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Marsilea qudrifolia Leaf Extract Ameliorates Pentylenetetrazol-Induced CA1 Neuronal Damage and Causing Anxiolytic Effects through Neurochemical Changes in Epileptic Rats

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ABSTRACT

Marsilea quadrifolia (MQ) is a unique plant with a high medicinal value showing anti-epileptic efficacy against various epilepsy models. Intraperitonial (I.P.) administration of Pentylenetetrazole (PTZ) causes neuronal damage and neurochemical changes displaying alteration in behavior. However, the mechanism of action remains unknown, but Antiepileptic Drugs (AEDs) are the mainstay of treatment. We hypothesized that the methanolic leaf extract of MQ (MQLE) may have a protective effect against Pentylenetetrazole (PTZ) induced anxiety like behaviour causing neuronal loss and neurotransmitter imbalance in the rat. The behavioral performance was tested in an open field and elevated plus-maze test after PTZ (35 mg/kg body weight) alternative administration (I.P.) for 30 days. The neurotransmitters and their metabolites were analyzed by HPLC with Electrochemical Detection (ECD), for the content of Noradrenaline (NE), Serotonin (5-HT) and 5-Hydroxy Indole Acetic Acid (5-HIAA); Dopamine (DA), and 3, 4-Dihydroxyphenylacetic Acid (DOPAC). Brain sections were taken for histopathological studies using cresyl violet staining. PTZ treated rats showed increased anxiety like behavior in the open field as well as elevated plus maze test and MQLE co-supplementation modulated the behavior. PTZ treated rats showed increased 5-HT and 5-HIAA with increased NA and unaltered DA and DOPAC content which can be restored by MQLE co-supplementation. The increased percentage count for pyknotic cells in the CA1 region of the hippocampus was reduced by MQLE co-supplementation in rats. These results showed the neuroprotective activities of MQLE on PTZ induced increased 5-HT and 5-HIAA content in the hippocampus causing anxiety like behavioral alteration. The PTZ induced neurotransmitter level changes with an increased percentage count of pyknotic cells in the CA1 region of the hippocampus may be ameliorated by MQLE causing anti-anxiety like behavior in wistar rats.

Keywords: Pentylenetetrazol; Open field test; Elevated-plus maze; Anxiety like behavior; 5-HT; 5-HIAA; DA; DOPAC; Pyknotic cells

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INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by irregular, excessive neuronal excitability, and recurrent seizures that affect millions of patients worldwide [1,2]. Epilepsy is a significant health concern for the human population and people with epilepsy carry a risk of premature mortality, with a life expectancy 10 years less than the

general population [3]. There is currently no cure or prevention for epilepsy and most, if not all of the approved antiepileptic drugs are not truly 'antiepileptic' but merely 'anti-seizures' [4]. Pentylenetetrazole (PTZ) is a convulsant interfering with GABA mediated inhibition used to model epileptic seizures in mice, rats, cats and primates [5]. Among all these models, pharmacological seizure induction is a common method used to generate an animal model for the investigation of the pathology of epilepsy [6]. PTZ suppresses the function of inhibitory synapses, leading to increased neuronal activity causing amnesia in the animal model and this regulation causes generalized seizures in animals [6,7]. A single injection of PTZ can induce acute seizures, especially status epilepticus and recurrent seizures [8]. Furthermore, seizure aggravation is accompanied by prolonged seizure duration and investigating the molecular mechanism regulating the seizure severity, latency and duration may be useful for screening anti-epileptic drugs [9-11].

