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Effect of probiotic supplementation on seizure activity and cognitive performance in PTZ-induced chemical kindling

Samaneh Bagheri [a], Ahzdar Heydari [a], Azam Alinaghipour [b], Mahmoud Salami [a,*]

[a] Physiology Research Center, Kashan University of Medical Sciences, Kashan, Islamic Republic of Iran
[b] Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran

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ABSTRACT

Epilepsy is one of the most common neurological disorders that severely affect life quality of many people worldwide. Ion transport in the neuronal membrane, inhibitory–excitatory mechanisms, and regulatory modulator systems have been implicated in the pathogenesis of epilepsy. A bidirectional communication is proposed between brain and gut where the brain modulates the gastrointestinal tract, and the gut can affect brain function and behavior. The gut microbiome takes an important role in health and disease where dysbiosis is involved in several neurological disorders. Probiotics as living microorganisms are beneficial to humans and animals when adequately administered. In the present work, we evaluated the effect of a probiotic bacteria mixture on seizure activity, cognitive function, and gamma-aminobutyric acid (GABA), nitric oxide (NO), malondealdehyde (MDA), and total antioxidant capacity (TAC) level of the brain tissue in the pentylenetetrazole (PTZ)-induced kindled rats. The Racine score and performance in water maze were considered as indices of the epileptic severity and the spatial learning and memory, respectively. We found that the probiotic supplementation substantially reduces seizure severity so that almost no probiotic-treated animals showed full kindling. The oral bacteriotherapy partially improved the spatial learning and memory in the kindled rats. The intervention decreased NO and MDA and increased TAC concentration of the brain. The probiotic treatment also increased the inhibitory neurotransmitter GABA. Our findings are the first preclinical report to show positive effect of probiotic bacteria on seizure-induced neurological disorders. Further investigation is required to answer the questions raised about the probable mechanisms involved.

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1. Introduction

Epilepsy is one of the most common neurological disorders with an occurrence of about 1% of population. This chronic disorder affects approximately 50 million people worldwide, and nearly 2.4 million people are diagnosed with epilepsy each year [1]. Disturbed ion transport or ion channel structure in the neuronal membrane, imbalanced inhibitory/excitatory transmissions, and altered regulatory modulator systems have been implicated in the pathogenesis of epilepsy [2–4]. Understanding the mechanisms underlying epileptogenesis helps in designing effective medications for therapy of epilepsy [5]. Aberrant synaptic transmission including gamma-aminobutyric acid (GABA) ergic system has been considered as the main mechanism involved in neuronal synchrony in seizures. In addition, impaired antioxidant defense mechanisms and increased lipid peroxidation are implicated in its pathogenesis [6–9]. Oxidative stress is also known to participate in pathways leading to neurodegeneration, which is the most important propagating factor in epileptogenesis and cognitive decline [10]. Pharmacoresistant epilepsy and associated comorbidities are two different conditions that interfere with the therapy of epilepsy. Antiepileptic drugs (AEDs) are the main feasible therapeutic methods used to treat epilepsy. At present, there are only 20 AEDs licensed globally, and there are many disadvantages associated with their use. Apart from the inevitable adverse effects, AEDs only restrain the symptoms of seizure rather than modify the development of epilepsy. Furthermore, AEDs are only regarded as effective in 60–70% of individuals with epilepsy [11].

The chemical kindling induced by pentylenetetrazole (PTZ), a $GABA_A$ receptor antagonist, is an indistinguishable model of clinically resistant epilepsy [12]. This model has become a pivotal and handy drug-resistant epilepsy model to explore oxidative stress, neurochemical alterations, and structural changes in brain [13].

Bidirectional communication between brain and gut, known as gut–brain axis, has long been recognized; the brain modulates the gastrointestinal tract by regulation of motility, secretion, absorption, and blood flow, and concurrently, the gut can affect brain function and behavior [14]. The scaffolding of the gut–brain axis includes the gastrointestinal tract, central nervous system (CNS), autonomic nervous system, enteric nervous system, neuroendocrine system, and immune system [14]. There has been increasing interest in the role of gut microbiome in health and disease. Indeed, intestinal microbiota composition varies

* Corresponding author.
E-mail address: salami-m@kaums.ac.ir (M. Salami).

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between healthy and diseased individuals for numerous diseases, and thus, targeting the intestinal microbiota might offer new possibilities for prevention and/or treatment of diseases.

Few studies have made a link between microbiota and epilepsy [15], however, the connection between dysbiosis and epilepsy remains restricted, mostly focusing on microbiome as a target for ketogenic diet.

Probiotics are defined as the living microorganisms that are helpful to humans and animals when adequately administered. The most commonly used probiotics are different species of Lactobacilli and Bifidobacteria that have been considered for their effects on CNS dysfunction in neurological disorders by increasing microbiota diversity and beneficial bacteria compositions [16]. Probiotics capably interact with the intestinal microbiome and provide health benefits [17]. In a series of clinical [18–20] and preclinical [21] studies, we evaluated the effect of probiotic supplementations on some brain disorders. Nothing is reported about the effect of probiotics on epileptic statuses or animal model seizures. Therefore, in the present work, we examined if a mixture of probiotic bacteria underlies epileptic activity of neuronal circuits in a PTZ-induced model of kindling.

