons and improved memory retention, likely due to its potential antioxidant properties, which safeguarded neurons in the frontal cortex and hippocampus of epileptic rats [23].

Despite the limited research on MQ's therapeutic properties, various properties have been attributed to it, including anticonvulsant, sleep-inducing, diuretic, anti-inflammatory, and sedative effects [24]. A study by Zahan et al. has demonstrated the plant's hypoglycaemic and in vitro antioxidant properties. This was demonstrated through evaluating total phenolic content, total antioxidant capacity, DPPH scavenging ability, and reducing power assay [25]. MQ also exhibits notable anti-cholinesterase activity. The cholinergic system, particularly in the hippocampus, is well-recognized for its critical role in learning and memory formation. Deficiencies in this system have been associated with the development of conditions like dementia and Alzheimer's disease, as evidenced by both experimental and clinical studies [26]. In this context, prior studies have demonstrated the anti-cholinesterase activity of MQ's methanolic extract, which elevates acetylcholine levels in the brain [27]. A recent investigation by Subramanian et al. delved into the neuroprotective potential of MQ in excitotoxicity. Their findings revealed that MQ mitigated monosodium glutamate-induced neurotoxicity [28].

### 4. CONCLUSION

The administration of 1-Triacontanol cerotate effectively alleviated the loss of pyramidal neurons in the medial prefrontal cortex and ameliorated memory deficits in chronically epileptic rats. It can be postulated that the anti-cholinesterase and antioxidant properties of *Marsilea quadrifolia* are likely the primary scientifically established reasons for its protective role against epilepsy-induced brain damage. Further studies are needed to elucidate the potential molecular mechanisms of the active component in improving epilepsy-induced cognitive deficits.

# **5. MATERIALS AND METHODS**

This study utilized adult male Wistar rats, two months of age, which were procured from the University's Central Animal House Facility. They were housed in controlled light and dark cycles of 12 hours with unrestricted access to food and water. All possible measures were taken to reduce the level of discomfort experienced by animals and limit the number of animals employed in the study. The study was approved by Institutional Animal Ethics committee (IAEC No. IAEC/KMC/23/2012). The experimental procedures were conducted in accordance with standardized protocols under the National Institute of Health Guidelines for the Care and Use of Mammals in Neuroscience and Behavioural Research.

## 5.1. Experiment design

The rats were allocated randomly to distinct groups, with ten rats in each group. Procedures involving animal preparation, the induction of chronic epilepsy through PTZ kindling, and the compound administration method were conducted following established protocols as outlined in a previous study [19]. Specific dosages of the compounds administered to each group are provided below.

- **I. Vehicle Control (VC):** For thirty days, animals in the VC group were administered 0.9% sodium chloride intraperitoneally and an equivalent volume of distilled water orally.
- **II. PTZ + Distilled Water (DW):** Animals in the PTZ+DW group were administered a dosage of 35 mg/kg of body weight of PTZ intraperitoneally, once every 48 hours. An equal volume of DW was given orally.
- **III. SV+ PTZ**: Animals in the SV+PTZ group received 200 mg/kg of body weight of SV 30 minutes before the administration of PTZ, every 48 hours for 30 days.
- IV. 1-Triacontanol cerotate 40mg (1TAC40) + PTZ: For 30 days, animals in the 1TAC40+PTZ group received 40 mg/kg of body weight of 1TAC orally, 30 minutes before the administration of PTZ, every 48 hours.
- V. **1-Triacontanol cerotate 80mg (1TAC80) + PTZ:** This group received 1TAC and PTZ in a similar manner to the 1TAC40 + PTZ group, but with a higher dosage of 1TAC at 80 mg/kg of body weight.

### 5.2. Isolation of active components

Isolation and administration of active components were performed as described in a previous study [19]. Initially, a total of 5 kg of shade-dried, coarsely powdered MQ leaves were extracted with methanol using a Soxhlet extractor in 750 g increments. Subsequently, the resultant extract was distilled to remove the solvent, resulting in a final extract of 9.09 g per 100 g of dried MQ leaves.

The methanolic extract was further fractionated into petroleum ether, dichloromethane, and nbutanol fractions, followed by solvent removal via distillation. The petroleum ether fraction (3.0 g) was subjected to column chromatography using a silica gel column. This process employed a stepwise elution gradient of petroleum ether and ethyl acetate. The outcome of this chromatographic process yielded a pure compound of 1TAC, presenting as a white powder with a melting point within the range of 82–84 °C. The quantification of 1TAC within the petroleum ether fraction was determined to be 0.76% w/w relative to the dry weight of the plant material.