The first few weeks in the post-natal life in the rat are a critical developmental period for numerous structural and functional changes, including increased behavioral capabilities and maturation of the neuromuscular junction, and changing phenotype [12]. First, the study of neurotoxic effects is important in the gestational phase of brain development because during the phase of neurodevelopment the Blood Brain Barrier (BBB) is incomplete and detoxification mechanisms are not as efficient as in the adult brain. Second, the acceptance that the plasticity of the developing brain is vastly different than that of the adult brain is slowly emerging [13,14]. The developing brain is often more sensitive than the adult brain to toxic insult and has been suggested to be associated with some developmental disabilities (learning disabilities, attention deficit hyperactivity disorder, dyslexia, autism spectrum disorders, and epileptic seizure) which are diagnosed in children at an alarmingly increasing rate [14]. For this reason, children are found to be more susceptible to neurotoxicity compared to adults [15-17]. About 40% of all epilepsies, especially during childhood, and adolescence, are idiopathic epilepsies. Several defects in ion channel or neurotransmitter genes or proteins that control brain excitability have been recently identified in some idiopathic epilepsy [18].

All the monoamines have been shown to have trophic roles and also the monoaminergic system act as a potential source of pathogenesis in epilepsy. Alternations in central monoamines have been reported in animals and human epileptic brain and increased monoamines and metabolite levels in the Cerebrospinal Fluid (CSF) [19,20]. For the major psychiatric disorders, therapeutic action occurs primarily in monoamine systems that mature between adolescence and young adult-hood; exposure before this point affects systems that are still immature and potentially vulnerable. Further proof of monoaminergic involvement in the pathogenesis of epilepsy is the evidence that depression, bipolar disorders, and other neuropsychiatric disorders are classically related to monoamine dysfunctions. The elevated levels of Noradrenaline (NA), 5-Hydroxytryptamine (5-HT) and Dopamine (DA) metabolites during epilepsy may represent an epiphenomenon, rather than a concerted strategy of local or distal neurons to contain an epileptogenic focus. Serotonin (5-HT) is a biogenic amine that involves a wide range of physiological functions including anxiety, depression, learning, and memory. Dysregulation of 5-HT release may contribute to the pathogenesis of depression and DA contributed to the seizure prone states of some genetic rodent models. But, whether the brain's aminergic system undergoes functional changes during epileptogenesis is not understood. The brain aminergic systems represent a potential target for the therapy of epilepsy and comorbid disorders like anxiety. The central nucleus of the amygdala and hippocampus in the brain play important roles in regulating emotional states as they relate to anxiolytic-like behavior.

PTZ has a central nervous system stimulant epileptogenic property. Previous studies showed that PTZ induced seizures caused pyknotic neurons in the hippocampus. It was indicated that a single attack of seizures leads to apoptotic neuronal death. It is well recognized that seizures are the outcome of an imbalance between central excitatory and inhibitory processes. It is also assumed that cerebral injury occurs following seizures, e.g. during epilepsy, but this phenomenon's underlying pathophysiology remains unknown. Theories regarding factors that may exacerbate brain damage include oxidative compromise, neuronal inflammation and the development of atypical axonal connections. Kindled seizures cause neuronal loss also in the limbic areas: CAl, CA3, Dentate Gyrus (DG) of the hippocampus, amygdala, and entorhinal cortex. Hence, in recent years, the main strategy of epilepsy treatment has been seeking promising drugs with neuroprotective activities that can prevent or delay disease progression and development.

The most common treatment strategy for epilepsy is the use of Anti-Epileptic Drugs (AEDs). All AEDs are designed to decrease the severity, duration, and frequency of epileptic seizures and create a balance in the neuronal network between excitation and inhibition. Although, these drugs can control or reduce epileptic seizures to some extent a large number of patients suffer from side effects of these antiepileptic drugs. Despite advanced medical management with

modern antiepileptic drugs more than 30% of patients continue to have drug resistant epilepsy with frequent seizures. The active component of many drugs and their action on neurochemical changes in PTZ induced epilepsy is yet to be established. In recent times there is increasing interest in traditional antiepileptic agents. Therefore, effective and safe therapy for epilepsy remains a daunting challenge and demand for new types of anticonvulsants still exists. In this perspective, *Marsilea quadrifolia* (MQ) is a common Indian hydrophytic fern referred to in Indian traditional medicine as sedative and anticonvulsant. The anti-epileptic effects of methanolic leaf extract of *Marsilea quadrifolia* (MQLE) in Maximal Electroshock (MES) and PTZ induced rat models of epilepsy. Therefore, in the present study, the aim was to test the efficacy of MQLE as an antiepileptic compound against PTZ induced anxiety related behavior and learning and memory impairment, affecting the monoamine neurotransmitter level with altered synaptic NMDA receptor subunit protein expression in the rat hippocampus.

