Article

J. Biol. Regul. Homeost. Agents. 2024; 38(6): 5087–5094
https://doi.org/10.23812/j.biol.regul.homeost.agents.20243806.405

A Study on the Role of Bifidobacterium Bifidum in Antagonizing Brain Tissue Injury and Inflammation in a Febrile Convulsion Model in Mice

Nan He[1,*,†], Yalong Wang[2,†], Tao Xu[3]

[1]Department of Pediatrics, Beijing Luhe Hospital, Capital Medical University, 101100 Beijing, China
[2]Department of Pediatrics, Maternal and Child Health Hospital of Tongzhou District in Beijing, 101100 Beijing, China
[3]Hunan Provincial Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations, Changsha Medical University, 410219 Changsha, Hunan, China
*Correspondence: henanluhe@163.com (Nan He)
[†]These authors contributed equally.
Submitted: 29 January 2024 Revised: 12 March 2024 Accepted: 20 March 2024 Published: 1 June 2024

Background: Febrile convulsions in children are often triggered by high fever and can lead to acute neurological episodes with potential long-term consequences on cognitive function. Probiotics, particularly bifidobacteria, have demonstrated promising potential in modulating immune function and intestinal health. However, their role in neurological disorders, including febrile convulsions, is yet to be fully explored. Therefore, this study aimed to investigate the therapeutic impacts of Bifidobacterium bifidum on mitigating brain tissue damage and inflammation utilizing a mouse model experiencing febrile convulsions.
Methods: To assess the impact of Bifidobacterium bifidum on febrile convulsions, a mouse model was established by administering dry yeast suspension and pentylenetetrazol solution. Using this model, we examined the changes in anal temperature, convulsion onset time, and convulsion duration. Histological analysis through Hematoxylin and Eosin (H&E) staining was employed to study the neuronal morphology in the mouse hippocampus. Furthermore, the levels of serum Cyclic Adenosine Monophosphate (cAMP) and Prostaglandin E2 (PGE_2) were assessed using Enzyme-Linked Immunosorbent Assay (ELISA). Additionally, protein expressions of cyclooxygenase-2 (COX-2), Inducible Nitric Oxide Synthase (iNOS), Gamma-Aminobutyric Acid Type A Receptor (GABAAR), and glial fibrillary acidic protein (GFAP) were determined through western blot analysis, while mRNA expressions of inflammatory markers Interleukin 1 beta (IL-1β), Interleukin 6 (IL-6), and Tumor Necrosis Factor alpha (TNF-α) in hippocampal tissue were determined by quantitative Polymerase Chain Reaction (qPCR).
Results: In comparison to the model group, mice in the Bifidobacterium group exhibited a significant reduction in anal temperature ($p < 0.05$), an increase in the time to onset of convulsions ($p < 0.05$), a shorter duration of convulsive episodes ($p < 0.05$), and an enhanced expression of GABAAR protein ($p < 0.05$). Additionally, the Bifidobacterium group showed lowered serum levels of cAMP and PGE_2 ($p < 0.05$), improved neuronal cell morphology in the hippocampus, and a decrease in the expression of COX-2, iNOS, and GFAP proteins in the brain tissue ($p < 0.05$). Furthermore, there was a substantial reduction in the hippocampal tissue levels of pro-inflammatory factors IL-1β, IL-6, and TNF-α mRNA ($p < 0.05$).
Conclusion: Bifidobacterium bifidum exhibits an effective antipyretic and anti-convulsant activity. Its mechanism may be attributed to its ability to reduce fever and inflammation in the brain.

Keywords: bifidobacteria; febrile convulsions; antipyretic; brain damage; inflammation

Introduction

Febrile convulsions are common neurologic emergencies in children, often induced by hyperthermia. Clinically, febrile convulsions manifest as acute episodes of coma and convulsions, usually associated with high fever, potentially causing long-term effects on the child's neurological and cognitive function [1]. Despite the growing understanding of febrile convulsions in clinical practices, their specific pathogenesis remains complex and multifaceted.

In recent years, probiotics have demonstrated a wide range of biological effects in regulating the host immune system and improving the balance of gut microbiota [2]. Among them, bifidobacteria, an important class of probiotics, have attracted more attention for their role in maintaining host health. Their ability to modulate intestinal diseases and immune responses has been recognized [3,4]. However, research on the role of bifidobacteria in neurological diseases remains relatively limited.

Moreover, emerging evidence suggests that probiotics regulate GABAergic neurotransmission, which is critical in determining susceptibility to seizure and epileptogenesis [5,6]. Probiotics have been shown to enhance GABAer-

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

gic inhibitory transmission, potentially reducing neuronal excitability and dampening seizure occurrences [7,8]. This study aimed to investigate the pharmacodynamic effects of Bifidobacterium bifidum in a mouse model of febrile convulsions, focusing on its potential mechanism in regulating brain tissue damage and inflammation. By constructing a mouse model of febrile convulsions, we evaluated the impact of Bifidobacterium bifidum on different parameters, including body temperature, convulsive latency, and duration of convulsions. Furthermore, this study investigated its effects on neuronal morphology, inflammatory factors, and neurotransmitter levels utilizing various biological techniques.