Furthermore, the purity of the compound was confirmed through high-performance thin-layer chromatography (HPTLC) analysis, employing a toluene: ethyl acetate (9.5:0.5) solvent system on silica gel 60 F 254 plates. This analysis revealed a purity of over 96%, based on the total peak area in the HPTLC analysis, confirming the compound's purity.

### 5.3. Step-through passive avoidance test

Upon completion of the one-month experimental phase, memory retention was assessed using a twocompartment passive avoidance apparatus, following the methodology outlined in a previous study [29]. The apparatus comprised a brightly illuminated large compartment and a small dark compartment. The experimental protocol encompassed three distinct stages: an initial exploratory assessment, a learning test involving aversive stimulation, and a final retention test. During the initial exploration test, a three-trial procedure was employed. Rats were placed in the centre of the large compartment, facing away from the entrance to the dark compartment, with the door connecting the two compartments left open. Rats were permitted to explore freely, and the latency to enter the dark compartment was recorded. Each rat completed three separate trials, with a 5-minute interval between each trial. Following the final exploration trial, the rat was placed in the larger compartment in a manner consistent with the trial sessions. Once the rat entered the dark compartment with all four paws crossing the threshold, the door was promptly closed, and three strong foot shocks (50 Hz, 1.5 mA, 1-second duration each) were administered at 5-second intervals. Retention tests were conducted 24 and 48 hours after the aversive conditioning session, and the latency to enter the dark compartment was recorded as a measure of retention memory. Extended latencies and aversion to the dark compartment were indicative of contextual memory formation.

# 5.4. Histological study

The animals from different groups were processed to detect the number of surviving cells in the medial prefrontal cortical region through CV staining. Following decapitation, the animals underwent transcardiac perfusion. The brains were carefully extracted and prepared for paraffin embedding. Using a rotary microtome (Leica RM2155, Germany), we obtained coronal brain sections measuring 40 µm in thickness. Subsequently, every sixth section underwent CV staining [23].

# 5.5. Quantification of pyramidal cells in the medial prefrontal cortical region

Viable pyramidal cells were enumerated using a light microscope set at a total magnification of 400X (Motic Image Plus 2.0, China). Counting was systematically carried out on every sixth section, and within each animal, a detailed analysis of three sections was performed along the spatiotemporal axis encompassing the entire prefrontal cortex. The spatial orientation of the axis, as well as the precise boundaries of the prefrontal cortex, were determined in accordance with the rat brain atlas [30]. Evaluation was conducted for both the right and left medial prefrontal cortices within each section. To maintain impartiality in the cell counting process, the microscope slides were coded by an investigator unaware of the experimental conditions, ensuring a double-blinded approach. Only cells displaying distinct nuclei and well-defined nuclear boundaries were considered. The quantification of viable cells was expressed as the number of cells within a specified cell field dimension, specifically a length of 125  $\mu$ m, as previously described [31,32].

### 5.6. Statistical Analysis

The study's findings are reported as means ± standard error of the means (SEM). Statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software, MA, USA), employing one-way analysis of variance (ANOVA). In instances where ANOVA yielded significant results, multiple Tukey's post hoc tests were conducted. A p-value below 0.05 was considered statistically significant.

Acknowledgements: The authors are grateful to staff of University's Central Animal House Facility and Physiology department, for their support during the study.

Author contributions: Concept – A.S., S.N.; Design – A.S., S.N.; Supervision – A.S., S.N.; Resources – A.S.; Materials – A.S., S.N.; Data Collection and/or Processing – A.S., C.S., S.S.; Analysis and/or Interpretation – A.S., C.S., S.S.; Literature Search – C.T., C.S., S.S.; Writing – C.T., C.S., S.S.; Critical Reviews – A.S., S.N., C.T., C.S., S.S.

Conflict of interest statement: The authors declared no conflict of interest

### REFERENCES

- [1] Singh G, Sander JW. The global burden of epilepsy report: Implications for low- and middle-income countries. Epilepsy Behav. 2020; 105: 106949. <u>https://doi.org/10.1016/j.yebeh.2020.106949</u>.
- [2] GBD 2016 Epilepsy Collaborators. Global, regional, and national burden of epilepsy, 1990–2016: A systematic analysis for the global burden of disease study 2016. Lancet Neurol. 2019; 18(4): 357–375. https://doi.org/10.1016/S1474-4422(18)30454-X.