2. Materials and methods

2.1. Animals

Two months old male Wistar rats (n = 40) were obtained from Animal House of Kashan University of Medical Sciences (KAUMS). Animals were housed in standard polypropylene cages under controlled laboratory conditions with 12 h light–dark diurnal cycle at 24 ± 2 °C and humidity of 50–60%. The animals had freely access to food and water except during the experiments. All experiments were performed under the protocol approved by the Animal Ethics Committee of KAUMS.

2.2. Experimental grouping

The experimental subjects were divided into five groups (n = 8 for each). One group was the normal control group (CON) that received carrier of the probiotics. Four groups of rats were kindled (as explained later) that include the groups receiving carrier of probiotics (PTZ), the probiotic supplement 24 days before kindling (PRO + PTZ) or during (PTZ + PRO) kindling. Also, a positive control group (VAP) intraperitoneally received 150 mg/kg valproic acid. Fig. 1 illustrates animal grouping and the experimental procedures throughout the study.

2.3. Induction of kindling

Chronic epileptic seizure was induced by administration of subconvulsive doses of PTZ (35 mg/kg). Pentylenetetrazole was freshly dissolved in saline (NaCl 0.9%) and injected intraperitoneally once every second day for 24 days. The convulsive behavior was video-monitored for 30 min. The intensity of the convulsions was registered according to modified Racine's scale as follows:

Stage 0 — No response;
Stage 1 — Hyperactivity, restlessness, and vibrissae twitching;
Stage 2 — Head nodding, head clonus, and myoclonic jerks;
Stage 3 — Unilateral or bilateral limb clonus;
Stage 4 — Forelimb clonic seizures; and
Stage 5 — Generalized tonic–clonic seizures with falling.

The kindling score and the latency of kindling were measured according to the method reported by Racine [22]. The vehicle control rats received saline at the same number of injections.

The effect of the probiotic treatment on the seizure severity before and during induction of kindling and % incidence of animals kindled at the end of 24 days were assessed.

2.4. Probiotic administration

Many studies have used Bifidobacteria and Lactobacilli preparations and mostly show improving some CNS functions. Doses of 10^9 and 10^{10} colony forming unit (CFU) for 2 weeks in animals and 4 weeks in humans have been sufficient to appear measurable effects [23]. The probiotic supplements (Pedilact, prepared by Zist Takhmir Company, I.R. Iran) were a mixture of three bacteria consisting of *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, and *Bifidobacterium infantis* (CFU ~ 10^9 for each). The probiotic-treated animals received 1 ml solution/day (a total of 3 × CFU ~ 10^9) of probiotic mixture via intragastric gavage. The control rats received 1 ml carrier of the probiotics (as below). The treatment lasted for 3 weeks.

2.5. Spatial learning and memory

The Morris water maze test was used to examine changes in the spatial learning and memory abilities of the animals as previously described [24]. The apparatus was a tank made of galvanized metal (150 cm in diameter, 70 cm in depth) that was filled with water (22 °C) up to 20 cm below the rim. The pool was divided into four equal quadrants named northeast, southeast, southwest, and northwest; each separated by 90° around the inner perimeter. A circular platform (10 cm in diameter) was submerged 1.5 cm below the water surface and was located in the center of one quadrant in fixed position throughout the experiment. The walls around the pool were pasted with the surrounding extramaze visual cues to provide spatial cues for the animal during trials.

The test consisted of two phases;: acquisition phase followed by probe trial phase. The first phase included four trials/day each for 90 s

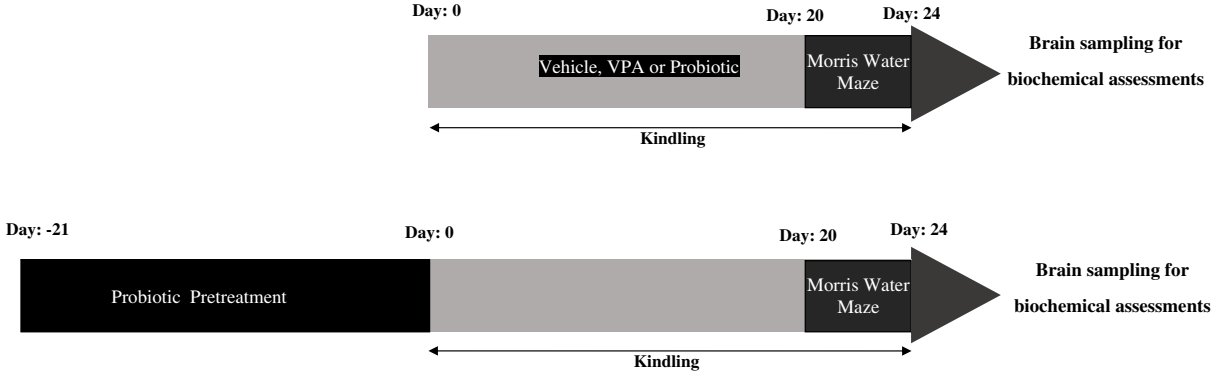

Fig. 1. The experimental schedule outlining animal grouping, and the time and duration of protocols applied.

