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ORIGINAL PAPER

The Beneficial Effect of Probiotics Supplementation on Penicillin-Induced Focal Seizure in Rats

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Abstract

The focal epilepsy is a chronic neurological brain disorder which affects millions of people in the world. There is emerging evidence that changes in the gut microbiota may have effects on epileptic seizures. In the present study, we examined the effect of probiotics on penicillin-induced focal seizure model in rats. Male Wistar Albino rats (n: 21) were randomly divided into three groups: control (no medication), penicillin and penicillin + probiotic. Probiotic VSL#3 (12.86 bn living bacteria/kg/day) was given by gavage for 30 days. The seizures were induced by intracortical injection of penicillin G (500 IU) into the cortex. An ECoG recordings were made for 180 min after penicillin G application. The spike frequency and the amplitude were used to assess the severity of seizures. Tumor necrosis factor (TNF-α), nitric oxide (NO) and interleukin (IL-6) levels in the brain were studied biochemically. Our results indicated that probiotic supplementation improved focal seizures through increasing the latency ($p < 0.001$) and decreasing the spike frequency ($p < 0.01$) compared to the penicillin group. Penicillin-induced seizure in rats significantly enhanced TNF-α ($p < 0.01$), NO ($p < 0.01$) and IL-6 ($p < 0.05$) compared to the control. Probiotic supplementation significantly decreased IL-6 ($p < 0.05$), TNF-α ($p < 0.01$) and NO ($p < 0.001$) compared to the penicillin group. When the body weights were compared before and after the experiment, there was no difference between the control and penicillin groups, but it was observed that the body weight decreased after probiotic supplementation in the penicillin + probiotic group. Probiotic supplementation may have anti-seizure effect by reducing proinflammatory cytokine and NO levels in epileptic rat brain.

Keywords Focal seizure · Nitric oxide · Penicillin · Probiotic · Proinflammatory cytokines · VSL#3

Introduction

Epilepsy is a chronic neurological disease that affects millions of people worldwide [1]. In addition, approximately 30% of patients with epilepsy are resistant to traditional antiepileptic drugs and new treatment approaches are needed to control epileptic seizures in patients suffering from this disease [2].

The human gut microbiome including ~ 100 trillion microbes such as fungi, bacteria, viruses and protozoa [3]. Studies investigating the role of the gut microbiome in health and disease are increasing day by day. The gut microbiota plays an important role in the brain function [4]. Recently, it has been shown that changes in the microbiome and the role of the gut microbiome are the basis of neurodegenerative diseases [5].

The composition of the gut microbiota may be altered by environmental factors such as medication, stress and diet [4]. It was reported that changes in the composition and amount of gut microorganisms may have an effect on the formation of epileptiform activity [4, 5]. Probiotics are the living microorganisms beneficial for animals and humans when supplemented adequately [6].

VSL#3 is a high-concentration probiotic mixture of eight live bacterial species including four strains of *Lactobacillus*, three strains of *Bifidobacterium* and one strain of *Streptococcus* [7, 8]. Different bacterial strains have various effects. For example, the strains of *Bacillus* genus can generate noradrenalin and dopamine while the *Lactobacilli* strains

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can generate γ-aminobutyric acid (GABA) and acetylcholine. *Bifidobacteria* strains, on the other hand, can produce GABA, and *Streptococcus* strain can generate serotonin (5-hydroxytryptamine, 5-HT) [9–12]. It was shown that the Streptococcus thermophilus can play an important role in defense system [7]. In most studies, use of VSL#3 was proven to be safe and well tolerated, but few studies investigated its effectiveness on epilepsy [8, 13].

The penicillin epilepsy model is widely used focal seizure models [14]. Penicillin is a GABA antagonist. When injected intracortical, it causes seizures that start focally and continue as generalized [15]. Therefore, the aim of the present study was to examine the effect of probiotic supplementation on epileptiform activity in experimental focal penicillin epilepsy model.

Material and Methods

Animal

Male Wistar Albino rats were used in the present study (n: 21). The day/night cycle is very important for the normal physiological behavior of these animals. For this reason, rats were housed in well-ventilated rooms with a 12-h light/12-h dark cycle, a relative humidity of 45–60%, and an average ambient temperature of 23 °C. Feed and water were provided to the rats ad libitum. All study procedures were approved by the Tokat Gaziosmanpaşa University Local Ethical Committee for Animal Experiments (HADYEK/2019–10).

Drugs

The study was performed in general anesthetized rats. Urethane (1.25 g/kg) was given intraperitoneally (i.p.). The urethane and Penicillin G potassium were purchased from Sigma Chemical Co. Probiotic VSL#3 (CD-Investments s.r.l/Rome/Italy) were given at a dose of 12.86 bn living bacteria/kg/day for 30 days by gavage. VSL#3 is a commercial probiotic mixture. It contains four strains of *Lactobacillus* (*Lactobacillus plantarum, L. acidophilus, L. delbrueckii subsp. bulgaricus* and *L. casei*), three strains of *Bifidobacterium* (*Bifidobacterium longum*, *B. breve* and *B. infantis*) and *Streptococcus salivarius subsp. thermophilus*. Probiotic VSL#3 and urethan were dissolved in saline while Penicillin G potassium was dissolved in sterile distilled water.

Experimental Protocol

Rats were divided randomly into three groups (n: 7) of control, penicillin and probiotic treatments. The body weights of all rats were determined on the first day of the experiment. Then, 1 ml of saline was given to the control and penicillin groups by gavage for 30 days. 12.86 bn living bacteria/kg was dissolved in 1 ml of saline and given to probiotic group by gavage for 30 days. On the 31st day of the experiment, the body weights were measured again before the rats were anesthetized (Fig. 1). Anesthesia was performed with urethane. The surgical procedure was carried out as described below and a tripolar electrode was placed on the skull. After the surgical procedure, basal recordings were taken from all rats for five minutes to observe whether there was a change in the ECoG recording in the rats. Then, sterile distilled water (2.5 µl) was applied intracortically to the control group, and recording was continued for 180 min. Epileptiform activity was created by intracortical application of penicillin (500 IU/2.5 µl) to the rats in the probiotic and penicillin groups, and recording was made for 180 min. After 180 min of recording in all three groups, rats were sacrificed under anesthesia and brain tissue was excised for biochemical analyses (Fig. 1).