MATERIALS AND METHODS

Chemicals

Pentylenetetrazol (PTZ, 97% purity as specified by the supplier) and Isoproterenol were obtained from (Sigma Aldrich Chemicals Co., St. Louis, MO, USA).

Experimental Animals

Male wistar rats (n=72) two months old as post-natal days 60 (PND60), weighing 150-200 g were taken from the animal care unit of Ravenshaw University, Cuttack. The experiment started with PND60 old rats, which were randomly assigned into different experimental groups (4 groups, 6 rats per group); control (vehicle treated), MQLE administered, PTZ treated and PTZ administered co-supplemented with MQLE. Rats were housed in different cages and cared for in purpose built transparent polycarbonate enclosures, in a climatically controlled room under a 12 h light dark cycle ad libiatum. Temperature and humidity were maintained at 25°C-28°C and 60-65%, respectively. Water was readily available; an illumination source simulating the night day pattern was established.

Preparation of Leaf Extract

Fresh, young, and healthy leaves of *Marsilea quadrifolia* (MQ) were collected from the local market and identified and authenticated by Dr. Soumendra Naik, head botany department of Ravenshaw University, Cuttack, Odisha, India. MQ leaves are shed dried crushed in an electrical grinder to get a free flowing powder and spread over a tray with shifting of materials daily to avoid the growth of fungus. This powder was subjected to extraction with methanol (95% v/v) at room temperature. The percentage yield of the extract was calculated (9-10 g/100 g of dried leaves of MQ). The extract solution was filtered (What man No1 filter paper) and vacuum dried at 50°C-60°C to get brown colored sticky mass and stored in the refrigerator.

Animal Treatment

The PTZ stock solutions (35 mg/kg body weight; I.P.) were freshly prepared by dissolving in 1 mL of saline water. A single dose of PTZ (sub-threshold for inducing mild seizure without convolution) was injected (I.P.) and MQLE extract (40 mg/kg b.w.) about 1 mL was suspended in 1% Carboxymethylcellulose (CMC) solution in distilled water in every 48 hours interval for 30 days. The control rats received saline water (1 mL/kg b.w <0.1%) only in the same way up to PND90. MQLE was administered to PTZ with the MQLE group about 30 minutes before PTZ administration. The behavioral experiments were carried out in the time window of the light cycle (10:00 to 11:30 a.m.) to avoid experimental deviations due to the diurnal cycle. All animal procedures and experiments were approved by the Ravenshaw university animal ethics committee (Regd. No.1927/Go/Re/S/16/CPCSEA). The animals were sacrificed after completion of the behavioral test at postnatal days 90 (PND 90). All brains were removed, washed with normal saline after dissection, weighted and then transferred for snap-frozen and stored at -80ºC for biochemical analysis and histopathological studies.

Behavioral Assay

Anxiety related behavior (Open field test): The open field test was used to study behavioral responses in rats that are placed in a novel and bright arena. Rats tend to avoid brightly illuminated areas. The test also measures a range of anxiety induced, locomotors activity, and exploratory behaviors. Open field activity was monitored in a circular arena (75 m diameter and 1.5 cm high) wall with grey color painting. For analysis, the inner zone is considered the center of the arena and an outer zone is made up of the remaining area along the side walls as the periphery. By placing the

animal on a specific side of the wall of the arena, the number of central and peripheral entries and time spent in the center was automatically monitored by ANY Maze video tracking system (Stoelting Co., USA, Version 6.1) for 5 minutes.