followed by a 10-minute break for 4 consecutive days. In each trial, the animal was released facing the wall in one of four predetermined starting points and allowed to swim for a maximum time of 90 s to find the hidden platform. After reaching and mounting the platform, the rat was allowed to rest for 20 s before the next trial was initiated. If the subject failed to find the platform within 90 s, it was manually guided to the platform by the researcher where it remained for 20 s. Each day, the test was started from different quadrant for each animal. In the acquisition phase, the time elapsed (escape latency) and the distance traveled to locate the platform were measured as learning scores. Following the completion of the trial, the rats were dried and returned to their home cage.

On the fifth day, the rats were introduced to the probe trial to evaluate the animals' retrieval performance of spatial memory. The platform was removed, and the rats were allowed to navigate the maze for 30 s. Spatial retention in the probe trial was measured concerning the duration of time spent and the distance traveled in the memorized region of the water maze. A video autotracking system (RADIAB-7, I.R. Iran) was used to monitor and save the animal navigations in the water maze.

2.6. Brain tissue processing

On the last day of treatment, 30 min after PTZ administration, the animals were deeply anesthetized and were transcardially perfused with 100 ml of cold saline for 30 min. Then, the rats were sacrificed, and the brain was immediately dissected and stored at −80 °C until assessments.

2.7. Biochemical assessments

The frozen brain samples were homogenized in an ice cold 10% (w/v) sodium phosphate buffer (KCl, 140 mmol/l and phosphate, 20 mmol/l; pH 7.4) and then centrifuged at 5000*g* for 5 min. The clear supernatant was used for estimation of GABA, nitric oxide (NO), malondealdehyde (MDA), and total antioxidant capacity (TAC) contents. The brain tissue GABA concentrations (as ng/g tissue) were quantified by the use of commercial enzyme-linked immunosorbent assay (ELISA) kit (GABA ELISA Kit, myBioSource, USA). Nitric oxide metabolites (nitrite/nitrate) in the brain tissue were measured by colorimetric method at 450 nm based on the Griess reagent after reduction of nitrate to nitrite by vanadium trichloride. The nitrite/nitrate concentrations are expressed as μmol/g tissue. Concentration of MDA, as the lipid peroxidation indicator, was evaluated using the thiobarbituric acid reactive substance method. The MDA level is expressed as μmol/g tissue. Ferric reducing ability of plasma (FRAP) assay was used for measuring TAC of blood. This method determines the ability of plasma to reduce Fe3+ to Fe2+. The complex between Fe2+ and tripyridyl-s-triazine gives a blue color with absorbance at 593 nm [25].

2.8. Statistical analysis

Two-way analysis of variance (ANOVA) (kindling and probiotic treatment as independent factors), followed by least significant difference (LSD) post hoc test was applied on the data pooled from seizure activities and behavioral performances. Fischer's exact probability test was used to evaluate the incidence % of the animals showing full kindling (stage 5) across the experiments. Differences were considered to be significant for values of $P < 0.05$. All data are presented as the means ± standard error mean (S.E.M).

3. Results

3.1. The effect of probiotic supplement on the seizure severity

According to the statistical analysis, the testing groups showed different seizure activity ($F_{4,407} = 71.965$, $P < 0.0001$). In the PTZ group, the score of seizure was gradually increased over the experiment reaching to 4 ± 0.37. The seizure severity in the PRO + PTZ showed a steady manner throughout the chronic chemical kindling with a maximum score of 1.85 ± 0.14. A difference was evident between the PTZ and PRO + PTZ groups ($P < 0.0001$). The PTZ + PRO group also demonstrated a decrease in the seizure severity that was almost steady over the experiment. This group gained a maximum score of 2.9 ± 0.29 showing a significant difference with the PTZ group ($P < 0.0001$). Valproate efficiently protected the animals against the effect of PTZ where the VAP group rats showed the least level of the kindling (1.5 ± 0.14 score) compared with the other kindled rats ($P < 0.0001$). Therefore, our data demonstrate that the probiotic administration decreases the level of epileptic activity (Fig. 2).

The incidence of full kindling in the tested groups was also considered. Fifty percent (4 out of 8) of animals in the PTZ group exhibited the score of 5 within 3 consecutive scorings. None of the kindled rats in the VAP and PTZ + PRO groups showed the score of 5 over the experiment. Only one rat showed the score of 5 in the PRO + PTZ group. The Fischer's exact test displayed a significant difference between the PTZ group compared with the VAP and PTZ + PRO groups ($P < 0.006$). Concerning the full kindling phase, no statistical difference was evident between the animals in the VAP and both probiotic-treated groups. These findings indicate that the probiotic supplementation substantially reduces the seizure severity.

3.2. Assessment of the cognitive performances

To evaluate if the probiotic supplement influences spatial learning and memory, the kindled animals were introduced to Morris water maze. The acquisition and retrieval probe phases were considered to indicate the learning and the memory consolidation, respectively.