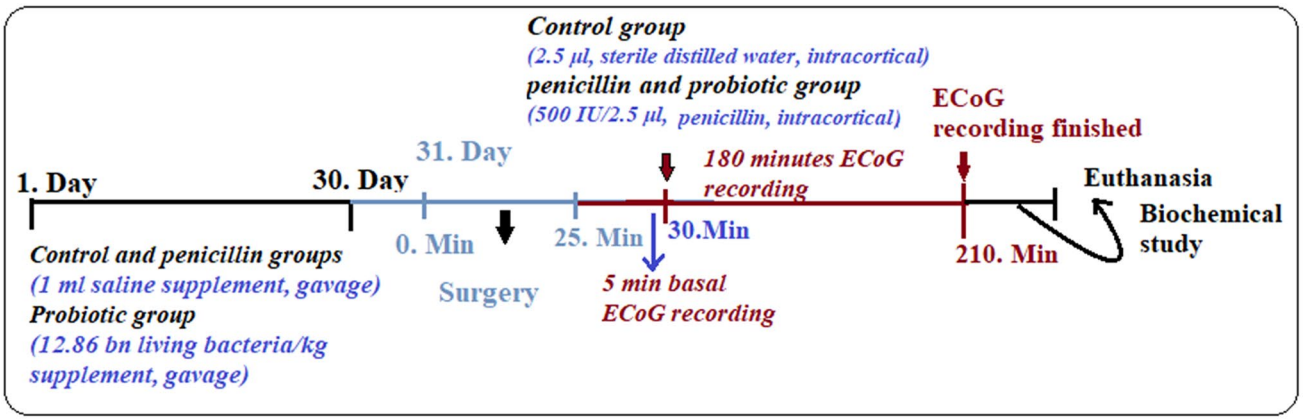


Fig. 1 Experiment timeline of all experimental groups

Surgical Procedure and Electrode Implantation

After 30 days, general anesthesia with urethane (1.25 g/kg) was applied to the rats. Then the animals were placed in the stereotaxic device. The scalp was opened and skull was cleared to show bregma. The points were determined with reference to bregma to place the bipolar electrodes. The three holes (0.2-mm diameter) were drilled on skull by microdrill. Two bipolar recording electrodes were placed on left parietal cortex (L + 4 mm; AP-3 mm) and left frontal cortex (L + 3.5 mm; AP + 3 mm). Ground electrode was placed to rat skin. A third hole was drilled for penicillin injection.

ECoG Recording

ECoG recording was performed using by MP-150 BioPac Systems. Baseline ECoG recordings were taken five minutes before the saline and penicillin (500 IU) administration. Also, epileptic focus was induced with 500 IU of the Penicillin G potassium application. Penicillin G was injected by a Hamilton microsyringe type 701 N in the left parietal cortex (from bregma: 3 mm rostral and 3 mm lateral). To inject penicillin G potassium into the cortex, the Hamilton microsyringe was inserted to a 3.2-mm depth to the skull surface without damaging blood vessels. 500 IU/2.5 µl penicillin G potassium was administered for five min at a rate of 0.5 µl/min. After penicillin injection ECoG recordings were performed for 180 min. Similarly, 2.5 µl sterile distilled water was applied to the rats in the control group using a Hamilton microsyringe into the cortex 3.2 mm depth of skull surface (rate: 0.5 µl/min), and recording was made for 180 min.

Biochemical Assays

Tissue samples were washed using 0.9% cold isotonic saline. Wet tissues were weighed, cut into small pieces with a scalpel, and homogenized using stainless steel balls in cold PBS (pH: 7.4). Tissues were centrifuged at 2500 rpm for 20 min. TNF-α, IL-6 and NO levels were determined using commercial quantitative sandwich ELISA kits (Bioassay Technology Laboratory) based on manufacturer's instructions. The results were normalized on the basis of wet tissue weight and expressed as µmol/L/g for NO and ng/L/g for TNF-α and IL6.

Statistical Analyses

All data were evaluated statistically using SPSS 20.0 software and GraphPad Prism (V:7, U.S.A.). The Kolmogorov Smirnov test was used to determine whether the groups had normal distribution. All data of the groups were found to have normal distribution. One-Way ANOVA and post-hoc Tukey or LSD tests were employed to reveal the statistical significance of differences among the groups. Paired sample-t test was used for body weights. Group values were expressed as mean ± standard error (SEM). Differences of $p < 0.05$ were considered statistically significant.

Results

Evaluation of ECoG Records

In the five-minute baseline recordings from all groups and the 180-min recording from the control group, and spike-like discharges representing epileptiform activity were not observed. The latent period is the period that occurs immediately after intracortical injection and continues until the onset of spike formation. Since no spike frequency was observed in the control group, the latent period spike frequency and average amplitude values were evaluated as 0 in the analyses of ECoG recordings. When the control group was compared to the penicillin and probiotic groups, it was observed that the length of the latent period, spike frequency and average amplitude values were significantly higher in the penicillin and probiotic groups compared to the control group ($p < 0.001$). A typical recording during basal brain activity of an anesthetized rat in the control group is illustrated in Fig. 2A.

Slow waves were significantly suppressed in the latent period after penicillin injection. The latency period was significantly longer in the probiotic group compared to the penicillin group ($p < 0.001$). In addition, it was found that the mean spike frequency decreased significantly in the probiotic group compared to the penicillin group ($p < 0.01$). However, there was no significant difference between the penicillin and probiotic groups for mean amplitude values ($p > 0.05$) (Fig. 3).