Materials and Methods

Establishment of Mouse Model and Drug Administration

The male SPF grade ICR mice (n = 18), weighing 22–24 g (ENSIWEIER, Chongqing, China) were acclimatized and fed for 7 days. Using a random number table method, the mice were allocated into three groups: a blank group, a model group, and a Bifidobacterium group, each comprising six mice. Furthermore, the blank and model groups received a gavage of 0.5% sodium carboxymethyl cellulose (CMC-Na) solution for 12 hours, whereas the Bifidobacterium group was administered with Bifidobacterium bifidum (formulated as Bifidobacterium tetragonum tablets, batch no. 100058345873, SHANGHAI PHARMA, Shanghai, China) at a dose of 2×10^9 CFU/pc. They were treated once a day consecutively for seven days. After the last administration, both the model and Bifidobacterium groups were injected subcutaneously with 20% dry yeast suspension (10 mL/kg) (CF20200324W2F33, Angie's Yeast Co., Ltd., Yichang, China) to cause a fever model [9], and the anal temperature was monitored following 6 and 12 hours of treatment. At the same time, the anal temperature of the blank group was also recorded. Immediately, the model and Bifidobacterium groups received intraperitoneal injections of pentylenetetrazol solution (60 mL/kg).

Furthermore, convulsive manifestation was recorded in each group within 30 minutes, and blood sample was collected from the retro-orbital venous plexus. Subsequently, the blood samples were centrifuged (2000 ×g) for 15 minutes at room temperature, and the resulting serum was collected for further analysis. Meanwhile, the mice were dissected on ice, and their hippocampal tissue were excised, respectively. These tissue specimens were stored in liquid nitrogen for subsequent experimentations. The study design involving animal experiments was approved by the Changsha Medical College Medical Ethics Committee (approval number: 2020074).

Behavioral Observation of Mice

We observed the occurrence, latency, and duration of convulsions among all three groups of mice. The occurrence of convulsive behaviors was recorded within 30 minutes following a previously described method [10,11]. The convulsive latency, the time from the last intraperitoneal injection of pentylenetetrazol solution to the initial appearance of a grade 2 reaction in mice, was recorded. Moreover, the convulsive duration was recorded as the time between the initial appearance of the grade 2 reaction and the last appearance of the grade 2 reaction. Following this, the mice were anesthetized using 1% pentobarbital sodium (40 mg/kg) (P-010-1ML, Merck, Kenilworth, NJ, USA). Upon achieving complete anesthesia, they were euthanized by cervical dislocation, and hippocampal tissue was harvested for subsequent analyses.

Hematoxylin and Eosin (H&E) Staining

Three mice in each group were randomly selected for cardiac perfusion after inhalation of isoflurane anesthesia. Initially, a specific volume of saline was injected to clear the blood for perfusion. Subsequently, after the liver and other organs turned grayish-white, a solution of 4% paraformaldehyde was gradually injected. Once the mice's limbs became stiff, the perfusion was stopped. The brains were removed, fixed, and set aside. The pathological manifestations of hippocampal neuronal injury were evaluated through H&E staining.

Enzyme-Linked Immunosorbent Assay (ELISA) for Serum Levels of Cyclic Adenosine Monophosphate (cAMP) and Prostaglandin E2 (PGE_2)

The blood was allowed to clot at room temperature for 20 minutes, followed by centrifugation at 4 °C and 3000 rpm for 20 minutes. The supernatant was collected, and the levels of cAMP (ab290713, Abcam, Cambridge, MA, USA) and PGE_2 (JL20578, Jianglai, Shanghai, China) were assessed using corresponding ELISA kits, following the manufacturer's instructions.

Evaluation of iN-OS, Glial Fbrillary Acidic Protein (GFAP), and GABAAR Protein Expression Levels in Mouse Brain Tissue Using Western Blot Analysis

The brain tissue was homogenized in RIPA solution containing protease and phosphatase inhibitors. Following centrifugation, the supernatant was extracted and subsequently underwent protein quantification using the BCA technique. The proteins were resolved through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto a polyvinylidene difluoride (PVDF) membrane. After blocking, the membranes were incubated overnight with primary antibodies, including anti-Inducible Nitric Oxide Synthase (iNOS) (1:500, A3774, abclonal, Wuhan, China), anti-GFAP (1:1000, A19058, abclonal, Wuhan, China), anti-Gamma-

Aminobutyric Acid Type A Receptor (GABAAR) (1:1000, A12568, abclonal, Wuhan, China), anti-GAPDH (1:5000, A19056, abclonal, Wuhan, China) at 4 °C. The next day, PVDF membranes were washed and incubated with the corresponding secondary antibody (1:3000, AS014, abclonal, Wuhan, China) for 2 hours. After washing the membranes, immunoblots were developed using ECL reagent, and the protein bands were analyzed through Image J software (version 1.8.0, National Institutes of Health, Bethesda, MD, USA).

