Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Arisaema cum Bile Mitigate Febrile Seizure in Rats via Inhibition of Neuroinflammation, Regulation of FXR and GABA Signaling Pathway

Xu Wu[1,2], Lian Zhong[3], Jing Wang[1,4,5], Qiao Zhang[1,4,5], Jing Sun[1,4,5], Changli Wang[1,4,5], Mengmeng Zhang[1], Chongbo Zhao[1,4,5]

[1]College of Pharmacy, Shaanxi University of Chinese Medicine, Xianyang, 712046, People's Republic of China; [2]School of Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine, Tianjin, 301617, People's Republic of China; [3]Sichuan Provincial Institute for Drug Control, Chengdu, 611137, People's Republic of China; [4]Shaanxi Provincial Engineering Technology Research Center for Traditional Chinese Medicine Decoction Pieces, Xianyang, 712046, People's Republic of China; [5]Traditional Chinese Medicine Processing Techniques Heritage Base (Shaanxi), National Administration of Traditional Chinese Medicine, Xianyang, 712046, People's Republic of China

Correspondence: Mengmeng Zhang; Chongbo Zhao, College of Pharmacy, Shaanxi University of Chinese Medicine, Qindu District, Xianyang, 712046, People's Republic of China, Email 2051140@sntcm.edu.cn; zhao_chongbo@126.com

Background: *Arisaema cum* bile (known as *DanNan Xing* in Chinese, DNX) is a traditional herbal medicine commonly used to treat febrile seizure (FS), but the underlying mechanism remains unclear.

Objective: To evaluate the therapeutic effect of DNX on hot water bath-induced FS rat model and further explore the potential mechanism.

Methods: The chemical constituents of DNX were determined via liquid chromatography-mass spectrometry (LC-MS). FS rat model was established using a hot water bath (45 ± 2 °C), and DNX (2.8 and 0.7 g/kg, i.g) were administered for two weeks. Based on behavior test (duration and latency), pathological changes in the hippocampal tissue, and the levels of inflammatory cytokines the therapeutic effect of DNX for FS was evaluated. Subsequently, the network pharmacology, 16S rRNA and non-targeted metabolomics analysis were combined analysis to explore the potential signaling pathway. Furthermore, the signaling pathway was verified using the RT-qPCR and immunohistochemistry assay.

Results: The DNX treatment showed effective therapy on hot water bath induced FS, as indicated by a shortened seizure duration time, prolonged seizure latency, reduced hippocampal neuron damage and neuroinflammatory factor levels (TNF-α, IL-1β, IL-6, and HMGB1). Neurotransmitters (GABA, Glu) are also significantly regulated. Moreover, the relative abundance of *Lactobacillus* and *Lachnospiraceae* was notably increased ($p < 0.01$), while that of *Tenericutes* decreased, compared to gut microbiota of FS rat. A total of 20 fecal differential metabolites were regarded as the potential biomarkers including GABA, CDCA, and UDCA for anti-FS of DNX, and combined network pharmacy the metabolic pathways of primary bile acids (BAs) biosynthesis and alanine, aspartate and glutamate metabolism were involved.

Conclusion: DNX possesses a therapeutic effect on FS through inhibiting neuroinflammation and regulation of FXR and GABA signaling pathway.

Keywords: *Arisaema cum* bile, febrile seizures, neuroinflammation, gut microbiota, fecal metabolites, FXR and GABA signaling

Introduction

Febrile seizures (FS) is a common type of seizure caused by fever, and usually occurs in young children aged from 6 months to 6 years.[1,2] Long-lasting repetitive FS increases risk by 6% to induce epilepsy.[3,4] In addition, FS, infection, and neurotrauma are induced activation of innate immune and subsequent inflammatory responses in the brain that are documented conditions associated with acute symptomatic seizures and with a high risk of developing epilepsy.[5] Therefore, neuroinflammation is one of the essential factors involved in FS development. As well as relieving neuroinflammation (TNF-α, IL-1β, IL-6, and HMGB1) is an effective strategy to protect nerve injury of FS.[6–9] Moreover, an