Biochemical Assay

Regarding the percentage of interleukin (IL)-6 (ng/L/g), tumor necrosis factor alpha (TNF-α) (ng/L/g) and nitric oxide (NO) (µ mol/L/g) parameters, penicillin injection significantly increased IL-6 ($p < 0.05$), TNF-α ($p < 0.01$) and NO levels ($p < 0.05$) compared to the control group. NO levels were significantly lower in probiotic group when compared to the control group ($p < 0.05$). IL-6 ($p < 0.05$), TNF-α ($p < 0.01$) and NO levels ($p < 0.001$) were significantly lower in probiotic group compared to the penicillin group. Data are shown in Fig. 4.

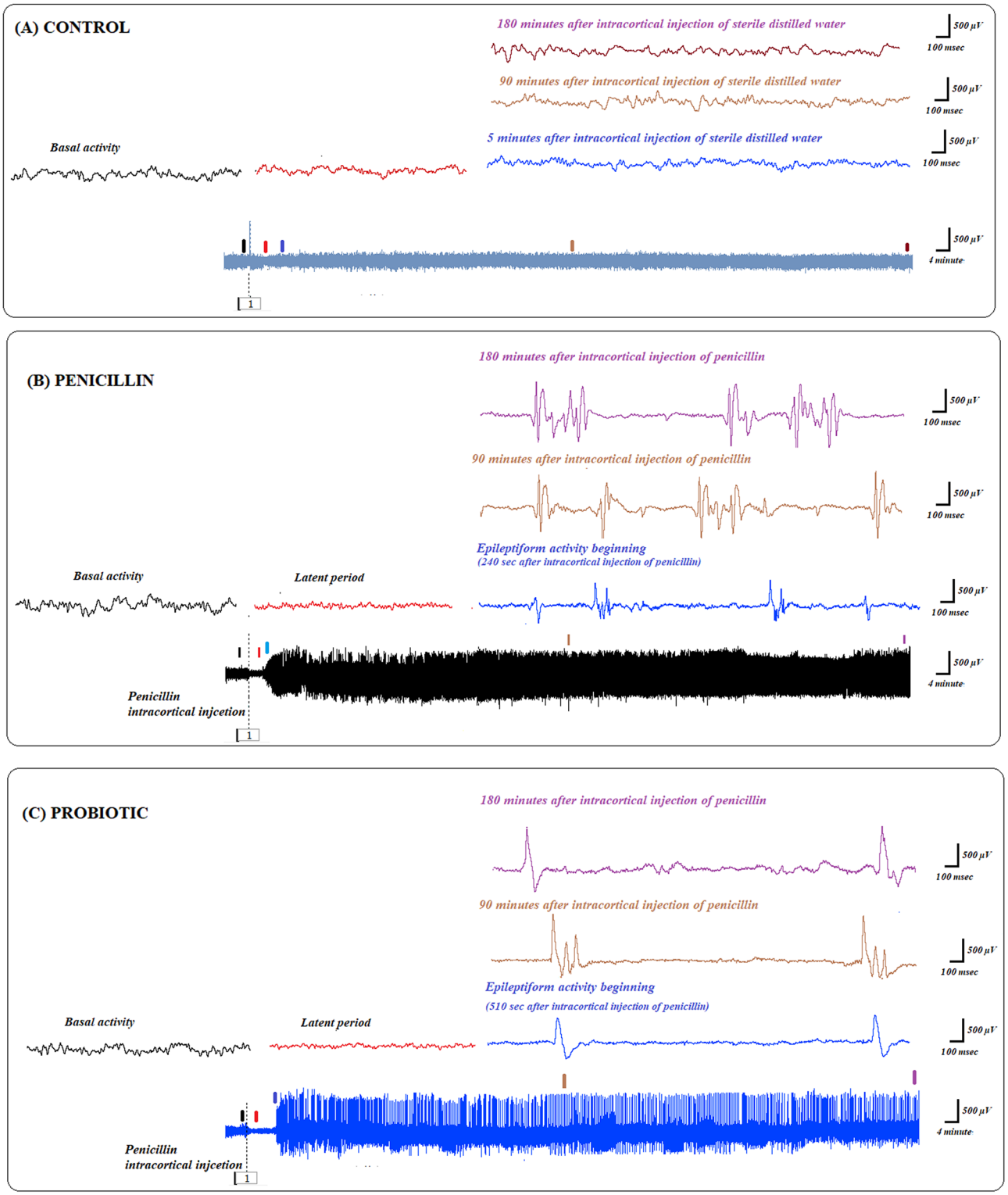


Fig. 2 A representative illustration of ECoG activity in **A** control, **B** penicillin, **C** probiotic groups