3.2.1. Acquisition phase

Analysis of the time elapsed to locate the hidden platform indicated a significant difference between the testing groups ($F_{4,779} = 2.725$, $P < 0.05$). The post hoc test indicated that the PTZ + PRO and PRO + PTZ rats overcame their PTZ counterparts ($P < 0.01$). The distance from the point of release to the hidden platform was also considered for evaluation of the maze steering. Our results demonstrated a considerable variation between the different groups ($F_{4,779} = 6.210$, $P < 0.001$). Post hoc analysis indicated that both probiotic-treated groups were superior on their PTZ counterparts ($P < 0.01$). On the other hand, the PRO + PTZ and PTZ + PRO groups had a lower maze performance when compared with either CON or VPA groups ($P < 0.01$). Fig. 3 illustrates performance of the animals in the acquisition phase of spatial cognition.

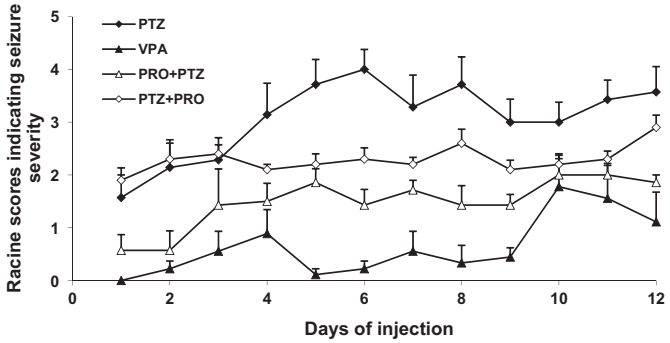

Fig. 2. The effect of the probiotic mixture on the seizure severity in the kindled rats. The mean Racine score in the PTZ group was the highest over the experiment. Valproic acid application greatly reduced the epileptic activity in the VPA. The probiotic treatment significantly diminished the level of seizure in the PRO + PTZ and PTZ + PRO, mostly in the former group. *P < 0.05, **P < 0.01, ***P < 0.001 compared with PTZ. #P < 0.05, ##P < 0.01 compared with VPA. ¶P < 0.05 compared with PTZ + PRO.

3.2.2. Probe trial phase

Statistical analysis applied on the time elapsed in the target quadrant proved a substantial difference among the testing groups ($F_{4,40} = 2.150$, $P > 0.05$). Posttest analysis indicated a high maze performance by the PTZ + PRO rats, which was varied significantly with the VPA and PRO + PTZ animals ($P < 0.01$). Regarding the distance traveled in the correct quadrant, we observed a general statistical difference between the animal groups ($F_{4,40} = 3.060$, $P < 0.05$). Within-group assessments showed that the PTZ + PRO group was superior to the CON and PTZ groups ($P < 0.05$). Also, the PTZ + PRO animals showed a higher performance than did their VPA and PRO + PTZ counterparts ($P < 0.01$). Fig. 4 depicts how the animals perform the retrieval memory in the probe trial test.

3.3. Biochemical assessments

3.3.1. The GABA concentration of the brain tissue

Measurement of level of the inhibitory neurotransmitter GABA in the brain tissue indicated no variation between the CON and PTZ groups. The probiotic treatment in the PTZ + PRO group considerably elevated the GABA concentration compared with the CON ($P < 0.001$), PTZ ($P < 0.01$), and VPA ($P < 0.001$) groups. Surprisingly, the GABA concentration was reduced in the VPA group in comparison with either CON ($P < 0.001$) or PTZ ($P < 0.01$) groups (Fig. 5).

3.3.2. The level of MDA in the brain tissue

The brain concentration of the oxidant MDA was evaluated as end product of lipid peroxidation. The MDA level was significantly increased in the PTZ animals when compared with their CON counterparts ($P < 0.05$). The brain concentration of MDA in both probiotic-treated groups was highly decreased in comparison with the PTZ group ($P < 0.001$). The MDA level of the brain was also diminished in the VPA-kindled rats ($P < 0.05$). The PTZ + PRO and PRO + PTZ animals showed a lower brain level of MDA even when compared with the CON group ($P < 0.05$, Fig. 6).