Received: 26 November 2024
Accepted: 21 April 2025
Published: 12 June 2025

increasing number of reports have shown intestinal flora disorders are associated with brain diseases including epilepsy, and Alzheimers, and regulating gut microbiota to alleviate neuroinflammation is contributing to combat these diseases.[10–13] Importantly, regulating gut microbiota is also beneficial to protect nerve injury and further against FS.[14] Currently, clinical guidelines for the FS recommend the use of antipyretics and antiepileptic drugs, such as sodium valproate, phenobarbital, and diazepam. These agents have proven to be effective through inhibiting the central nervous system and they frequently inhibit neuronal excitation rather than effective down-regulation of neuroinflammation. Besides, long-term use of these agents often leads to a series of sides effects, such as high mortality rates and cognitive decline.[15–17]

Of note, it is essential to explore more effective agents with exceptional efficacy and fewer adverse effects for FS. An increasing number of evidence have shown that FS can be successfully treated by using traditional Chinese herbal medicine (TCHM) prescriptions, extracts, or components derived from TCHM.[18–20] *Arisaema rhizome* is a TCHM for relieving convulsion and spasm. The plant is shown in Figure 1A and medicament portion (blocky rhizomes) is shown in Figure 1B. Interestingly, it is commonly processed with cattle bile to be changed to *Arisaema cum* Bile (known as *DanNan Xing*, DNX, Figure 1C), which achieves the purpose of reducing toxicity and increasing efficacy including anti-febrile convulsions.[21] For centuries, DNX was traditionally used in treatment of central nervous system diseases such as FS, and stroke clinically, due to its efficacy of medicine characteristics.[22] Especially, it has been used for the treatment of FS since the Qing dynasty according to documentation in the medical monographs of *You You Ji Cheng*,[23] it was used as the minister drug of the traditional prescriptions to treat FS description in *Xiao Er Tai Ji Wan*.[24] In recent years, many chemical constituents have been composed of DNX, including flavonoids, phenols, nucleosides, sugars, and bile acids (BAs). These components not only exert a protective effect on FS but also suppressed the expression of toll-like receptor 4, nuclear factor kappa-B (NF-κB), interleukin-1β (IL-1β) and interleukin-6 (IL-6), and thus inhibited the neuroinflammation in the brain induced by FS.[25,26] Modern pharmacological studies have revealed that DNX plays a protective role in FS by regulating neurotransmitter disorders and inhibiting neuroinflammation.[27] And our previous study showed that DNX possess an anti-FS effect through regulating neurotransmitter disorder and gut microbiota.[14] Thereby, the mechanism of DNX on regulating gut microbiota, fecal metabolism, and neuroinflammation in the process of anti-FS is still unclear and further to demonstrate in this study. Therefore, the protective effect of DNX on FS were explored by using