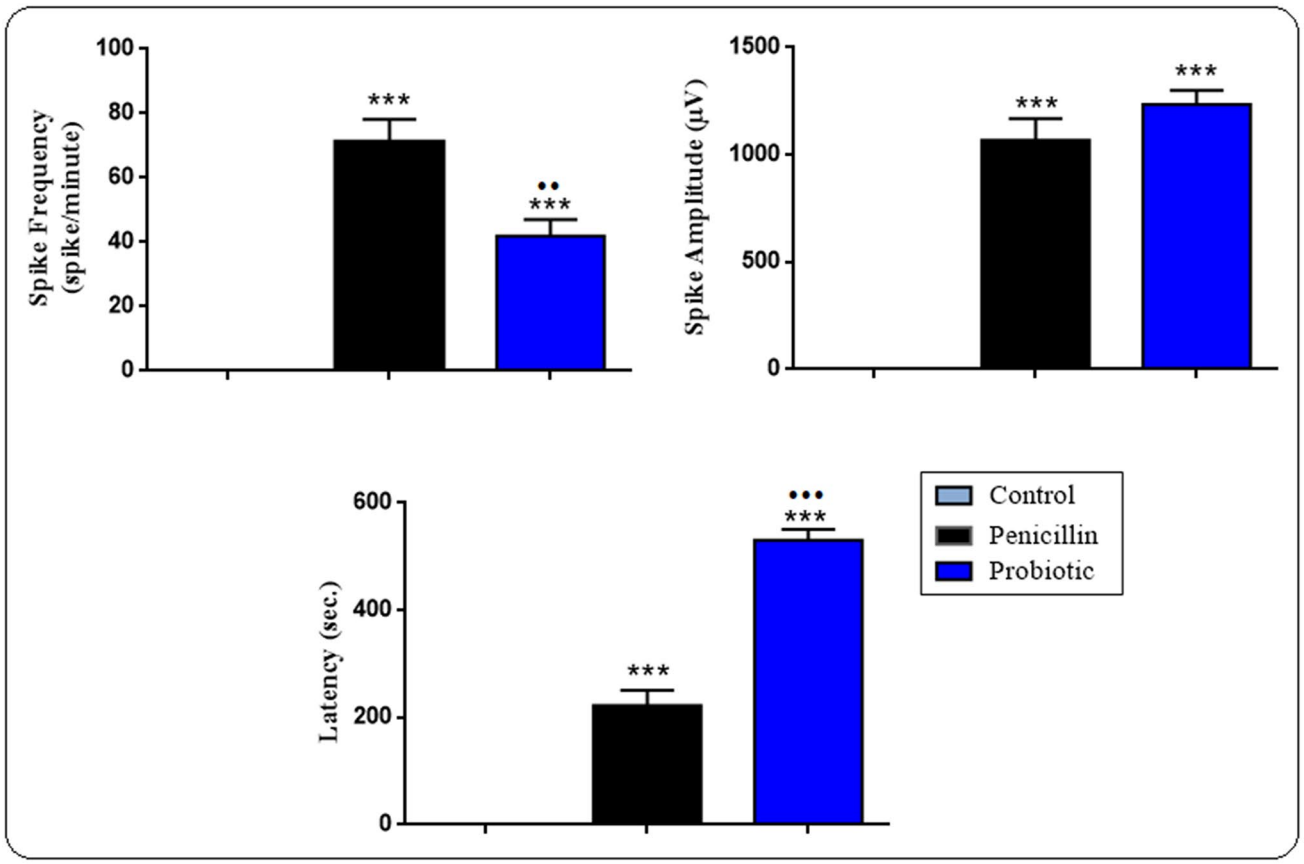


Fig. 3 Probiotic supplementation increased the latency and decreased the spike frequency compared to the penicillin group (••$p < 0.01$; •••$p < 0.001$). Spike, spike-wave discharges which are indicative of epileptiform activity were not observed in the control group. ***$p < 0.01$, penicillin group compared to the control group

Body Weight Assay

The body weights of all groups were determined on the first day of the experiment, and probiotic supplementation was started (day 1 of the experiment). Probiotic supplementation was continued for 30 days. On the 31st day of the experiment, body weights were measured again in all groups before the rats were anesthetized.

No significant difference was found between the body weights on the 1st day and the 30th day in the control and penicillin groups. In the probiotic group, on the other hand, the difference was significant ($p < 0.05$), (Fig. 5). The fact that the control and penicillin groups received only saline for 30 days may have caused no change in body weights of the rats in these groups. In the probiotic group, taking a probiotic supplement for 30 days may have caused a decrease in body weight.

Discussion

The major finding of the current study was that probiotic VSL#3 supplementation reduced spike frequency significantly in penicillin-induced focal seizures. Additionally, our data showed that VSL#3 limited the increases of TNF-α, IL-6 and NO levels in epileptic rats. Also, VSL#3 supplementation decreased body weight of the rats in the probiotic group.

In some experimental studies on neurodegenerative diseases, both saline and probiotic administration alone formed two separate groups. In the study of Athari et al., the saline-only group was compared with the probiotic-only group, and it was observed that there was no statistical difference for the levels of oxidative stress biomarkers [superoxide dismutase (SOD), catalase activity and malondialdehyde (MDA)] in