Approach avoidance behavior (The Elevated-plus maze): For further testing the anxiety like behaviors, rats were exposed to the elevated plus-maze task, where the entry to open arm avoidance can be validated as a translational measure of anxiety like as well as activity of the animal. The elevated plus-maze consisted of two opposite open arms (50 cm long × 10 cm wide) and two enclosed arms (50 × 10 × 40 cm) that extended from a common central platform (10 × 10cm), elevated 75 cm above the floor. The entire apparatus was constructed of a plastic rim with grey paintings and placed in the center of a room that was lit with fluorescent lights. Rats tend to avoid the open areas, especially when they are brightly lit, favoring darker, and more enclosed spaces.

Animals were transported to a plus-maze test laboratory 10 minutes before to facilitate adaptation to novel surroundings. Then, rats were placed individually onto the center of the apparatus facing an open arm, and the time spent on and entries onto each arm were detected for 5 min. The apparatus was connected with dual infrared sensor beams positioned at the entry to the arm interfaced with a PC controlled by ANY maze video tracking System and software (Stoelting Co., USA, Version 6.1). The maze was wiped and clean with 30% alcohol and dried after each trial. The spatial and temporal distribution of behavior was calculated as percent total for both frequency (percent open entries, *i.e.* 100^{*}(open arm entry/(open arm entry+closed arm entry)) and duration (percent time spend on open, *i.e.* 100^{*}(open arm time/(open arm time+closed arm time)) data.

HPLC analyses (measurement of monoamines and their metabolites): Frozen brain tissue was dissected out for the hippocampal region and the homogenized by sonification in 0.5 ml of 0.2 M $HCLO_4$ to the sample per 100 mg wet tissue containing Isoproterenol (ISO solution, 1 µg/ml (x 1000 dilution of 1mg/ml standard stock) as an internal standard substance. The homogenized tissue was then kept in the ice bath for 30 min and centrifuged for 2 min at 20,000Gx, and the supernatant was added with 1 M sodium acetate to adjust the pH to 3.0. Of this, 10 µl was injected onto a separation column (Eicompak SC-5ODS, ID 3.0 × 100 mm) in an HPLC-ECD system, for measurement of amines and their metabolites. The mobile phase used with this aliquot (0.1 M Citrate-Acetate buffer (pH 3.5) allowed for the separation of neurotransmitter like Norepinephrine (NA), Dopamine (DA), 3,4-Dihydroxyphenylacetic Acid (DOPAC), 5-Hydroxyindoleacetic acid (5-HIAA) and 5-hydroxytryptamine (5-HT). Output from the detector was plotted and measured using a power chrome plotting integrator, which also was used to calculate the peak areas.

Histopathological analysis: Cresyl Violet (CV) staining was carried out to study the structural damage in pyramidal neurons in the hippocampal CA1 brain region after PTZ administration for two months old rats. The whole-brain samples were carefully removed isolated and processed further at 4ºC and subjected to post-fixation in 4% paraformaldehyde for 24 hours followed by 30% sucrose solution treatment overnight. Serial cryosectioning (Leica CM3050S) of the hippocampal brain region was carried out. After being stained with 0.1% CV, alternate sections were taken and observed under requisite magnification in a bright field phase microscope (Olympus, BX43 F).

Statistical Analysis: All data are presented as group means ± S.E.M. Body weight and behavioral studies were subjected to a one-way analysis of variance (ANOVA) followed by Dennett's test. Monoamines and metabolite levels data were analyzed by ANOVA compared with other groups as a percentage of control values. In all cases, the significance level was considered to be $p<0.05$.

RESULTS

Anxiety like behavior and exploratory locomotion in the Open Field Test (OFT): To study further the PTZ effects concerning anxiety like behavior, one additional test was performed based on the natural aversion of rats to open or unprotected spaces: The center of the field. In the open field test, rats administered 35 mg/kg b.w. of PTZ, the one way ANOVA revealed a statistically significant difference between all groups $F(3, 23)=16.80$, $p<0.0001$) for more time on the central area in the center in comparison to the control group (Figure 1). The PTZ treated rats showed more anxiety like behavior in comparison to the control rats. However, as the MQLE co-supplemented groups, compared to the PTZ treated groups showed significant decrease in stay time in the center showing anxiolytic like behavioral outcome. Additionally, the OFT was also used to assess the locomotor activity in rats by estimating their distance traveled in the arena, but the findings remained non-significant in rats treated with PTZ in comparison to control groups.