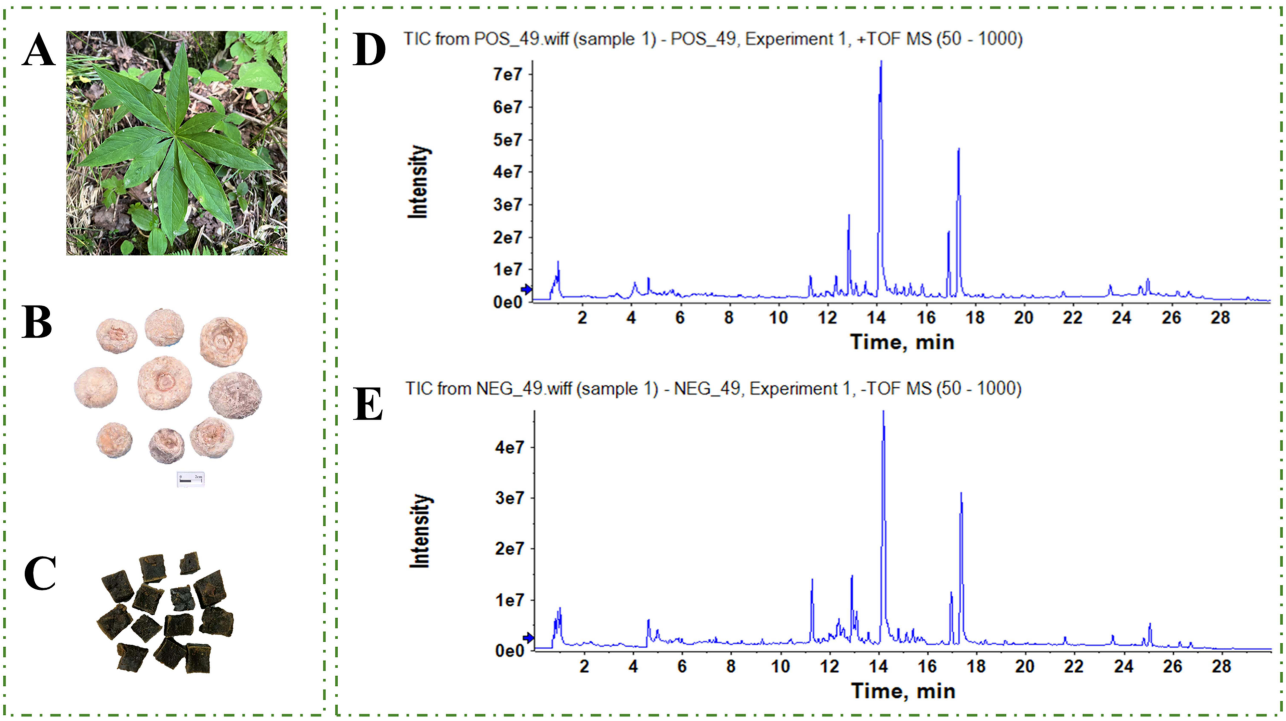

Figure 1 The representative picture of herbal plant and decoction pieces of DNX and the total ion chromatogram of DNX. Herbal plant (**A**); TNX (**B**); DNX (**C**) and total ion flow chromatogram of DNX in the positive ion (**D**) and negative ion mode (**E**), respectively.

a hot water bath rat model, and behavior tests, brain damage were detected. Then, the 16SrRNA sequencing was used to study species and abundance of gut microbiota. The biomarkers and potential signaling pathway were analyzed to utilize fecal metabolomics combining network pharmacy, which was further verified by using RT-qPCR and immunohistochemistry.

Materials and Methods

Chemicals and Reagents

Molpure® Cell/Tissue Total RNA Kit (No. 19221ES50) was obtained from YEASEN (Shanghai, China). PrimeScript RT reagent Kit (No. RR047A, 100 Preps), and TB Green™ Premix Ex Taq™ II (Tli RNaseH Plus) (No. RR820A, 200Rxns) were obtained from TakaraBioInc (Beijing, China). The Elisa Kits of GABA (No. 20221010), Glu (No. 20221110), TNF-α (No. 20221201), IL-1β (No. 20221010), IL-6 (No. 20221110), and High mobility group box 1 (HMGB1) (No. 202201012) were obtained from Jiangsu Meimian Industrial Co., Ltd. (Nanjing, China). DNX (No. 20220316) was obtained from Sichuan BaiSheng Pharmaceutical Co., Ltd. (Chengdu, China), which was identified by Prof. B.Y. Yang based on each herb documented in China Pharmacopoeia (Part I, 2020 Version).

DNX Sample Preparation

The DNX powder (300 g) was soaked in water (3000 mL) for 30 min and then heated until boiling (100 °C) and continuing for 60 min, a total of 3 times. Then filter with gauze, discard the residue and save the filtrate. The filtrate was collected and vacuumed concentration to 0.28 g/mL and 0.07 g/mL and stored at 4 °C for DNX treatment. According to 10 mL/kg gavage, the dose was 2.8 g/kg and 0.7 g/kg, respectively.[27,28]