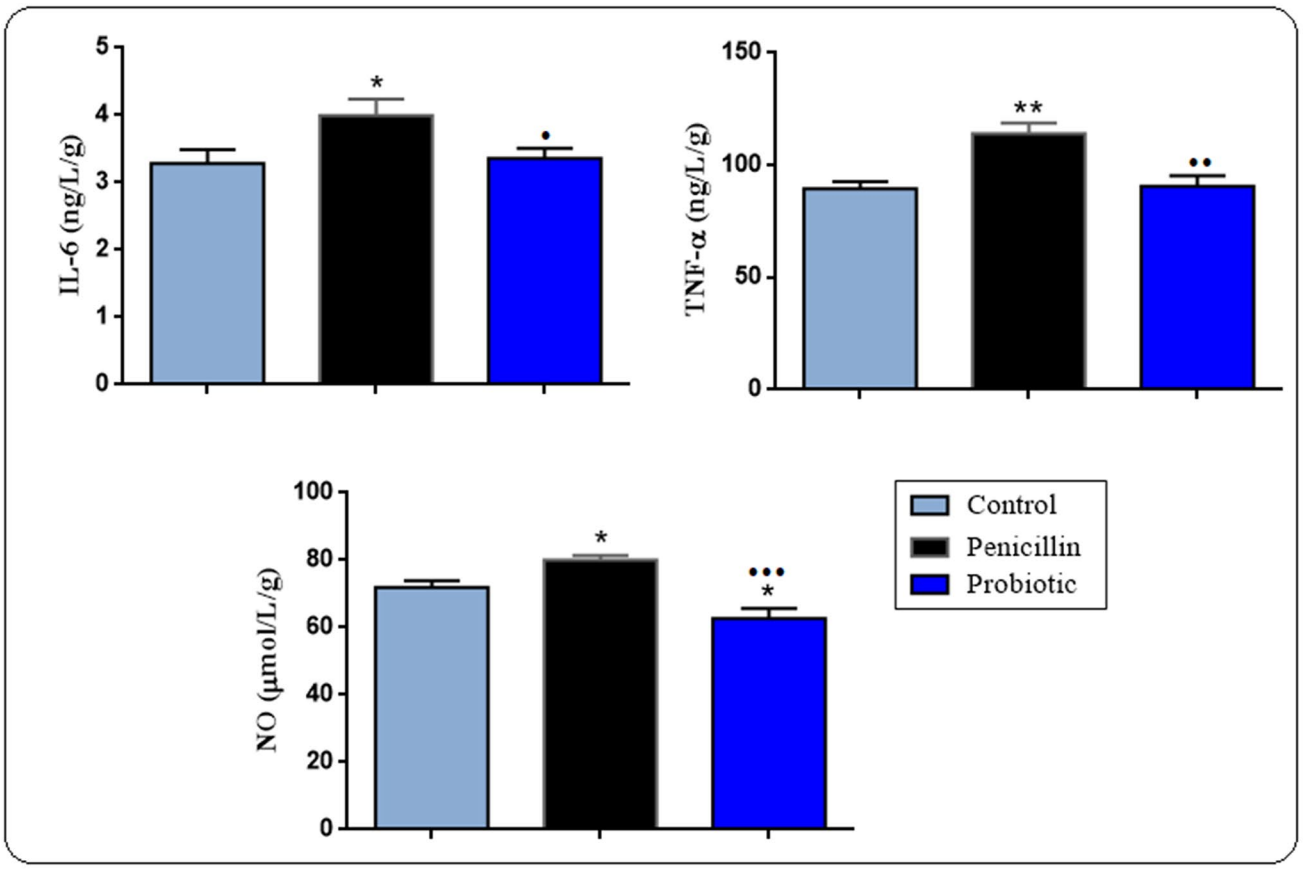


Fig. 4 IL-6 (ng/L/g), TNF-α ng/L/g) and NO (mmol/L/g) levels were significantly higher in penicillin group compared to the control group. NO levels were significantly lower in probiotic group compared to the control group (*p < 0.05; **p < 0.01). IL-6, TNF- α and NO levels were significantly lower in probiotic group compared to the penicillin group (•p < 0.05; ••p < 0.01; • • •p < 0.001)

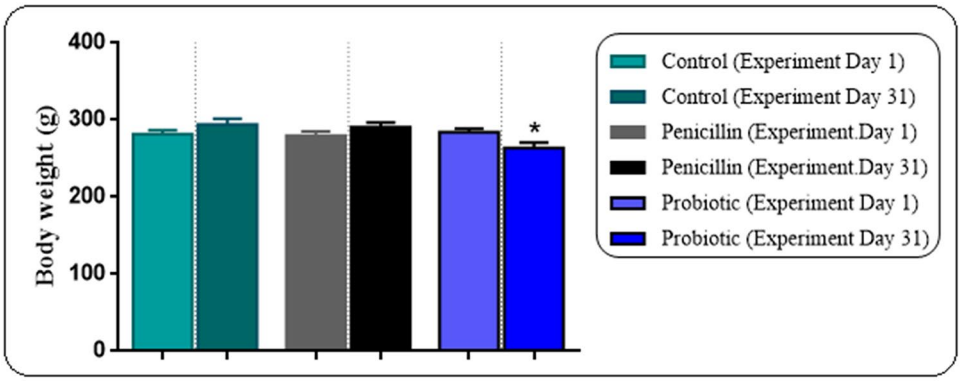


Fig. 5 Body weight (g) of the rats in study groups on the first and 31st day of the experiment (*p < 0.05)

the brain [16]. In the study of Asl et al. [17], the group that was given drinking water was compared with the group that took only probiotic supplement, and it was shown that there was no change in plasma MDA and total antioxidant capacity (TAC) levels. In another study, it was observed that there was no difference between the groups taking drinking water and probiotics only for the values of dopamine, serotonin, acetylcholine, acetylcholine esterase, nitric oxide (NO) and blood glucose in plasma [18]. In addition, in hematoxylin–eosin and immunohistochemical studies, no difference was found between the saline-only and probiotic-only groups [18]. In the study by Dwyer et al., the group that took only probiotic supplement and the group that took only drinking water were compared, and no change was observed between the two groups for the number of T helper cells, TNF-alpha (TNF-α), interleukin (IL)-6, IL-B1, IL-10 levels and glial

fibrillary acidic protein (GFAP) at the level of microglia [19]. When all studies are evaluated together, it was shown that probiotic supplementation alone does not affect oxidative stress markers, does not lead to changes in neurotransmitters and does not affect proinflammatory cytokines. Also considering the previous studies, a saline only group was established in order not to use too many animals. For this reason, the saline group was used as the control group in the present study.

Gut microbiota or gut flora consists of microorganisms such as various bacteria, archaea and fungi [20]. The gut microbiota might have a critical role in many neurodegenerative diseases especially due to its two-way communication with the gut-brain axis [20]. Changes in the gut microbiota were shown to play a role in the pathogenesis of depression, anxiety, schizophrenia, Alzheimer's disease, autism and Parkinson's disease by causing irregularities in the gut-brain axis [21–24]. Studies investigating the relationship between the gut microbiome and epilepsy are very limited. Many studies indicated that restructuring of the gut microbiota with a ketogenic diet, probiotic supplementation and fecal microbiota transplantation was shown to reduce seizure activity in drug-resistant epilepsy [25–27]. Medel-Matus et al. [28] showed that microbial transplantation in animals reduced epileptic seizures in amygdala kindling epilepsy model. A study comparing the patients with < 4 seizure episodes per year and patients with > 4 seizure episodes per year reported higher amounts of *Bifidobacterium* and *Lactobacillus* bacteria in patients with fewer seizures [29]. Another study indicated that supplementation with a probiotic mixture (*Lactobacillus reuteri, Bifdobacterium infantis*, and *Lactobacillus rhamnosus*) significantly decreased the severity of PTZ-induced epileptic seizures [6]. Previous studies showed that bacteria of *Bifidobacterium* and *Lactobacillus* genera could have protective effects against epileptic seizures [6, 13, 29]. VSL#3 consists of eight bacterial strains including *Bifidobacterium* and *Lactobacillus* genus of bacteria [7, 8], and may have a protective effect against the epileptic seizures. In line with this, a study demonstrated that the VSL#3 probiotic supplementation significantly decreased kindling development and increased latency in PTZ- kindling model of epilepsy [13]. In the present study, similar to the previous ones [6, 13], VSL#3 probiotic supplementation reduced the spike frequency in penicillin-induced focal seizure model.