Detection of Inflammatory Factors Interleukin 1 beta (IL-1β), Interleukin 6 (IL-6), and Tumor Necrosis Factor alpha (TNF-α) mRNA Expression Level in Mouse Hippocampal Tissue Using Quantitative Polymerase Chain Reaction (qPCR) Method

Total RNA was extracted from mouse hippocampal tissue utilizing the Trizol (R0016, Beyotime, Shanghai, China) method, and subsequently quantified using a NanoDrop Ultra-Micro UV Spectrophotometer. The samples were prepared and amplified using Hieff qPCR SYBR Green Master Mix (11203ES08, Yeasen, Shanghai, China). The relative expression level of mRNA was assessed using the $2^{-\Delta\Delta Ct}$ method. *GAPDH* was used as an internal reference. The primer sequences used in qPCR were as follows: *IL-1β*-F, 5′-GGAGAACCAAGCAACGACAAAATA-3′; *IL-1β*-R, 5′-TGGGGAACTCTGCAGACTCAAAC-3′; *IL-6*-F, 5′-GTTGCCTTCTTGGGACTGAT-3′; *IL-6*-R, 5′-GCCATTGCACAACTCTTTTCT-3′; *TNF-α*-F, 5′-CTGCCCCGACTACGTGCTCCTCA-3′; *TNF-α*-R, 5′-AGTTGGTCCCCCTTCTCC-3′; *GAPDH*-F, 5′-TGAAGCAGGCATCTGAGGG-3′; *GAPDH*-R, 5′-CGAAGGTGGAAGAGTGGGAG-3′.

Statistical Methods

Statistical analyses were conducted using GraphPad Prism software (version 9.0, GraphPad Software, San Diego, CA, USA). The data were expressed as $\bar{x} \pm s$. Moreover, an independent sample *t*-test was used to compare the two groups. Whereas the multiple group comparisons were performed through a one-way analysis of variance (ANOVA) followed by Tukey's test, to examine intergroup differences. Statistical significance was determined at a *p*-value < 0.05.

Results

Effect of Bifidobacterium Bifidum on the Change of Anal Temperature in Mice with Febrile Convulsions

The initial rectal temperature across all groups of mice showed no significant variation. However, following 6 and 12 hours of exposure to 20% dry yeast suspension, a significant increase in rectal temperature was observed in the model group compared to the blank group ($p < 0.05$), confirming the successful establishment of the febrile mouse model. In contrast, the rectal temperature in the Bifidobacterium group was significantly lower than that in the model group ($p < 0.05$), demonstrating the fever-reducing effects of Bifidobacterium (Fig. 1).

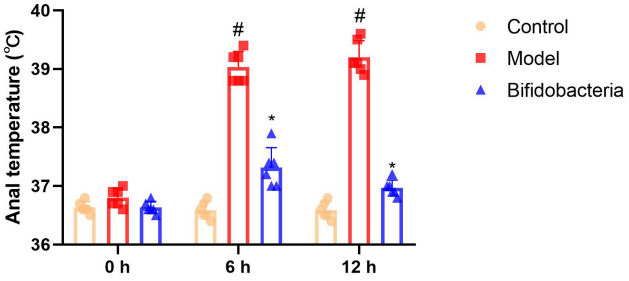

Fig. 1. Effect of Bifidobacterium bifidum on anal temperature of mice with febrile convulsions. Anal temperatures of mice in three groups at 0 h, 6 h and 12 h, respectively. # signifies a *p*-value < 0.05 in comparison to the Control group, while * denotes a *p*-value < 0.05 relative to the Model group. N = 6.

Bifidobacterium Bifidum Regulates the Latency of Convulsive Seizures and Duration of Convulsions in Mice with Febrile Convulsions

We observed that no convulsive manifestations occurred in the blank group but convulsive manifestations were found in the model group. Compared to the model group, the Bifidobacterium group exhibited significantly prolonged convulsive latency ($p < 0.05$) and shortened convulsion duration ($p < 0.05$, Fig. 2).

Effect of Bifidobacterium Bifidum on GABAAR and Serum cAMP and PGE_2 Levels in Brain Tissue of Mice with Febrile Convulsions

Western blot analysis revealed that Bifidobacterium bifidum treatment significantly upregulated GABAAR protein expression in brain tissues ($p < 0.05$), indicating the anti-convulsant effects of Bifidobacterium bifidum. Furthermore, ELISA findings revealed a significant increase in the serum cAMP and PGE_2 levels within the model group compared to the blank group ($p < 0.05$). In contrast, these levels were significantly reduced in the Bifidobacterium bifidum group, compared to the model group ($p < 0.05$). These findings suggest that the antipyretic impact of Bifidobacterium bifidum might be linked to its ability to suppress the release of endogenous mediators like serum cAMP and PGE_2 in mice (Fig. 3).