Chemical Component Profiling of DNX

The Exion LC AD UPLC (Shimadzu, Japan) system equipped with Triple TOF 5600 quadrupole electrostatic field orbitrap high-resolution mass spectrometer (AB SCIEX, USA) was used to identify the chemical component profiling of DNX. Chromatographic separation was achieved using an Acquity™ HSS T3 column (Waters Co., USA, 1.8 μm, 2.1×100 mm) and the injection volume was 2 μL. Acetonitrile (A) and deionized water containing 0.1% formic acid (B) were used as mobile phases. The gradient elution program was as follows: 0–1.5 min: 5% A; 1.5–10 min: 5–50% A; 10.0–23.0 min: 50–85% A; 23–28 min: 85% A-95% A; 28–30 min: 95% A. The flow rate was 0.3 mL/min, and the column temperature was 40 °C.

The electrospray ion source (ESI^+/ESI^-) was used, and the capillary voltage of 2.5 Kv, the ion source temperature of 150 °C were set. The mass scanning range was 50–1000 *m/z*, and the collision energy was 10–30 eV. The components of DNX were determined by comparing their relative chromatographic elution behavior (retention time), mass spectrometry fragment patterns with those recorded in reference.

Animals

As previously described method,[29–31] in this study the SPF male SD rats (14-day-old, 35 ± 5 g) were purchased from Chengdu Dashuo Experimental Animal Co., Ltd. (No. SCXK (川) 2020–030, Chengdu, China) that the separation operation of rat pups to obtain and separate the pups from their dams was standardized by the company. All the experiments were approved by the Ethics Committee of Shaanxi University of Chinese Medicine (approval No. SUCMDL 20220309003), and all experiments conformed to the "Guidelines for the Care and Use of Laboratory Animals."[32]

Establishment of the FS Model and Treatment Design

To investigate the anti-FS effects of DNX, the 40 male rats were randomly divided into control group, model group, positive group, DNX-high dose group, and DNX-low dose group. The positive group was administered with diazepam 10 mg/kg (i.g). The DNX-high dose group, and DNX-low dose group were administered with water extract 2.8 g/kg (i.g) and 0.7 g/kg (i.g), respectively. The control group and model group administered with an equal volume of salt solution

(0.9%) 10 mL/kg (i.g) as well. In brief, the rats in the control group were placed in water (37 °C), while the other rats were placed in a cylindrical-shaped tank ($\pi \times 15^2$ cm × 40 cm) with hot water (45 ± 2°C) to establish the FS model. The temperature of the hot water was monitored in real time to ensure the stability of the water bath. Meanwhile, during the experiment, the rats were ensured to breathe freely, and the water level line was kept in a suitable position to avoid drowning. Observing the convulsion and death of rats. The FS model was induced once every two days, a total of 14 times. All the rats were given with corresponding drugs for 1 h before water bath, then observe the level of convulsions as follow: (I) facial muscle twitch only, (II) nodding, (III) unilateral forelimb spasm, (IV) bilateral forelimb spasm while standing, (V) generalized tonic-clonic seizure.[27] After the observation, the rats were taken out and the hair was dried by blower and put back in the feeding box.

After the last water bath for 2 h, the seizure latency, seizure duration, and mortality of the rats were evaluated as previously described.[33] Subsequently, after anesthesia, blood and hippocampus tissues were collected to detect inflammatory factor level and neurotransmitter level. The colon tissues were collected and hippocampus tissues for Hematoxylin-eosin (H&E) staining, in which hippocampus tissues were furthered to Nissl staining analysis. Meanwhile, hippocampus and colon tissues were used for immunohistochemical and real-time quantitative reverse transcription PCR (RT-qPCR) analysis. Additionally, colon contents were collected for microbial and metabolomics analysis. The animal experimental protocol is presented in Figure 2A.

H&E and Nissl Staining

The rats' brain and colon tissues were fixed with 4% paraformaldehyde (Guo Yao Group Chemical Reagent Co., Ltd, No. 20200320) for 24 h. After paraffin embedding and coronal sectioning, the tissues were cut into 5 μm sections and then stained with H&E. In addition, the neuronal damage was further assessed by Nissl staining, which were evaluated by an experienced pathologist who was blinded to the treatment protocols.