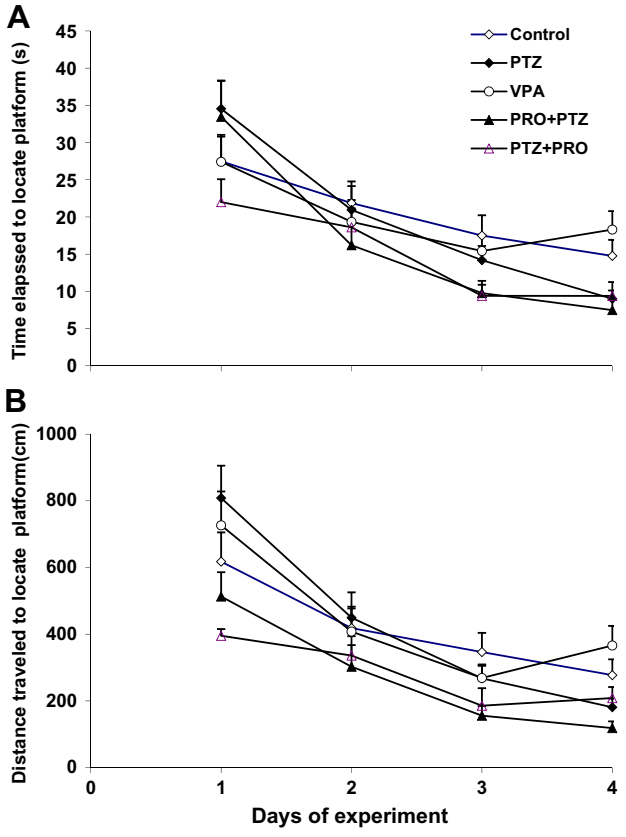

Fig. 3. The water maze navigation of the testing groups across four days of acquisition phase. A) The PTZ + PRO rats required less time to learn location of the hidden platform than did the PTZ animals ($P < 0.01$). B) In comparison with the PTZ rats both probiotic treated groups traveled less distance to find the hidden platform ($P < 0.01$).

3.3.3. The level of NO in the brain tissue

Concentration of NO in the brain tissue remained unchanged in the PTZ rats compared with the CON ones. However, the brain level of NO demonstrated a substantial reduction in either PTZ + PRO or PRO + PTZ animals when compared with the PTZ and CON groups ($P < 0.001$). The NO concentration was also mildly decreased in the VAP rats in comparison with the PTZ and CON groups ($P < 0.05$). Further, we observed a lower concentration of NO in the probiotic-treated animals compared with the valproate-treated ones ($P < 0.05$, Fig. 7).

3.3.4. The level of TAC in the brain tissue

The chemical kindling had no substantial effect of the TAC level of the brain. Valproic acid administration led to a slight decrease in the VPA group compared with the CON one ($P < 0.05$). Whereas simultaneous kindling and probiotic treatment highly elevated the brain concentration of TAC in the PTZ + PRO rats compared with the other testing groups ($P < 0.001$), the pretreatment with the probiotic mixture in the PRO + PTZ was ineffective on the antioxidant index (Fig. 8).

4. Discussion

Our findings in the present study demonstrated that treatment of chemically kindled animals with a mixture of probiotic bacteria substantially diminished epileptic activities. Such an effect was reflected in the Racine score levels as well as the number of the animals that reached full kindling. The intervention also had a favorable effect on the cognitive performances of the experimental subjects where it positively influenced both learning and memory consolidation. The data from the biochemical measurements support improvements in the epileptic and cognitive behaviors. The oxidant factors MDA and NO were reduced in the probiotic-administered groups. Also, the antioxidant index TAC was enhanced in a group of probiotic-treated rats. Concentration of the inhibitory neurotransmitter GABA was increased only in PTZ + PRO group. Importantly, almost majority of either behavioral or biochemical assessments were mostly highlighted in the PTZ + PRO group; in which the kindling and probiotic administration occurred simultaneously.

gamma-Aminobutyric acid as a bioactive component of pharmaceuticals and foods is produced by various microorganisms. Of probiotics, Lactobacilli are considered the main producers of GABA among bacteria [26]. Also, some strains of Bifidobacteria are shown to produce GABA [27]. These bacteria are able to produce GABA from its precursor monosodium glutamate [28]. Glutamic acid decarboxylase (GAD) enzymes are necessary for catalyzing the reaction. GAD genes are found in the gut microbiome as well as GABA-producing probiotic strains and influence health and behavior in animal models [29–31]. Therefore, it seems that increased level of GABA neurotransmitter in the probiotic-treated kindled rats might be contributed to alleviation of the kindling effects. However, we found a slight decrease in the level of GABA neurotransmitter in the VPA-treated PTZ group. Consistent result was reported by Safar et al. where they found that VPA decreased concentration of GABA [32]. A study of Guzman et al. also indicated that GABA concentration is not sensitive to VPA [33]. Considering the present results and those reported by other researchers, it seems that VPA may display its anticonvulsant properties through various mechanisms [34]. It should be noted that the role of the other main brain neurotransmitters must not be ignored where, along with inhibition of inhibitor GABA neurotransmitters, PTZ-induced convulsions that proceed into generalized form also start by activation of glutamate receptors [35].

Growing body of evidence indicates that certain pathologies, related to an altered microbiome, are linked to mood, stress, behavior, and cognition [14]. It is well known that PTZ-induced kindling is associated