Effect of Bifidobacterium Bifidum on Neuronal Cell Morphology

Compared to the blank group, the brain tissue from the model group mice showed abnormalities in the overall structure of the hippocampus. The arrangement of neuron cells was irregular, indicating that convulsive seizure had

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

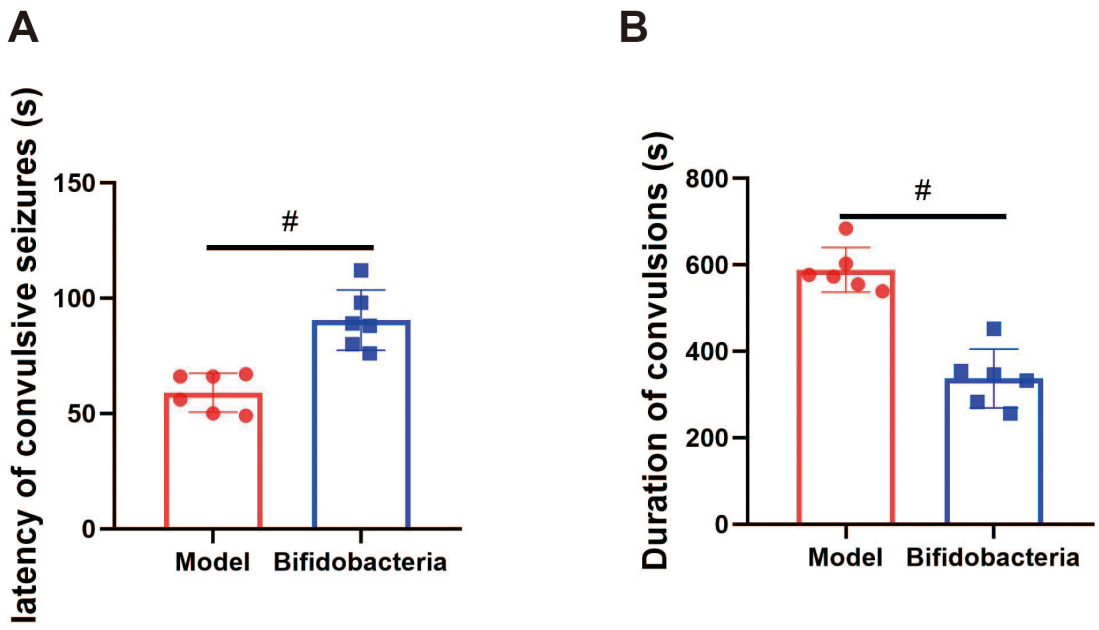

Fig. 2. Effect of Bifidobacterium bifidum on convulsive seizure latency and convulsive duration in febrile convulsive mice. (A) Convulsive seizure latency. (B) Convulsive duration. # indicates $p < 0.05$. N = 6.

damaged the normal morphology and structure of neuron cells. Moreover, compared to the model group, the morphology of hippocampal neurons in the Bifidobacterium bifidum group was significantly improved, with relatively orderly arrangement and distinct nuclei. These observations demonstrate that Bifidobacterium bifidum can effectively enhance the morphology of hippocampal neuronal cells and protect them from damage induced by convulsions, indicating its specific neuroprotective effects (Fig. 4).

Regulation of Inflammatory Factors and Functional Proteins by Bifidobacterium Bifidum in Hippocampal Tissues of Febrile Convulsed Mice

The expression levels of inflammatory factors such as IL-1β, IL-6, and TNF-α were significantly higher in the model group compared to the blank group ($p < 0.05$). However, the expression levels of these factors were significantly reduced in mice treated with bifidobacteria compared to the model group ($p < 0.05$). These observations suggest that bifidobacteria can decrease the release of inflammatory factors in hippocampal tissue and exhibit an antagonizing effect on brain tissue inflammation.

Furthermore, western blot analysis revealed an elevated expression of iNOS and GFAP proteins in the brain tissue of the model group compared to the blank group ($p < 0.05$). In contrast, the expression levels of iNOS and GFAP proteins in the brain tissue of the mice treated with Bifidobacterium were significantly reduced compared to the model group ($p < 0.05$). These findings indicated that Bifidobacterium effectively reduced the expression of these proteins, mitigated the inflammatory response, and ultimately led to a reduction in brain tissue damage (Fig. 5).

Discussion

Febrile seizures are a common convulsive disorder among children, and recent research suggests a correlation between this disease and the immune-inflammatory process [12]. During febrile convulsions, the body's inflammatory response induces the release of inflammatory mediators such as cAMP and PGE3, leading to a febrile response. This response can result in neuronal excitation in the hypothalamus and other brain regions, leading to abnormal discharges and subsequent convulsions [13–15]. The literature shows a close association between neuronal cell inhibition and activation in the onset of convulsions. The inhibitory neurotransmitter GABA can inhibit the postsynaptic potential after binding with GABA receptors, thereby reducing neuronal excitability and inhibiting convulsions [16,17]. In this study, Bifidobacterium bifidum treatment significantly increased the level of GABAAR protein in the brain tissue of mice experiencing febrile convulsions. This finding suggests that Bifidobacterium bifidum might attenuate the onset of febrile convulsions by enhancing inhibitory signaling within the GABAergic system. This result aligns with previous ideas regarding the positive modulatory effects of probiotics on the nervous system, suggesting the potential role of the GABAergic system in the anti-convulsant effects of Bifidobacterium [18,19]. These results demonstrated that Bifidobacterium treatment improved the morphological integrity of hippocampal neurons in febrile convulsant mice, attenuating both abnormal arrangement and number of neurons. This observation is consistent with previous reports indicating the neuroprotective effects of probiotics in neurological disorders [20]. Bifidobacteria could