In the present study, the anti-seizure effect of probiotic VSL#3 supplementation can be explained by several mechanisms. Firstly, in many studies, the relationship between the immune system and gut bacteria was extensively investigated [8, 12]. The contribution of inflammation to the formation of epileptic seizures is well known [30]. The increasing proinflammatory cytokines including TNF-α and IL-6 promote the onset of epilepsy [30]. In regard to this, studies demonstrated that the expression of IL-6 and TNF-α was elevated in most epileptic patients compared to the control subjects [31, 32]. Also, different studies found that enhanced level of IL-6 was associated with epileptic conditions [33]. Samland et al. [34] reported that overproduction of IL-6 caused a remarkable increase in glutamatergic agonist-induced epileptic seizures. Proinflammatory cytokines increase the permeability of the blood brain barrier (BBB), thereby leading to inflammation in central nerve system [30, 35]. These effects promote development of epileptic seizures by increasing the excitability of neurons [30]. In the present study, it was observed that TNF-α and IL-6 levels were high in rats with penicillin-induced seizures. Also, our data showed that probiotic supplementation reduced TNF-α and IL-6 levels in epileptic rat brains. Many studies reported that probiotics exert anti-inflammatory effects, and decrease proinflammatory cytokines [25, 36, 37]. In addition, a clinical study showed that probiotic mixture supplementation reduced the number of epileptic seizures by more than 50% and lowered concentrations of IL-6 in patients with epilepsy [25]. According to the data of the presented study, VSL#3 probiotic may have had an anti-seizure effect in the seizure model induced by penicillin through reducing proinflammatory cytokines.

Secondly, proconvulsant or anticonvulsant properties of NO donors/NO inhibitors were shown in many experimental models of epilepsy (e.g., penicillin-induced, electrically or pharmacologically-evoked convulsions) [38, 39]. A clinical study indicated that nitric oxide synthase had higher expression levels in the brain of patients with epilepsy [40]. Increasing NOS or NO levels in epileptic brains were shown in various experimental models of epilepsy including maximal electroshock stimulation, PTZ-induced epilepsy model, kainic acid-induced convulsion model [41–44]. In an interesting study, it was demonstrated that NO increased in cortex by 302% in the 30th minute and by 633% in the 90th minute after kainic acid-induced convulsion [43]. In the present study, NO level in the brain was measured 180 min after the seizure with penicillin. It was concluded that longer duration of seizure may have increased NO level.

Many studies reported that the inhibition of NOS reduced BBB disruption by upregulating zonula occludens-1 [45–47]. Increased NO level in the brain cause oxidative stress [43]. Disruption of BBB and oxidative stress cause seizures. Therefore, NO and neuroinflammation reducing in central nerve system could play important roles in controlling the epileptic seizures. Bagheri et al. [6] demonstrated that probiotic supplementation enhanced the antioxidant capacity of the brain by significantly reducing malondialdehyde and nitric oxide concentrations in PTZ-induced epileptic rats [6]. Additionally, similar to previous studies, our data showed that probiotic VSL#3 supplementation reduced NO level in epileptic rat brains.

Previous studies demonstrated that probiotics supplementation modify the secretion of neurotransmitters and hormones, and reduce inflammatory factors. Thus preventing food intake triggers lead to weight loss [48]. Also, probiotics supplementation increases the production of short-chain-fatty acids that influence the energy homeostasis and appetite [49]. In addition, probiotic supplementation reduces the total triglycerides, LDL cholesterol and total cholesterol levels, while they enhance the level of HDL cholesterol [50, 51]. Many studies have reported that some probiotic species, such as Bifidobacterium and Lactobacillus, have an anti-obesity effect when consumed for 4–6 weeks [52–54]. Probiotic VSL#3 contains Lactobacillus and Bifidobacterium bacterial species. Probiotic VSL#3 was shown to reduce the body weight and fat mass during a high-fat diet compared to the control (placebo) [55, 56]. Moreover, another study showed that VSL#3 supplementation was able to prevent diabetes in high-fat diet-induced obesity model by reducing weight gain and inhibiting insulin resistance [57]. It was shown in experimental studies that VSL#3 could prevent obesity and glucose intolerance through increasing the level of proopiomelanocortin and reducing the agouti-related protein (AgRP) and neuropeptide Y levels in the hypothalamus, which are related to energy metabolism [57, 58]. Our results indicated that the body weight of rats after probiotic supplementation (for one month) was less than the body weight measured before probiotic supplementation. The richness of the bacterial diversity contained in VSL#3 and the long-term supplementation to rats may have caused the weight loss in rats. Anti-epileptic drugs often increase body weight and may cause various chronic diseases [59, 60]. VSL#3 is the only probiotic recognized as a type of medicinal food [8]. For this reason, it may be useful in weight control of patients with epilepsy.

Conclusion

The findings of the present study suggested that penicillin-induced epileptiform activity in rats was associated with elevated levels of NO, TNF- α and IL-6 in brain tissue. Probiotic VSL#3 supplementation reduced the seizure activity by reducing pro-inflammatory cytokines and NO levels in penicillin-induced focal epilepsy model.