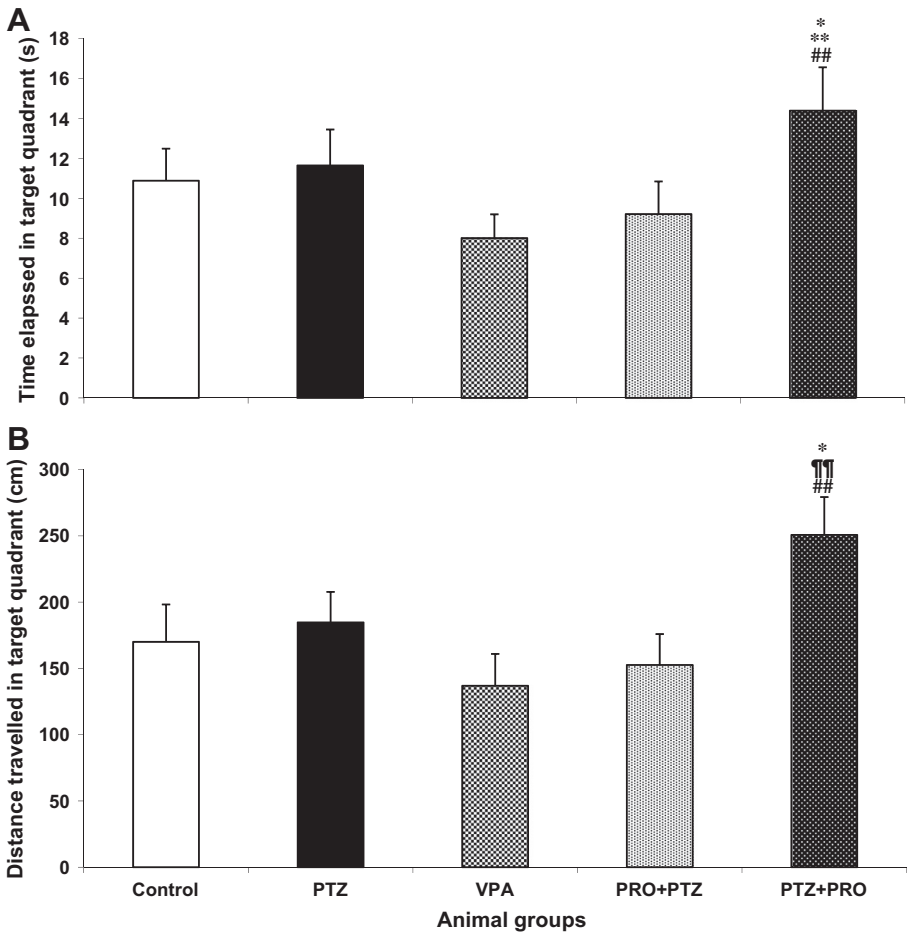

Fig. 4. Histograms to show how the different animal groups consolidated the learned target during the probe test. A) The PTZ + PRO rats significantly overcame the VPA and PRO + PTZ groups in the time elapsed in the target quadrant. B) Concerning the distance passed in the correct quadrant the PTZ + PRO animals showed to be superior to all testing groups. *P < 0.05 versus CON and PTZ; **P < 0.01 versus VPA; ##P < 0.01 versus PRO + PTZ.

with a variety of behavioral, neurophysiological, and neurochemical alterations. Epilepsy is known to be associated with cognitive dysfunction and, hence, impaired cognitive performance is reported in PTZ kindling [36]. Hence, it is important to have antiepileptic therapeutic paths with positive effects on epilepsy-associated cognition disturbances.

Although evidence indicating a link between gut microbiota and brain function is emerging [37], however, data addressing direct effects of probiotics on improving impaired learning and memory are scarce. Reports of a few studies are in accordance with our findings [38,39]. Gareau reported that intestinal dysbiosis in germ-free animals (containing no microbiota), bacterial infection with an enteric pathogen, and administration of probiotics all can modulate cognitive behaviors including learning and memory [40]. Our previous clinical and experimental findings prove that probiotic supplements may restore cognitive disorders [19,21]. Although the present findings provide further proof for favorable effect of probiotic on the cognitive

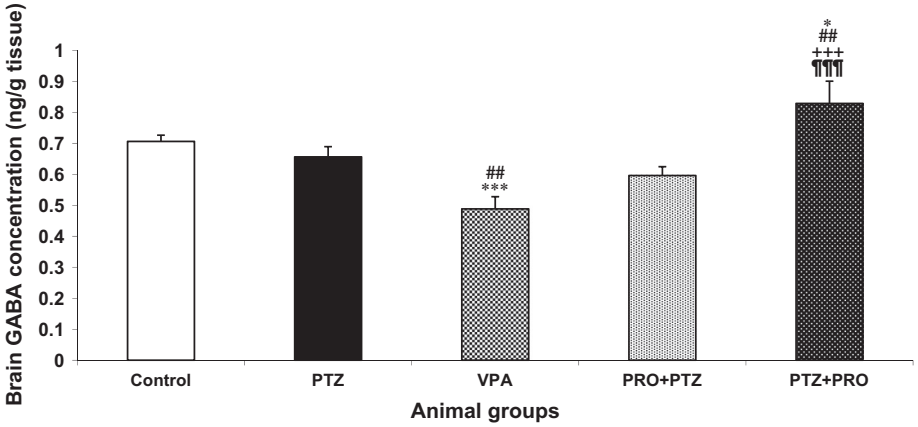

Fig. 5. Effect of probiotic on the brain level of GABA in the kindled rats. The GABA concentration was increased only in the PTZ + PRO rats. The tissue level of GABA was slightly reduced in the VPA group. The GABA concentration was unchanged in the other groups. *P < 0.05 versus control group; ***P < 0.00 1 versus Control group; ##p < 0.01 versus PTZ group; ¶¶¶P < 0.001 versus VPA group; +++P < 0.001 versus PRO + PTZ group.