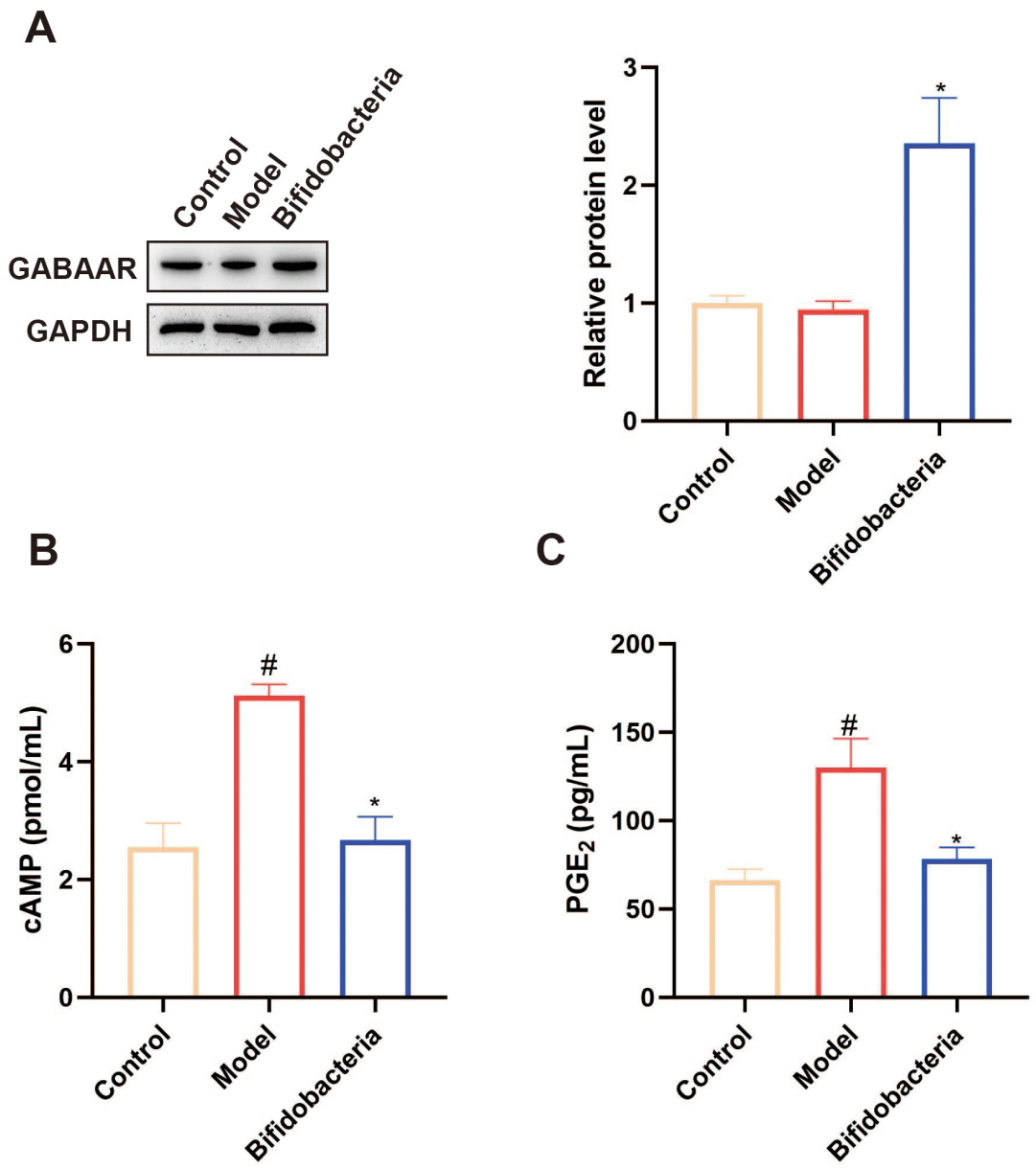

Fig. 3. Regulation of Gamma-Aminobutyric Acid Type A Receptor (GABAAR), Cyclic Adenosine Monophosphate (cAMP), and Prostaglandin E2 (PGE_2) expression by Bifidobacterium bifidum in febrile convulsed mice. (A) GABAAR protein expression was assessed through western blot analysis. (B,C) The expression levels of cAMP and PGE_2 were determined using Enzyme-Linked Immunosorbent Assay (ELISA). # signifies a p-value < 0.05 in comparison to the Control group, while * denotes a p-value < 0.05 relative to the Model group. N = 3.

potentially attenuate convulsion-induced neuronal damage by protecting neuronal morphology and structure.