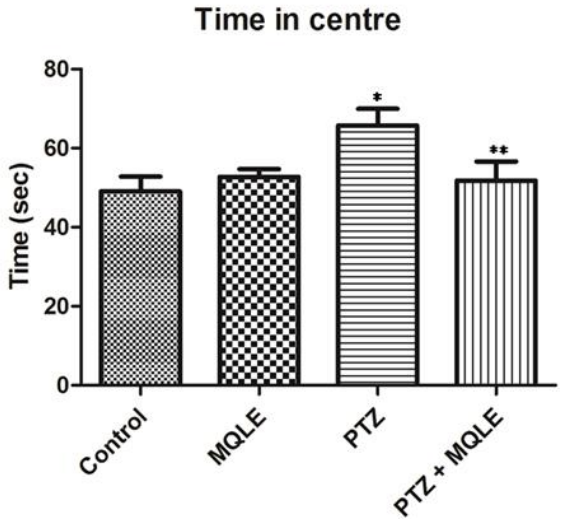

Figure 1: Time in center

Effects of PTZ on anxiety like behavior on the Elevated Plus-Maze (EPM): To study further the PTZ induced effects on anxiety, one additional test was performed that was based on the natural aversion of rats to open or unprotected spaces: the elevated plus-maze. In EPM, one-way ANOVA demonstrated a significant difference between all groups for open arm time ($F3, 23=3.302$, $p<0.05$) after rats administered 35 mg/kg of PTZ. In the EPM, PTZ treated rats spent significantly longer stay time on the open arm ($p<0.05$) in comparison to control groups (Figure 2). The more pronounced anxiolytic effect of *Marsilea quadrifolia* leaf extract (MQLE) was observed in the combination of MQLE co-supplemented groups after PTZ administration e.g. significantly ($p<0.0001$) decreased stay time in the open arm, the unprotected zones of the plus-maze. These result suggested that administration of PTZ (I.P.) produced significant increase in anxiety like behavior in comparison to control groups. These increases in anxiety like behaviour was ameliorated by MQLE co-supplementation that exhibiting the anxiolytic like behavioral effect of the leaf extract showing the potential protective effect against PTZ induced increased anxiety like behavioral activity in wistar rats.