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Data Availability The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

1. World Health Organization (WHO) (2019) Epilepsy. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/epilepsy
2. Chen B, Detyniecki K, Choi H et al (2017) Psychiatric and behavioral side effects of anti-epileptic drugs in adolescents and children with epilepsy. Eur J Paediatr Neurol 21(3):441–449
3. Holmes M, Flaminio Z, Vardhan M et al (2020) Cross talk between drug-resistant epilepsy and the gut microbiome. Epilepsia 61(12):2619–2628
4. Lum G, Olson C, Hsiaso E (2020) Emerging roles for the intestinal microbiome in epilepsy. Neurobiol Dis 135:104576
5. Catanzaro R, Anzalone M, Calabrese F et al (2014) The gut microbiota and its correlations with the central nervous system disorders. Panminerva Med 57(3):127–143
6. Bagheri S, Heydari A, Alinaghipour A, Salami M (2019) Effect of probiotic supplementation on seizure activity and cognitive performance in PTZ-induced chemical kindling. Epilepsy Behav 95:43–50
7. Douillard FP, Mora D, Eijlander RT et al (2018) Comparative genomic analysis of the multispecies probiotic-marketed product VSL#3. PLoS ONE 13:e0192452
8. Cheng FS, Pan D, Chang B et al (2020) Probiotic mixture VSL# 3: an overview of basic and clinical studies in chronic diseases. World J Clin Cases 8(8):1361
9. Lyte M (2011) Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. BioEssays 33(8):574–581
10. Barrett E, Ross RP, O'Toole PWC et al (2012) gamma-Aminobutyric acid production by culturable bacteria from the human intestine. J Appl Microbiol 113(2):411–417
11. Dinan TG, Stilling RM, Stanton C, Cryan JF (2015) Collective unconscious: how gut microbes shape human behavior. J Psychiatr Res 63:1–9
12. Yue Q, Ca, M, Xiao B et al (2021). The Microbiota–Gut–Brain Axis and Epilepsy. Cell Mol Neurobiol 1–15
13. Tahmasebi S, Oryan S, Mohajerani HR et al (2020) Probiotics and Nigella sativa extract supplementation improved behavioral and electrophysiological effects of PTZ-induced chemical kindling in rats. Epilepsy Behav 104:106897
14. Fisher RS, Acevedo C, Arzimanoglou A et al (2014) ILAE official report: a practical clinical definition of epilepsy. Epilepsia 55(4):475–482
15. Aygun H, Ayyildiz M (2021) Vortioxetine increases absence-like seizures in WAG/Rij rats but decreases penicillin-and pentylenetetrazole-induced seizures in Wistar rats. Epilepsy Behav 116:107797
16. Azm SAN, Djazayeri A, Safa M, Azami K, Ahmadvand B, Sabbaghziarani F, Vafa M (2018) Lactobacilli and bifidobacteria ameliorate memory and learning deficits and oxidative stress in β-amyloid (1–42) injected rats. Appl Physiol Nutr Metab 43(7):718–726
17. Asl ZR, Sepehri G, Salami M (2019) Probiotic treatment improves the impaired spatial cognitive performance and restores synaptic plasticity in an animal model of Alzheimer's disease. Behav Brain Res 376:112183