We observed that Bifidobacterium treatment significantly reduced anal temperature, prolonged convulsive latency, and shortened convulsive duration in febrile convulsive mice, indicating its antipyretic effect. This finding aligns with the results of prior research investigating the immunomodulatory effects of probiotics [21]. Our results support the possibility that Bifidobacterium bifidum could suppress the febrile response in febrile convulsion model mice by affecting the immune system. Prolonged febrile convulsive episodes can cause hippocampal neuronal cell damage, inducing an inflammatory response within the brain, as well as activation of astrocytes, characterized by the overexpression of GFAP. GFAP is one of the best markers for astrocyte activation following CNS stress, and astrocytes usually respond to such stress within the nervous system, suggesting that bifidobacteria could inhibit the febrile response by affecting the immune system [22,23]. In our study, Bifidobacterium treatment reduced the expression levels of inflammatory factors in the hippocampal tissues of febrile convulsions mice. Additionally, it resulted in the downreg-

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

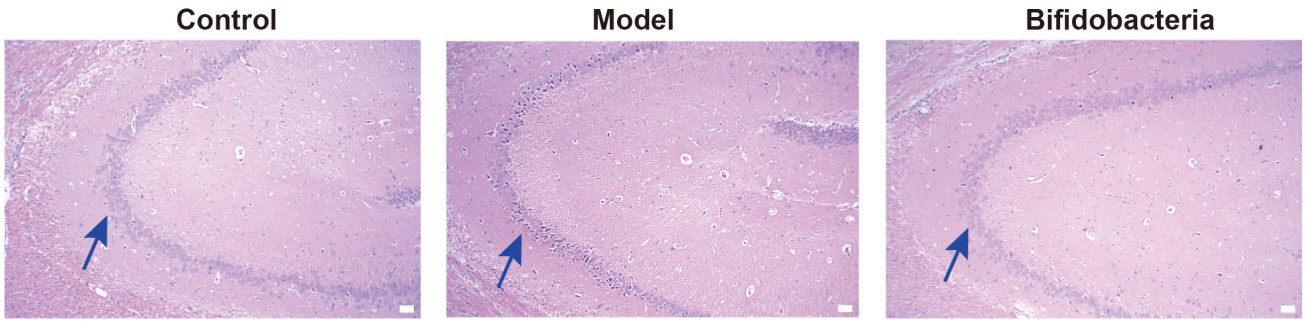

Fig. 4. Effect of Bifidobacterium bifidum on the morphology of neuronal cells in the hippocampal region of febrile convulsed mice. Arrows indicate neuronal cells in the hippocampal region. N = 3. Scale bar = 50 μm.