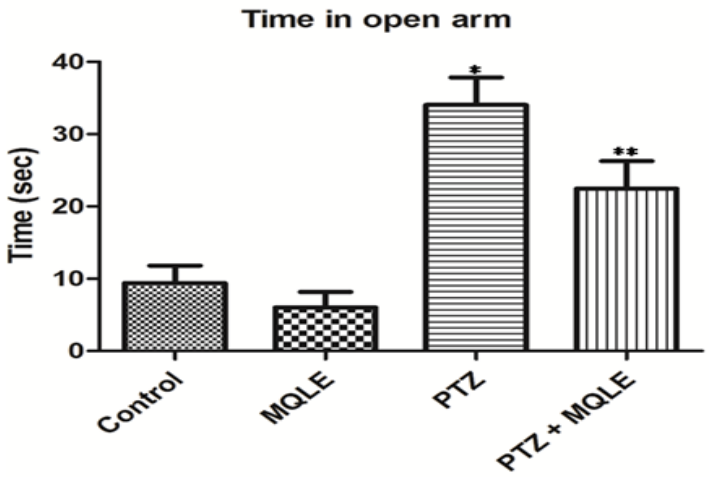

Figure 2: Time in open arm

Concentration of neurotransmitters and their metabolites: The total concentration of 5-HT, DA, and NA in the hippocampus of the control and PTZ administered experimental groups were shown in Figure 3. A significant increase of 5-HT and a highly significant increase of NA ($p<0.05$) were found in the hippocampus comparing control and PTZ administered experimental groups. No significant differences were found in DA and DOPAC concentration. The concentration of 5-HT in the hippocampus exhibits a statistically significant increase in the PTZ administered experimental group compared to the control ($p<0.05$). The same was observed regarding NA concentration ($p<0.05$); no significant differences in DA concentration were observed between the two groups. In the hippocampus, the concentration of 5-HT and NA significantly increased ($p<0.05$) in the experimental group after I.P. administration of PTZ (35 mg/kg b.w.); but there were no significant differences found between different groups in DA and DOPAC concentration of epileptic rats. These results demonstrate that PTZ-induced increase in frequency of ion channel opening in response to NA and 5-HT neurotransmitters and MQLE co-supplementation was correlated to a significant ($p<0.001$) reduction in both the neurotransmitter level and the 5-HT metabolites (5-HIAA) concentration in the hippocampal region of the brain.

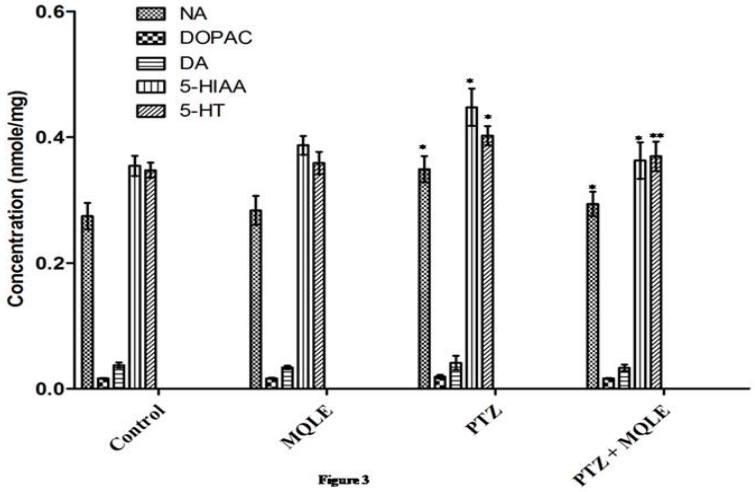

Figure 3: The total concentration of 5-HT, DA, and NA in the hippocampus of the control and PTZ administered experimental groups

Cresyl violet staining for histopathological studies: Histopathological studies showed a remarkable increase in percentage change of pyknotic neuronal cell count in the hippocampal region of epileptic rat brain in comparison to PTZ administered groups when compared with the control group (Figure 4). The distribution of pyramidal neuronal cells in hippocampal CA1 regions was examined under a low (x10) power objective after PTZ administration to rats at PND60. Mostly, round, clear, medium or large neurons with distinct nuclei and cytoplasm is evenly filled with Nissl substance were counted. Cells with darkly stained cytoplasm, shrunken cells and cells with fragmented nuclei were excluded from the count. Though pyknosis was observed on PTZ treated rats, it was significantly augmented in PTZ administered co-supplemented with MQLE. Histogram showing representative images with clear indication toward deeply stained pyknotic neuronal cells in the CA1 region of the hippocampus and percentage change in neuronal cell count in graphs (F_3, 35=40.78, $p<0.05$) after PTZ administration and PTZ administered co-supplemented with MQLE groups. Neurons with deeply stained nuclei (red arrows) showing pyknosis were counted in all groups under higher magnification using cellSens imaging software. These results were expressed in percentage change showing a significant increase in pyknotic neuronal cell count after PTZ administration compared to the control groups in the CA1 hippocampal region of the epileptic rat brain. The percentage change in pyknotic cell counts was markedly decreased ($^*p<0.05$) in all parts of the hippocampus after MQLE co-supplementation.