18. Beltagy DM, Nawar NF, Mohamed TM, Tousson E, El-Keey MM (2021) Beneficial consequences of probiotic on mitochondrial hippocampus in Alzheimer's disease. J Compl Integrat Med
19. Dwyer Z, Chaiquin M, Landrigan J, Ayoub K, Shail P, Rocha J, Hayley S (2021) The impact of dextran sodium sulphate and probiotic pre-treatment in a murine model of Parkinson's disease. J Neuroinflammation 18(1):1–15
20. Westfall S, Lomis N, Kahouli I et al (2017) Microbiome, probiotics and neurodegenerative diseases: deciphering the gut brain axis. Cell Mol Life Sci 74(20):3769–3787
21. Foster JA, McVey Neufeld KA (2013) Gut-brain axis: how the microbiome influences anxiety and depression. Trends Neurosci 36:305–312
22. Nemani K, Hosseini Ghomi R et al (2015) Schizophrenia and the gut-brain axis. Prog Neuropsychopharmacol Biol Psychiatry 56:155–160
23. Kohler CA, Maes M, Slyepchenko A et al (2016) The gut-brain axis, including the microbiome, leaky gut and bacterial translocation: mechanisms and pathophysiological role in Alzheimer's disease. Curr Pharm Des 22:6152–6166
24. Vuong HE, Hsiao EY (2017) Emerging roles for the gut microbiome in autism spectrum disorder. Biol Psychiatry 81:411–423
25. Gómez-Eguílaz M, Ramón-Trapero JL, Pérez-Martínez L, Blanco JR (2018) The beneficial effect of probiotics as a supplementary treatment in drug-resistant epilepsy: a pilot study. Benefic Microbes 9(6):875–881
26. Fan Y, Wang H, Liu X, et al (2019) Crosstalk between the ketogenic diet and epilepsy: from the perspective of gut microbiota. Mediators Inflamm 8373060.
27. Citraro R, Lembo F, De Caro C et al (2021) First evidence of altered microbiota and intestinal damage and their link to absence epilepsy in a genetic animal model, the WAG/Rij rat. Epilepsia 62(2):529–541
28. Medel-Matus JS, Shin D, Dorfman E, Sankar R, Mazarati A (2018) Facilitation of kindling epileptogenesis by chronic stress may be mediated by intestinal microbiome. Epilepsia Open 3(2):290–294
29. Peng A, Qiu X, Lai W et al (2018) Altered composition of the gut microbiome in patients with drug-resistant epilepsy. Epilepsy Res 147:102–107
30. Vezzani A, Lang B, Aronica E (2015) Immunity and inflammation in epilepsy. Cold Spring Harb Perspect Med 6(2):a022699
31. Lehtimaki KA, Keranen T, Huhtala H et al (2004) Regulation of IL-6 system in cerebrospinal fuid and serum compartments by seizures: the effect of seizure type and duration. J Neuroimmunol 152(1–2):121–125
32. Choudhary A, Varshney R, Kumar A et al (2021) A prospective study of novel therapeutic targets interleukin 6, tumor necrosis factor α, and interferon γ as predictive biomarkers for the development of posttraumatic epilepsy. World Neurosurg X, 12.
33. de Vries EE, van den Munckhof B, Braun KP et al (2016) Inflammatory mediators in human epilepsy: a systematic review and meta-analysis. Neurosci Biobehav Rev 63:177–190
34. Samland H, Huitron-Resendiz S, Masliah E et al (2003) Profound increase in sensitivity to glutamatergic-but not cholinergic agonist-induced seizures in transgenic mice with astrocyte production of IL-6. J Neurosci Res 73(2):176–187
35. Kelly JR, Kennedy PJ, Cryan JF et al (2015) Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. Front Cell Neurosci 9:392
36. Liu X, Cao S, Zhang X (2015) Modulation of gut microbiotabrain axis by probiotics, prebiotics, and diet. J Agricultural and Food Chem 63:7885–7895
37. Fedorak RN, Feagan BG, Hotte N et al (2015) The probiotic VSL# 3 has anti-inflammatory effects and could reduce endoscopic recurrence after surgery for Crohn's disease. Clini Gastroenterol Hepatol 13(5):928–935
38. Banach M, Piskorska B, Czuczwar JS, Borowicz K (2011) Nitric oxide, epileptic seizures, and action of antiepileptic drugs. CNS Neurol Disord Drug Targets 10(7):808–819
39. Marangoz C, Ayyildiz M, Ağar E (1994) Evidence that sodium nitroprusside possesses anticonvulsant effects mediated through nitric oxide. NeuroReport 5(18):2454–2456
40. González-Hernández T, García-Marín V, Perez-Delgado MDM et al (2000) Nitric oxide synthase expression in the cerebral cortex of patients with epilepsy. Epilepsia 41(10):1259–1268
41. Bashkatova V, Narkevich V, Vitskova G, Vanin A (2003) The influence of anticonvulsant and antioxidant drugs on nitric oxide level and lipid peroxidation in the rat brain during penthylenetetrazole-induced epileptiform model seizures. Prog Neuro-Psychopharmacol Biol Psychiatry 27(3):487–492
42. Bashkatova VG, Yu VG, Narkevich VB et al (2000) Nitric oxide content measured by ESR-spectroscopy in the rat brain is increased during pentylenetetrazole-induced seizures. J Mol Neurosci 14:183–190
43. Milatovic D, Gupta RC, Dettbarn WD (2002) Involvement of nitric oxide in kainic acid-induced excitotoxicity in rat brain. Brain Res 957(2):330–337
44. Puttachary S, Sharma S, Verma S et al (2016) 1400W, a highly selective inducible nitric oxide synthase inhibitor is a potential disease modifier in the rat kainate model of temporal lobe epilepsy. Neurobiol Dis 93:184–200
45. Leal EC et al (2007) Inducible nitric oxide synthase isoform is a key mediator of leukostasis and blood-retinal barrier breakdown in diabetic retinopathy. Invest Ophthalmol Vis Sci 48:5257–5265
46. Librizzi L et al (2012) Seizure-induced brain-borne inflammation sustains seizure recurrence and blood–brain barrier damage. Ann Neurol 72:82–90
47. Yang S et al (2013) Hemoglobin-induced nitric oxide synthase overexpression and nitric oxide production contribute to blood–brain barrier disruption in the rat. J Mol Neurosci 51:352–363
48. Aoun A, Darwish F, Hamod N (2020) The influence of the gut microbiome on obesity in adults and the role of probiotics, prebiotics, and synbiotics for weight loss. Prevent Nutrition Food Sci 25:113–123
49. Wici ´nski M, ¸ebalski G, Goł ¸ebiewski J, Malinowski B (2020) Probiotics for the treatment of overweight and obesity in humans—a review of clinical trials. Microorganisms 8:1148
50. Collado MC, Surono IS, Meriluoto J et al (2007) Potential probiotic characteristics of Lactobacillus and Enterococcus strains isolated from traditional dadih fermented milk against pathogen intestinal colonization. J Food Prot 70(3):700–705
51. Daniali M, Nikfar S, Abdollahi M (2020) A brief overview on the use of probiotics to treat overweight and obese patients. Expert Rev Endocrinol Metab 15:1–4
52. Chen L, Zou Y, Peng J et al (2015) Lactobacillus acidophilus suppresses colitis-associated activation of the IL-23/Th17 axis. J Immunol Res 2015:1–10
53. Barengolts E (2016) Gut microbiota, prebiotics, probiotics, and synbiotics in management of obesity and prediabetes: review of randomized controlled trials. Endocr Pract 22(10):1224–1234
54. Ejtahed H-S, Angoorani P, Soroush A-R et al (2019) Probiotics supplementation for the obesity management; a systematic review of animal studies and clinical trials. J Funct Foods 52:228–242
55. Osterberg KL, Boutagy NE, McMillan RP et al (2015) Probiotic supplementation attenuates increases in body mass and fat mass during high-fat diet in healthy young adults. Obesity 23:2364–2370
56. Boutagy NE, Neilson AP, Osterberg KL et al (2015) (2015) Probiotic supplementation and trimethylamine-N-oxide production following a high-fat diet. Obesity (Silver Spring) 23:2357–2363

57. Yadav H, Lee JH, Lloyd J et al (2013) Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. J Biol Chem 288:25088–25097
58. Belgardt BF, Okamura T, Brüning JC (2009) Hormone and glucose signalling in POMC and AgRP neurons. J Physiol 587:5305–5314
59. Biton V (2003) Effect of antiepileptic drugs on bodyweight. CNS Drugs 17(11):781–791
60. Chen B, Choi H, Hirsch LJ et al (2015) Cosmetic side effects of antiepileptic drugs in adults with epilepsy. Epilepsy Behav 42:129–137

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