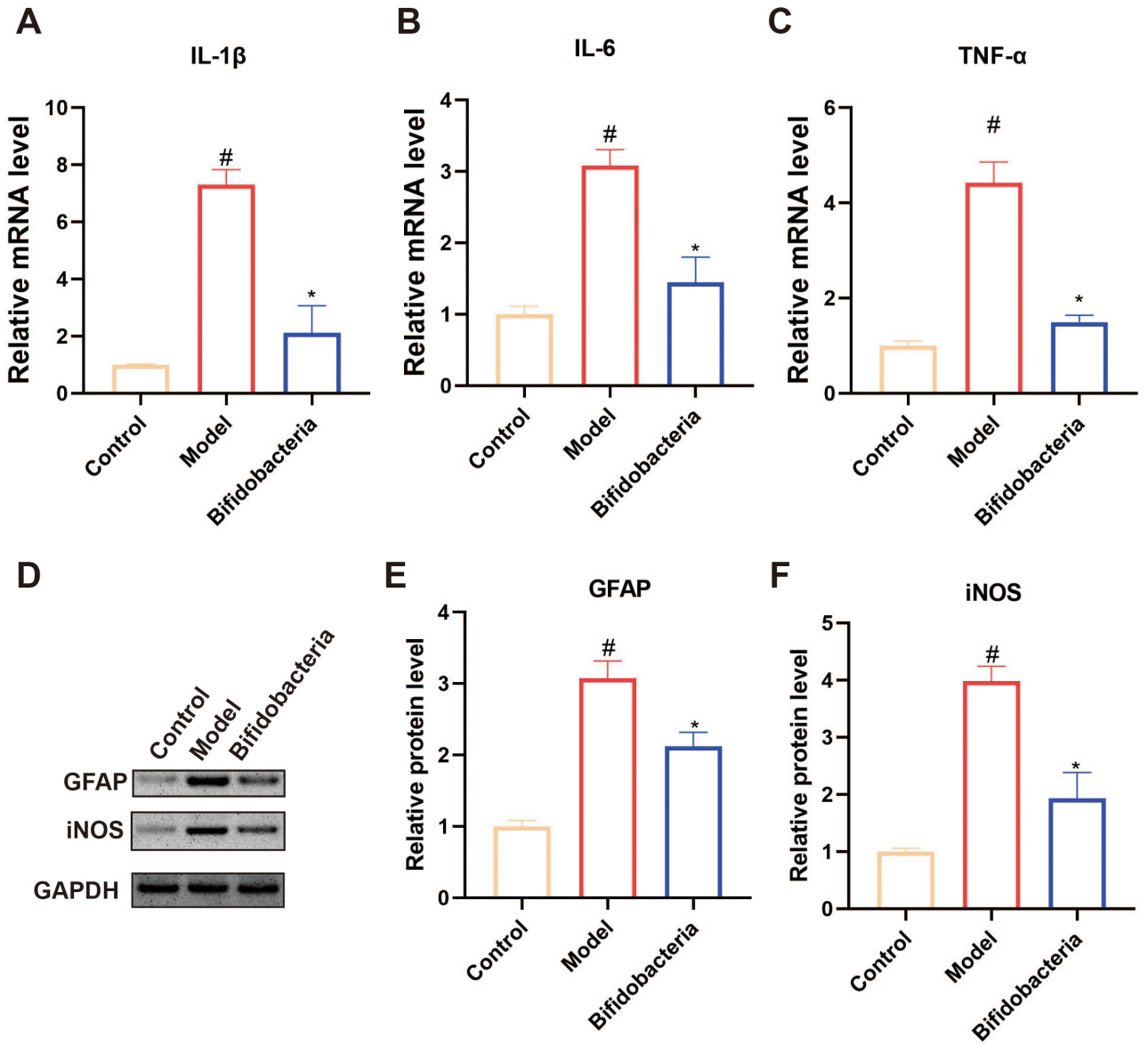

Fig. 5. Regulation of Interleukin 1 beta (IL-1β), Interleukin 6 (IL-6), and Tumor Necrosis Factor alpha (TNF-α), glial fibrillary acidic protein (GFAP), and Inducible Nitric Oxide Synthase (iNOS) expression by Bifidobacterium bifidum in febrile convulsed mice. (A–C) Quantitative Polymerase Chain Reaction (qPCR) for IL-1β, IL-6, TNF-α expression. (D–F) Western blot analysis of GFAP and iNOS expression. $^{\#}$ signifies a p-value < 0.05 in comparison to the Control group, while * denotes a p-value < 0.05 relative to the Model group. N = 3.

ulation of the relative expression of iNOS and GFAP proteins in brain tissues. Bifidobacterium bifidum appears to play a role in antagonizing brain tissue inflammation by inhibiting the onset of inflammatory responses and attenuating the release of inflammatory mediators in neural tissues. Our findings support the antipyretic, anti-inflammatory, and neuroprotective roles of Bifidobacterium bifidum in neurological disorders. These findings support further exploration into the potential mechanisms of bifidobacteria in treating neurological diseases.

While the study yielded some interesting findings, it is crucial to acknowledge its limitations. Firstly, the mouse model, although capable of simulating febrile convulsions, still has its limitations, and the applicability of its results in humans needs to be verified. Secondly, the molecular mechanism underlying the action of bifidobacteria was not extensively explored in this study, presenting a direction for future research. Future research directions could include an in-depth exploration of the molecular mechanisms of bifidobacteria regulating neuronal and inflammatory processes, as well as evaluation of their potential application in humans. Additionally, conducting multi-center, large-sample clinical studies will help comprehensively assess the efficacy of bifidobacteria in treating neurological disorders.

Conclusion

Bifidobacterium bifidum treatment significantly reduced anal temperature, prolonged convulsive latency, and shortened convulsive duration in febrile convulsive mice. Additionally, Bifidobacterium treatment improved the morphology and structure of hippocampal neurons and attenuated the release of inflammatory factors by upregulating GABAAR protein expression, showing antipyretic, anticonvulsant, neuroprotective, and anti-inflammatory effects. Overall, this study lays the foundation for the potential application of Bifidobacterium bifidum in treating neurological diseases and provides valuable insights for future research and clinical applications.

Availability of Data and Materials

All experimental data included in this study can be obtained by contacting the first authors if needed.

Author Contributions

NH and TX designed the research study. NH, TX and YLW performed the research. NH, TX and YLW analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was reviewed by the Changsha Medical College Medical Ethics Committee (2020074).

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Hao JR, Xu Q, Zhang QR, Xie XY, Weng YF, Yang F, *et al*. Magnetic resonance imaging morphological study of the effects of juvenile febrile convulsions on the brain structure of medial temporal lobe epilepsy. Zhonghua Yi Xue Za Zhi. 2020; 100: 2121–2125. (In Chinese)

[2] Wang X, Liu Y, Kang N, Xu G. Wide identification of chemical constituents in fermented licorice and explore its efficacy of anti-neurodegeneration by combining quasi-targeted metabolomics and in-depth bioinformatics. Frontiers in Neuroscience. 2023; 17: 1156037.

[3] O'Callaghan A, Bottacini F, O'Connell Motherway M, van Sinderen D. Pangenome analysis of Bifidobacterium longum and site-directed mutagenesis through by-pass of restriction-modification systems. BMC Genomics. 2015; 16: 832.