- [3] Cho CH. New mechanism for glutamate hypothesis in epilepsy. Front Cell Neurosci. 2013; 7: 127. https://doi.org/10.3389/fncel.2013.00127.
- [4] Gelfuso EA, Reis SL, Pereira AMS, Aguiar DSR, Beleboni RO. Neuroprotective effects and improvement of learning and memory elicited by erythravine and 11α-hydroxy-erythravine against the pilocarpine model of epilepsy. Life Sci. 2020; 240: 117072. <u>https://doi.org/10.1016/j.lfs.2019.117072</u>.
- [5] DiNuzzo M, Mangia S, Maraviglia B, Giove F. Physiological bases of the K+ and the Glutamate/GABA hypotheses of epilepsy. Epilepsy Res. 2014; 108(6): 995–1012. <u>https://doi.org/10.1016/j.eplepsyres.2014.04.001</u>.
- [6] Landi S, Petrucco L, Sicca F, Ratto GM. Transient cognitive impairment in epilepsy. Front Mol Neurosci. 2018; 11: 458. <u>https://doi.org/10.3389/fnmol.2018.00458</u>.
- [7] Colciaghi F, Finardi A, Nobili P, Locatelli D, Spigolon G, Battaglia GS. Progressive brain damage, synaptic reorganization and NMDA activation in a model of epileptogenic cortical dysplasia. PLoS One. 2014; 9(2): e89898. https://doi.org/10.1371/journal.pone.0089898.
- [8] Shin EJ, Jeong JH, Chung YH, Kim WK, Ko KH, Bach JH, Hong JS, Yoneda Y, Kim HC. Role of oxidative stress in epileptic seizures. Neurochem Int. 2011; 59(2): 122–137. <u>https://doi.org/10.1016/j.neuint.2011.03.025</u>.
- [9] Wu Q, Zhao CW, Long Z, Xiao B, Feng L. Anatomy based networks and topology alteration in seizure-related cognitive outcomes. Front Neuroanat. 2018; 12: 25. <u>https://doi.org/10.3389/fnana.2018.00025</u>.
- [10] Rogawski MA, Löscher W, Rho JM. Mechanisms of action of antiseizure drugs and the ketogenic diet. Cold Spring Harb Perspect Med. 2016; 6(5): a022780. <u>https://doi.org/10.1101/cshperspect.a022780</u>.
- [11] Bromfield EB, Cavazos JE, Sirven JI, editors. An Introduction to Epilepsy [Internet]. West Hartford (CT): American Epilepsy Society; 2006. Available from: https://www.ncbi.nlm.nih.gov/books/NBK2508/
- [12] Eddy CM, Rickards HE, Cavanna AE. The cognitive impact of antiepileptic drugs. Ther Adv Neurol Disord. 2011; 4(6): 385-407. <u>https://doi.org/10.1177/1756285611417920</u>.
- [13] Perucca P, Gilliam FG. Adverse effects of antiepileptic drugs. Lancet Neurol. 2012; 11(9): 792-802. https://doi.org/10.1016/S1474-4422(12)70153-9.
- [14] Park SP, Kwon SH. Cognitive effects of antiepileptic drugs. J Clin Neurol. 2008; 4(3): 99-106. https://doi.org/10.3988/jcn.2008.4.3.99.
- [15] Choudhary N, Singh V. Insights about multi-targeting and synergistic neuromodulators in ayurvedic herbs against epilepsy: Integrated computational studies on drug-target and protein-protein interaction networks. Sci Rep. 2019; 9(1):10565. <u>https://doi.org/10.1038/s41598-019-46715-6</u>.
- [16] Manchishi SM. Recent advances in antiepileptic herbal medicine. Curr Neuropharmacol. 2018; 16(1): 79-83. https://doi.org/10.2174/1570159X15666170518151809.
- [17] Levira F, Thurman DJ, Sander JW, Hauser WA, Hesdorffer DC, Masanja H, Odermatt P, Logroscino G, Newton CR. Premature mortality of epilepsy in low- and middle-income countries: a systematic review from the mortality task force of the international league against epilepsy. Epilepsia. 2017; 58(1): 6–16. <u>https://doi.org/10.1111/epi.13603</u>.
- [18] Snehunsu A, Mukunda N, Satish Kumar MC, Sadhana N, Naduvil Narayanan S, Vijay Kapgal K, Avinash H, Chandrashekar BR, Raghavendra Rao K, Nayak BS. Evaluation of anti-epileptic property of *Marsilea quadrifolia* Linn. in maximal electroshock and pentylenetetrazole-induced rat models of epilepsy. Brain Inj. 2013; 27(13–14): 1707–1714. https://doi.org/10.3109/02699052.2013.831121.
- [19] Snehunsu A, Ghosal C, Kandwal M, Yadav PK, Nayak BS, Rao KR, Kamath SU, Sahoo P, Srinivasan KK, Naduvil Narayanan S, Kumar S, Joseph A. 1-Triacontanol cerotate, isolated from *Marsilea quadrifolia* Linn. ameliorates reactive oxidative damage in the frontal cortex and hippocampus of chronic epileptic rats. J Ethnopharmacol. 2015; 172: 80–84. https://doi.org/10.1016/j.jep.2015.06.020.
- [20] Seghatoleslam M, Alipour F, Shafieian R, Hassanzadeh Z, Edalatmanesh MA, Sadeghnia HR, Hosseini M. The Effects of *Nigella sativa* on neural damage after pentylenetetrazole induced seizures in rats. J Tradit Complement Med. 2016; 6(3): 262–268. <u>https://doi.org/10.1016/j.jtcme.2015.06.003</u>.
- [21] Pahuja M, Mehla J, Kumar Gupta Y. Anticonvulsant and antioxidative activity of hydroalcoholic extract of tuber of Orchis mascula in pentylenetetrazole and maximal electroshock induced seizures in rats. J Ethnopharmacol. 2012; 142(1): 23–27. <u>https://doi.org/10.1016/j.jep.2012.04.006</u>.
- [22] Mortazavi F, Ericson M, Story D, Hulce VD, Dunbar GL. Spatial learning deficits and emotional impairments in pentylenetetrazole-kindled rats. Epilepsy Behav. 2005; 7(4): 629–638. <u>https://doi.org/10.1016/j.yebeh.2005.08.019</u>.
- [23] Snehunsu A, Nayak SB, Kandwal M, Piyali A, Adiga M, Sahoo P, Medabala T, Rao KR, Joseph A. 1-Triacontanol cerotate isolated from *Marsilea quadrifolia* Linn. safeguards hippocampal CA3 neurons and augments special memory deficit in chronic epileptic rats. Int J Morphol. 2019; 37(1): 265–272. <u>https://doi.org/10.4067/S0717-95022019000100265</u>.
- [24] Sahu S, Dutta G, Mandal N, Goswami AR, Ghosh T. Anticonvulsant effect of *Marsilea quadrifolia* Linn. on pentylenetetrazole induced seizure: A behavioral and EEG study in rats. J Ethnopharmacol. 2012; 141(1): 537–541. <u>https://doi.org/10.1016/j.jep.2012.02.039</u>.
- [25] Zahan R, Ripa F A, Alam M B, Haque M A, Mosaddik M A, Nahar L. Hypoglycemic and in vitro antioxidant effects of methanolic extract of *Marsilea quadrifolia* plant. Pharmacogn J. 2011; 3(26): 86–92. <u>https://doi.org/10.5530/pj.2011.26.14</u>.
- [26] Hasselmo, ME. The role of acetylcholine in learning and memory. Curr Opin Neurobiol. 2006; 16(6): 710-715. https://doi.org/10.1016/j.conb.2006.09.002.