ELISA Analysis

The levels of Gamma-aminobutyric acid (GABA), Glutamate (Glu), Tumor necrosis factor alpha (TNF-α), IL-6, IL-1β and High mobility group box 1 (HMGB1) in the serum and hippocampus were detected by ELISA kit according to the manufacturer's instructions. After unfreezing, the hippocampus tissues were homogenized with ice normal saline at 4 °C, and then centrifuged at 3000 g for 10 min. The absorbance was measured at 450 nm using a microplate reader (800TS, USA).

Network Pharmacology Analysis

The potential components of DNX were collected and the related targets of components were obtained by using Swiss Target Prediction (http://www.swisstargetprediction.ch/). Additionally, the keywords of "febrile convulsion" and "febrile seizures" were input into the Genecards (https://www.genecards.org/) database to search the known targets related to FS. After overlapping the components and FS related targets, a protein-protein interaction (PPI) network was constructed by using the STRING database (https://string-db.org/),[34] and Cytoscape software (v3.10.1) was used to visualize. Furthermore, a network of DNX-components-disease-targets was constructed using Cytoscape (v3.10.1) software. The three topological features of "degree", "betweenness", and "closeness" were calculated to identify the key targets of DNX for treating FS. Finally, the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway was analyzed, and the molecular docking simulation was performed to assess the affinity of potential components of DNX and the key targets.

16S rRNA Sequencing Analysis

Colon content from each of three groups (Control, Model, and DNX 2.8 g/kg) were collected for 16SrRNA sequencing analysis. Firstly, the total microbial genomic DNA was extracted using the Power Soil DNA Isolation kit (Qiagen) according to manufacturer's instructions. The quality of DNA extraction was verified using 1.0% agarose gel electrophoresis, and the DNA concentration and purity were determined using NanoDrop2000. Then, the hypervariable region of the 16SrRNA gene V3-V4 was amplified by PCR using the primers 338F (5′-