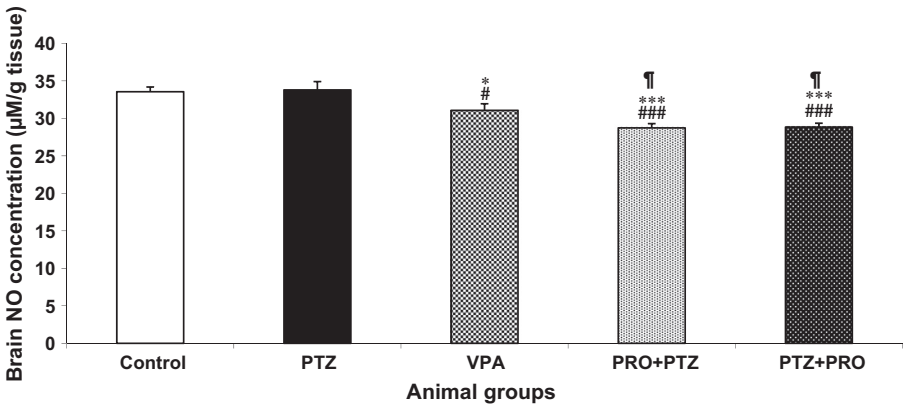

Fig. 6. Effect of probiotic on NO level of the brain tissue in the kindled rats. The NO concentration was decreased in both probiotic administered rats compared with the CON, PTZ, and VPA groups. The NO level was also decreased in the VPA animals in comparison with the CON and PTZ rats. *P < 0.05 versus Control group; ***P < 0.001 versus Control group; #P < 0.05 versus PTZ group; ###P < 0.001 versus PTZ group ¶P < 0.05 versus VPA group;.

aspects during an abnormal condition of brain, regardless, we are still in introductory status to judge if and how the probiotics affect learning and memory.

Plenty reports indicate that brain damages caused by oxidative stress are importantly involved in pathogenesis of some neurological disorders, including epilepsy [41–44]. Increasing evidence indicates that elevated oxidative stress, decreased antioxidant enzyme activity, and repetitive seizure attacks play an important role in neuronal death [45]. It is proposed that deficient antioxidant protection mechanisms and increased lipid peroxidation end products are reasoned for seizural activity [6–9]. Therefore, antioxidant agents have been expected to treat epilepsy [46]. Based on studies, AEDs attempt to adjust the antioxidant/oxidant balance in patients with epilepsy [9,47].

Nevertheless, the effect of AEDs on antioxidant status of epilepsy has been controversial where both effective [9,47,48] and ineffective [7,41,49] reports about the effect of AEDs on the antioxidant status of patients with epilepsy are present. In the present study, we asked if the probiotic bacteria underlie the brain concentration of the two oxidative stressors NO and MDA. We found that both NO and MDA level of the brain was decreased in the probiotic-administered animals. We also considered the TAC level of the brain as an indicator of antioxidant. The probiotic treatment elevated the TAC level of the brain tissue in the kindled rats.

Nitric oxide synthase (nNOS) is broadly expressed in neurons of the brain, where it produces NO. This enzyme is structurally associated with N-methyl-D-aspartic acid (NMDA) receptors [50]. It is demonstrated that PTZ kindling activates NMDA receptor [51], which, in turn, induces nNOS activity [52]. Hence, it is proposed that, during epileptic condition, nNOS is stimulated through NMDA receptor activation, which, in turn, enhances NO.

Malondealdehyde, as an oxidative factor, is an indicator of lipid peroxidation [46]. It affects ion exchange through cell membranes to cause cross-linking of membrane compounds, which has undesirable effects such as altered ion permeability and enzymatic activity. Some studies have suggested that, indeed, AEDs regulate the oxidant/antioxidant balance in patients with epilepsy [9,47].

Results of a meta-analysis revealed that probiotic treatment counteracts plasma concentration of oxidative stressors [53]. In an in vitro study, *Lactobacillus plantarum* C88 consumption increased TAC but decreased MDA in liver cells [54]. Also, in a clinical trial, probiotic supplementation increased plasma TAC and decreased MDA [55]. Increased level of TAC and decreased level of MDA by probiotic treatment were demonstrated in the study of Asemi and his colleagues [56]. In our previous studies, we found that while probiotic intake decreases plasma level of MDA in the patients with multiple sclerosis and Alzheimer's disease, the intervention is ineffective on TAC and NO concentration [18].