[4] Hao H, Zhang X, Tong L, Liu Q, Liang X, Bu Y, *et al*. Effect of Extracellular Vesicles Derived from *Lactobacillus plantarum* Q7 on Gut Microbiota and Ulcerative Colitis in Mice. Frontiers in Immunology. 2021; 12: 777147.

[5] Mazzoli R, Pessione E. The Neuro-endocrinological Role of Microbial Glutamate and GABA Signaling. Frontiers in Microbiology. 2016; 7: 1934.

[6] Casertano M, Fryganas C, Valentino V, Troise AD, Vitaglione P, Fogliano V, *et al*. Gut production of GABA by a probiotic formula: an in vitro study. Beneficial Microbes. 2024; 15: 67–81.

[7] Ciltas AC, Toy CE, Güneş H, Yaprak M. Effects of probiotics on GABA/glutamate and oxidative stress in PTZ- induced acute seizure model in rats. Epilepsy Research. 2023; 195: 107190.

[8] Tette FM, Kwofie SK, Wilson MD. Therapeutic Anti-Depressant Potential of Microbial GABA Produced by *Lactobacillus rhamnosus* Strains for GABAergic Signaling Restoration and Inhibition of Addiction-Induced HPA Axis Hyperactivity. Current Issues in Molecular Biology. 2022; 44: 1434–1451.

[9] Meng L, Gao H, Chen B, Liu PP, Shan GS, Zhang F, *et al*. Simultaneous Determination of Five Chromones of Radix *Saposhnikoviae* Extract in Rat Plasma by UPLC-MS/MS: Application to a Comparative Pharmacokinetic Study in Normal and Febrile Rats. Journal of Analytical Methods in Chemistry. 2019; 2019: 6454252.

[10] Racine RJ. Modification of seizure activity by electrical stimulation: cortical areas. Electroencephalography and Clinical Neurophysiology. 1975; 38: 1–12.

[11] Racine RJ. Modification of seizure activity by electrical stim-

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

ulation. II. Motor seizure. Electroencephalography and Clinical Neurophysiology. 1972; 32: 281–294.

[12] Jiang L, Yuan P. Febrile seizures: some issues related to the diagnosis and treatment. Chinese Journal of Contemporary Pediatrics. 2015; 17: 539–542. (In Chinese)

[13] Cassim S, Qulu L, Mabandla MV. Prenatal stress and early life febrile convulsions compromise hippocampal genes MeCP2/REST function in mid-adolescent life of Sprague-Dawley rats. Neurobiology of Learning and Memory. 2015; 125: 195–201.

[14] Byeon JH, Kim GH, Kim JY, Sun W, Kim H, Eun BL. Cognitive Dysfunction and Hippocampal Damage Induced by Hypoxic-Ischemic Brain Injury and Prolonged Febrile Convulsions in Immature Rats. Journal of Korean Neurosurgical Society. 2015; 58: 22–29.

[15] Dubé CM, Brewster AL, Richichi C, Zha Q, Baram TZ. Fever, febrile seizures and epilepsy. Trends in Neurosciences. 2007; 30: 490–496.

[16] Rubio T, Viana R, Moreno-Estellés M, Campos-Rodríguez Á, Sanz P. TNF and IL6/Jak2 signaling pathways are the main contributors of the glia-derived neuroinflammation present in Lafora disease, a fatal form of progressive myoclonus epilepsy. Neurobiology of Disease. 2023; 176: 105964.

[17] Sanz P, Garcia-Gimeno MA. Reactive Glia Inflammatory Signaling Pathways and Epilepsy. International Journal of Molecular Sciences. 2020; 21: 4096.

[18] Zhang C, Li G, Lu T, Liu L, Sui Y, Bai R, *et al*. The Interaction of Microbiome and Pancreas in Acute Pancreatitis. Biomolecules. 2023; 14: 59.

[19] Cuesta CM, Guerri C, Ureña J, Pascual M. Role of Microbiota-Derived Extracellular Vesicles in Gut-Brain Communication. International Journal of Molecular Sciences. 2021; 22: 4235.

[20] Chu C, Yu L, Li Y, Guo H, Zhai Q, Chen W, *et al*. *Lactobacillus plantarum* CCFM405 against Rotenone-Induced Parkinson's Disease Mice via Regulating Gut Microbiota and Branched-Chain Amino Acids Biosynthesis. Nutrients. 2023; 15: 1737.

[21] Wynn JL, Neu J, Moldawer LL, Levy O. Potential of immunomodulatory agents for prevention and treatment of neonatal sepsis. Journal of Perinatology: Official Journal of the California Perinatal Association. 2009; 29: 79–88.

[22] Choi M, Lim C, Lee BK, Cho S. Amelioration of Brain Damage after Treatment with the Methanolic Extract of *Glycyrrhizae Radix et Rhizoma* in Mice. Pharmaceutics. 2022; 14: 2776.

[23] Zhang S, Wu M, Peng C, Zhao G, Gu R. GFAP expression in injured astrocytes in rats. Experimental and Therapeutic Medicine. 2017; 14: 1905–1908.