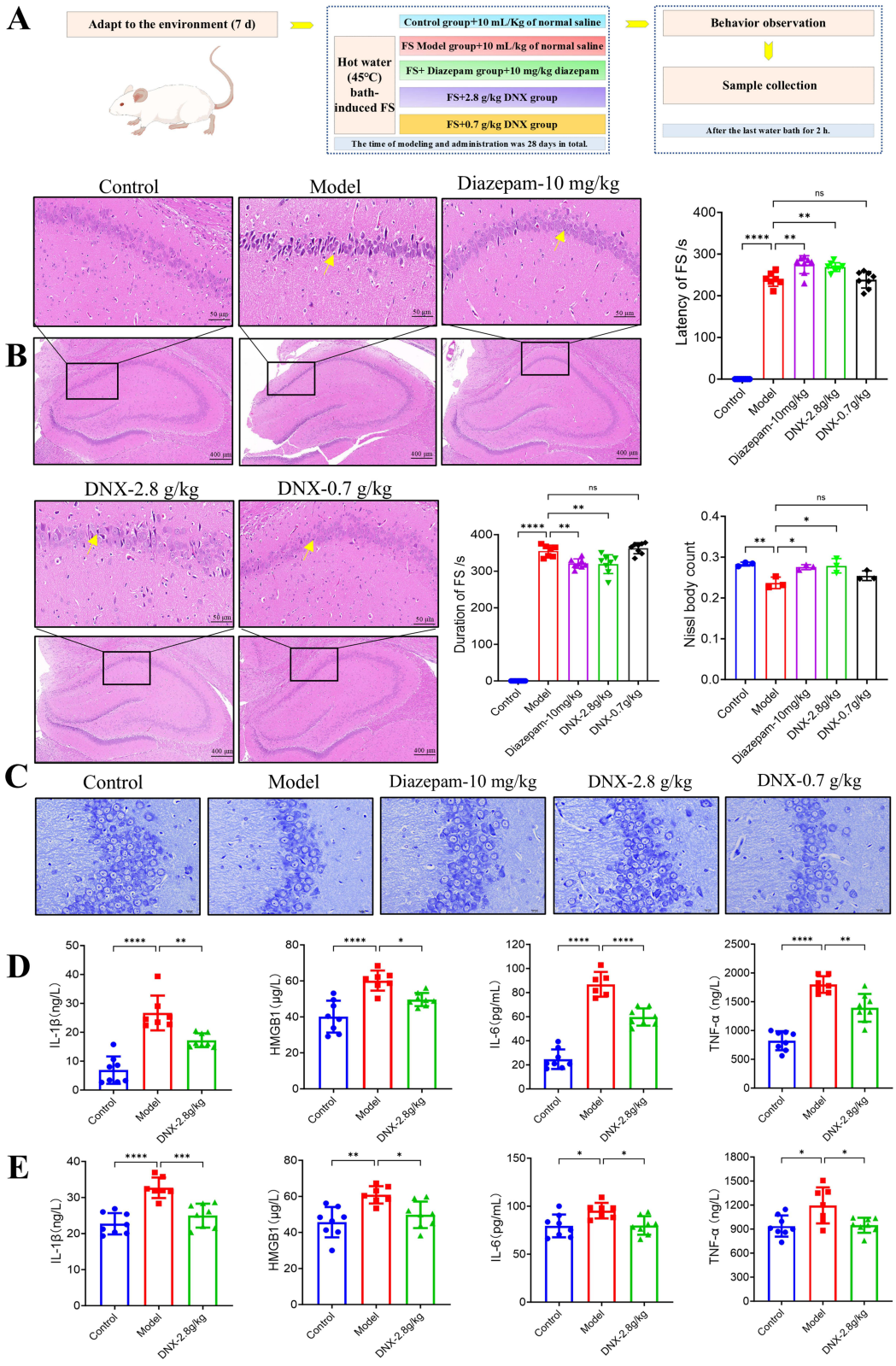

Figure 2 Effect of DNX on the behavior test, hippocampal neuron, and levels of pro-inflammatory cytokines in the FS rat model. Experimental design procedure (**A**), n=8 or n=7; H&E staining (**B**), Nissl staining (**C**), n=3; pro-inflammatory cytokines and neurotransmitter disorder in the serum (**D**) and hippocampus (**E**), n=8 or n=7. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns means no statistical difference.

https://doi.org/10.2147/JIR.S508690

ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR amplification cycling conditions were shown as follows: initial denaturation at 98 °C for 5 min, followed by 25 cycles of denaturation at 98 °C for 30s, annealing at 53 °C for 30s and extension at 72 °C for 45s, a single extension at 72 °C for 5 min, and a final 4 °C step. Finally, the amplification products were purified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA), and the paired-end sequence were performed on an Illumina NovaSeq PE300 platform (Illumina, San Diego, USA). Microbiome bioinformatics and sequence data analyses were performed with QME2 2019.4 and R packages (v3.2.0).[35]

Metabolite Analysis

Each colon content sample was weighed (5 mg) and 25 μL of water was added for homogenizing with zirconia beads. Then, the 120 μL methanol containing internal standard was added to obtain a good metabolite extraction. Fecal metabolic profiling was acquired by an Ultra Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS) (ACQUITY UPLC-Xevo TQ-S, Waters Corp., Milford, MA, USA). Chromatographic separation was achieved using an ACQUITY UPLC BEH C_{18} 1.7 μM VanGuard pre-column (2.1 × 5 mm) and ACQUITY UPLC BEH C_{18} 1.7 μM analytical column (2.1 × 100 mm) maintained at 40 °C. The flow rate was set as 0.4 mL/min, and the injection volume was 5.0 μL. The mobile phase consisted of water-containing 0.1% formic acid (A) and acetonitrile: IPA (70: 30) (B). The gradient elution program was set as follows: 0–1 min, 95% A; 1–11 min, 95–22% A; 11–13.5 min, 22–5% A; 13.5–14 min, 5–0% A; 14–16 min, 0% A; 16–16.1 min, 0–95% A; 16.1–18 min, 95% A.