Some other substances have also shown both antiepileptic and antioxidant properties. Mehvari et al. reported that vitamin E administration caused a significant decrease in the frequency of seizures in patients with epilepsy along with a higher TAC, catalase, and glutathione [57]. Rathor et al. found that *Aloe vera* leaf extract administration

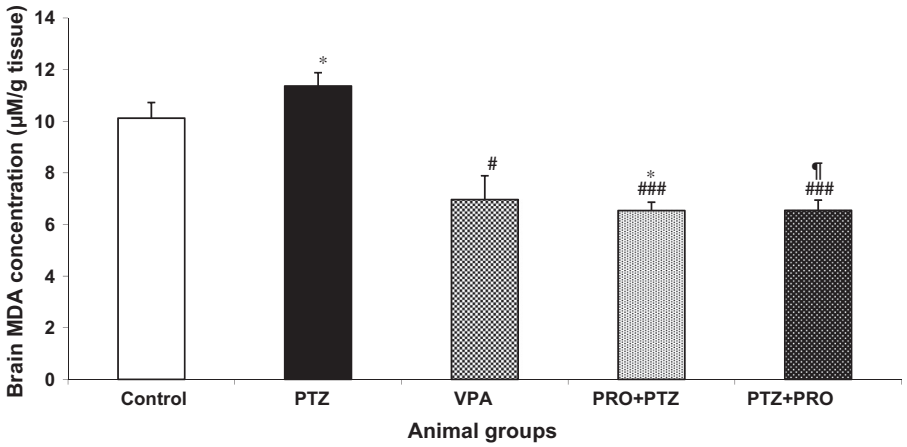

Fig. 7. Effect of probiotic on MDA level of the brain tissue in the kindled rats. While, in comparison with the CON rats, the tissue concentration of MDA increased in the PTZ animals it was decreased in the probiotic treated and VPA rats. * P < 0.05 versus Control group; # P < 0.05 versus PTZ group; ### P < 0.001 versus PTZ group; ¶ P < 0.05 versus VPA group.

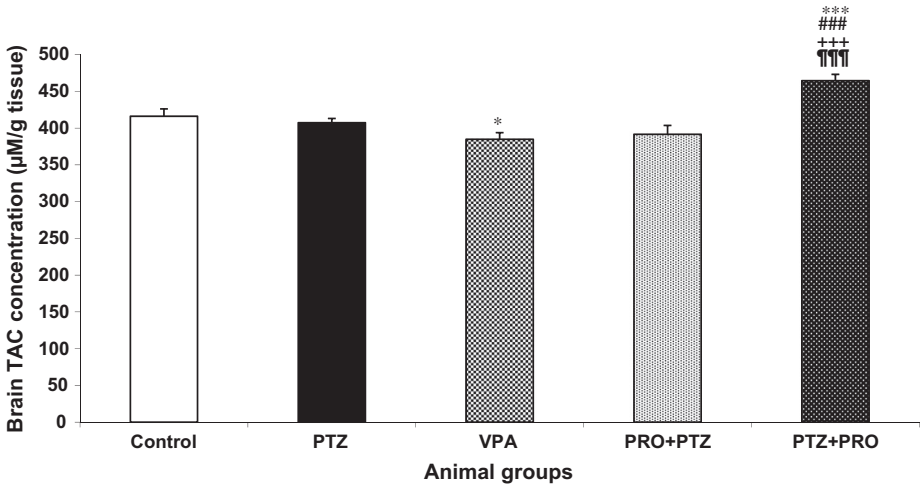

Fig. 8. Effect of probiotic on TAC level of the brain tissue in the kindled rats. The total antioxidant was increased in the PTZ + PRO animals that significantly differed compared with all of the other groups. *P < 0.05 versus Control group; ***P < 0.001 versus Control group; ###P < 0.001 versus PTZ group; ¶¶¶P < 0.05 versus VPA group; +++P < 0.001 versus PRO + PTZ group.

was associated with increased latency to onset and decreased duration of clonic convulsion, reduced brain levels of MDA, and increased levels of Glutathione (GSH) [58]. Magnesium supplementation displayed the anticonvulsant effects and enhanced the brain level of GSH and TAC in PTZ-induced kindled animals [32].

Evidence indicates that probiotics inhibit oxidative stress via reducing inflammation and increasing antioxidant enzymes [59]. Davis and Milner showed that intestinal microbiota provides additional enzymatic activities involved in the transformation of dietary compounds leading to increased bioavailability of dietary antioxidant [60]. Importantly, antioxidative stress role of probiotics is exerted through restoring gut microbiota [61,62]. Concomitantly, evidence shows that perturbations in the gut microbiome increase susceptibility to epilepsy [15].

Thus, we concluded that improvement of antioxidant/oxidant ratio might be another way by which the probiotic supplementation prevents the kindling-induced adverse effects.

5. Conclusion

The present work, as the first study evaluating the effect of probiotic supplementation on an animal model of epilepsy, revealed that the probiotic bacteria substantially reduce seizure severity. The oral bacteriotherapy also partly improved the spatial learning and memory in the kindled rats. While the imbalance of inhibitory/excitatory neurotransmission and the antioxidant/oxidant agents is known to be among the main reasons of epileptic seizure, our findings uncovered that the probiotic treatment reasonably increases the GABA activity and improves the antioxidant/oxidant balance in the kindled rats. Nevertheless, our findings are preliminary, and further preclinical and clinical studies are warranted to provide better scientific support confirming full potential of probiotics as a therapeutic strategy in management of epilepsy and epilepsy-associated complications.

Conflict of interest

No conflict of interest.

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