- [27] Bhadra S, Mukherjee PK, Bandyopadhyay A. Cholinesterase inhibition activity of *Marsilea quadrifolia* Linn. an edible leafy vegetable from west Bengal, India. Nat Prod Res. 2012; 26(16): 1519–1522. https://doi.org/10.1080/14786419.2011.565006.
- [28] Subramanian A, Tamilanban T, Sekar M, Begum MY, Atiya A, Ramachawolran G, Wong LS, Subramaniyan V, Gan SH, Mat Rani NNI, Wu YS, Chinni SV, Fuloria S, Fuloria NK. Neuroprotective potential of *Marsilea quadrifolia* Linn against monosodium glutamate-induced excitotoxicity in rats. Front Pharmacol. 2023; 14: 1212376. https://doi.org/10.3389/fphar.2023.1212376
- [29] Chen CC, Shen JW, Chung NC, Min MY, Cheng SJ, Liu IY. Retrieval of context-associated memory is dependent on the Ca(v)3.2 T-type calcium channel. PLoS One. 2012; 7(1): e29384. <u>https://doi.org/10.1371/journal.pone.0029384</u>.
- [30] Hess A, Kreitz S, Brune K. Functional atlas of the rat brain. In: Meinzer HP, Handels H, Horsch A, Tolxdorff T. (eds) Bildverarbeitung für die Medizin 2005. Informatik aktuell. Springer, Berlin, 2005, pp.73-77.
- [31] Govindaiah S, Rao BS, Raju TR, Meti BL. Loss of hippocampal CA1 neurons and learning impairment in subicular lesioned rats. Brain Res. 1997; 745(1-2): 121–126. <u>https://doi.org/10.1016/S0006-8993(96)01135-3</u>.
- [32] Wood ER, Mumby DG, Pinel JPJ, Phillips AG. Impaired object recognition memory in rats following ischemiainduced damage to the hippocampus. Behav Neurosci. 1993; 107(1): 51-62. <u>https://doi.org/10.1037/0735-7044.107.1.51</u>.