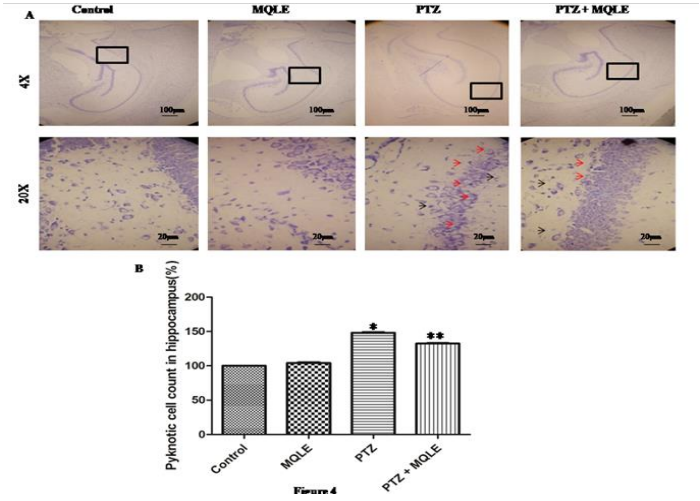

Figure 4: Cresyl violet staining for histopathological study

DISCUSSION

Epilepsy is a chronic disease that occurs due to an imbalance between inhibitory and excitatory activities of neurons in the brain. Although there is a large number of a model available for screening of the anticonvulsant activity, we used i.p. methods of exposure to PTZ for direct assessment of anti-epileptic effects of MQLE on the nervous system function. Our results indicate that MQLE has an antiepileptic property that can protect against seizure-induced behavioral changes with hippocampal neuronal damage. In the present study, PTZ administration elicited an increase in stay time in the center and time in open arms of two pharmacologically well-validated exploration-based tests: open field and elevated plus-maze. The plus-maze test is a paradigm used for validating the open arm: closed arm entries as a measure of anxiety-like behavior due to the effect of drugs. Studies have reported that the monoaminergic and GABAergic systems are involved in epilepsy.

The anxiety-like behavioral effects exerted by PTZ administration were reflected by increased Spatio-temporal measures, such as increased open-arm time and decreased closed-arm time, which may be attributable to a facilitatory action of 5-HT in the etiology of anxiety and alteration of mood after MQLE co-supplementation. This parameter, which changes independent of the percentage of time spent in the center in an open field test and open arm time in a plus-maze test, was generally accepted as the best parameter of anxiety-like behavior. There was no significant difference in general activity after PTZ exposure in both open fields (total distance traveled) and elevated plus-maze (closed arm and total arm entries). Our study found that MQLE may ameliorate the anxiety-like behavioral alteration in PTZ-induced epileptic rats similar to the study showing significant anti-epileptic efficacy of MQ against various epilepsy models. In the present study, PTZ-treated groups showed a significant increase ($p<0.05$) in brain 5-HT and 5-HIAA concentrations with an elevated NA level in the hippocampus, which was restored after MQLE co-supplementation, suggesting an anticonvulsants activity against epileptic seizure. The brain region like the CA1 region of the hippocampus is involved in the regulation of cognition and mood performance and MQLE co-supplementation may exert its effects *via* inhibitory monoamine oxidase enzyme activity increasing 5-HT and NA levels in rat brain. Therefore, the extent of toxicity induced by PTZ may be sufficient to alter the homeostatic state of the growing complex neuronal system leading to integrated neurobiological functions and behavioral responses specifically related to the release of neurotransmitters and their metabolite (NA, 5-HT, and 5-HIAA) in the hippocampus of rats.

We have diagnosed neuronal cell loss in the hippocampus after PTZ administration using a cresyl violet stain. PTZ administered rats showed anxiety behavior exhibiting neuronal loss through pyknosis that causes neurodegeneration

and neurochemical changes in the hippocampus of rats. In addition, increased levels of the neurotransmitter like NA and 5-HT with its metabolites 5-HIAA were analyzed after PTZ administration that was specifically decreased in PTZ administered with MQLE co-supplementation groups, indicating its involvement in the developmental processes. One of the possible mechanisms of hippocampal neuronal loss after PTZ administration is that high levels of 5-HT-like neurotransmitter release in the hippocampus lead to excitotoxic damage to vulnerable neuronal populations. This concept is supported by animal studies in which administration of PTZ results in loss of neurons, whereas co-supplementation of MQLE prevents neuronal damage. Another possible scenario is that accumulation of neurotransmitters and their metabolites after MQLE co-supplementation to PTZ administered rats can result in depletion of serotonin and its metabolites protecting the most extensive damage to the hippocampus of the brain at least partial compensatory antiepileptic mechanisms. The pyramidal cells were typically layered and were having large, round, transparent, intact nuclei in the rats of control groups. In control groups we found the normal neuronal cell bodies appearing with distinct nuclei and nucleoli whereas PTZ administration showed degenerating cell bodies having pyknotic nuclei and vacuolar spaces in the pyramidal neurons of the hippocampus, this finding agrees with the observations of recent studies. MQLE co-supplementation with PTZ showed marked improvement in the histological appearance of neurons in the CA1 region of the hippocampus causing alteration in behavior. The possible explanation is that high levels of neurotransmitter release may cause loss of inhibition by neurons due to impaired vesicular release to receptors.

CONCLUSION

The results of the present study show increases in pyknotic cell count representing neurodegeneration in rat brain, especially in the CA1 region of the hippocampus, and change in the neurotransmitter and its metabolites could be a possible explanation for the observed anxiolytic-like effects of MQLE following PTZ administration causing neurodegeneration in CA1 region of the hippocampus in Wistar rats. The neuronal damage can be ameliorated by the MQLE due to its antiepileptic properties that may be attributable to its impacts on the level of neurotransmitters and its metabolites. Therefore, further studies are necessary to clarify the unique pharmacological profile of MQLE to our understanding; due to its beneficial effect on a chronic animal model of epilepsy.

ACKNOWLEDGMENTS

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REFERENCES

[1] Grabowska-Grzyb A, Jędrzejczak J, Nagańska E, et al. *Epilepsy Behav*. **2006**;8(2):411-417.

[2] Sadek B, Alaa A, Ojha SK, et al. *Curr Pharm Des*. **2020**;26(15):1693-1711.

[3] Beghi E. *Neuroepidemiology*. **2020**;54(2):185-191.

[4] Shimada T, Yamagata K. *J Vis Exp*. **2018**;136: e56573.

[5] Park JH, Cho H, Kim H, et al. *Neuroscience*. **2006**;140(2):673-684.

[6] Kandratavicius L. *Neuropsychiatr Dis Treat*. **2014**;10:1693-1705.

[7] Rogawski MA, Löscher W, Rho JM, et al. *Cold Spring Harb Perspect Med*. **2016**;6(5):a022780.

[8] Claycomb RJ, Hewett SJ, Hewett JA, et al. *Epilepsia*. **2011**;52(2):273-283.

[9] Fang M, Xi ZQ, Wu Y, et al. *Med Hypotheses*. **2011**;76(6):871-876.

[10] Marson A, Jacoby A, Johnson A, et al. *Lancet* **2005**;365(9476):2007-2013.

[11] Schmidt D, Loscher W. *Epilepsy Curr*. **2009**;9(2):47-52.

[12] Gaitatzis A, Johnson AL, Chadwick DW, et al. *Brain*. **2004**;127(11):2427-2432.

[13] Brown LA, Khousbouei H, Goodwin SJ, et al. *Neurotoxicology*. **2007**;28(5):965-978.

[14] Costa LG, Aschner M, Vitalone A, et al. *Annu Rev Pharmacol Toxicol*. **2004**;44:87-110.

[15] Neubauer BA, Gross S, Hahn A, et al. *Dtsch Arztebl Int*. **2008**;105(17):319-328.

[16] Guerrini R. *Lancet.* **2006**; 367:499-524.

[17] Hosford DA. *J Clin Neurophysiol.* **1999**;16(4):306-313.

[18] Löscher W. *Epilepsy Res*. **2002**;50(1-2):105-123.

[19] Kurian MA, Gissen P, Smith M, et al. *Lancet Neurol.* **2011**; 10(8):721-733.

[20] Bhagya V, Srikumar BN, Raju TR, et al. *J Neurosci Res.* **2015***;*93(1):104-120.