Both the positive and negative ion mode were applied and the parameters of the mass spectrometer are set as follows, capillary voltage 1.5Kv (ESI+) and 2.0Kv (ESI^-), source temperature 150 °C, desolvation temperature 550 °C, desolvation airflow 1000 L/Hr. Then the MassLynx (v4.1, Waters, Milford, MA, USA) was utilized to analyse original data file form UPLC-MS/MS, and the peak integration, calibration and quantification of each metabolite were performed. Finally the iMAP (v1.0; metabo-Profile, Shanghai, China) platform was used for statistical analysis, in which orthogonal partial least squares discriminant analysis (OPLS-DA) for multivariate statistical analysis, as well as univariate analyses were based on whether the data were normally distributed.[36] The VIP > 1, and $p < 0.05$ were identified as significant metabolic differences.

Immunohistochemical Staining

The brain and colon sections were stained with antibodies of gamma-aminobutyric acid types A receptor ($GABA_AR$, No. DF8548, dilution 1:100, Wuhan, China), Glutamate lonotropic Receptor AMPA Type Subunit 1 (GRIA1, No. 67642, dilution 1:100, America), Farnesoid X receptor (FXR, 25055-1-AP, dilution 1:100, Wuhan, China). The relative optic density of $GABA_AR$, GRIA1 and FXR were analyzed by Indica labs (Halo 101-WL-HALO-1, USA).

RT-qPCR Analysis

Total RNA of the rat's hippocampus and colon was both extracted using a Molpure® Cell/Tissue Total RNA Kit (No. 19221ES50, YEASEN), and the concentration of purity of which were measured using a TB Green™ Premix Ex Taq™ (Tli RNaseH Plus) (No. RR820A, Thermo Fisher Scientific, Waltham, MA, USA). The RT-qPCR was performed on an ABI 7500 FAST system (Quant Studio™ 3, Thermo Fisher, MA, USA), and the conditions were as follows: Pre-denaturation 95 °C, 30s, denaturation 95 °C, 5s, annealing 55 °C, 30s, with 40 cycles, then its extended and to collected fluorescence for 72 °C, 30s. The $2^{-\Delta\Delta Ct}$ method was used to analyze the target gene expression, with β-actin as the internal control. The primer sequences were presented in Table 1.

Correlation Network Analysis

Neuroinflammatory factors, intestinal microbial communities, and metabolic markers were significantly intervened via DNX in the treatment of FS. All above these factors were utilized for correlation analysis by using SPSS 25.0 Pearson correlation analysis, and further the network was constructed to employ Cytoscape (v3.10.1) software, which the $p < 0.05$, $r > 0.8$ were performed significant correlation.

Table 1 Primer Sequences of the Genes Used for RT-qPCR

Gene	Forward Primer (5′→3′)	Product Size (bp)
β-actin	F: GGGAAATCGTGCGTGACATT R: GCGGCAGTGGCCATCTC	76
$GABA_AR$	F: AGTGCCAGAAATTCCCTCCCAAAG R: CAATCAGAGCCGAGAACAGAAGG	94
FXR	F: CGTCGGAAGTGCCAGGATTGC R: CGCTGTCCTCATTCACTGATC	160
HMGB1	F: AGGCTGACAAGGCTCGTTATGAAAG R: GGGCGGTACTCAGAACAGAACAAG	139
IL-1β	F: AATCTCACAGCAGCATCTCGACAAG R: TCCACGGGCAAGACATAGGTAGC	98
IL-6	F: ACTTCCAGCCAGTTGCCTTCTTG R: TGGTCTGTTGTGGGTGGTATCCTC	110
TNF-α	F: CACCACGCTCTTCTGTCTACTGAAC R: TGGGCTACGGGCTTGTCACTC	150

Statistical Analysis

Statistical analysis was performed using SPSS 25.0 (SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by Dunn's multiple comparison tests was used, and p-value < 0.05 was considered statistically significant. Results were expressed as means ± standard deviation (SD).

Results

Compounds Analysis of DNX

As shown in Figure 1D and E, a total of 57 compounds were identified from DNX, which mainly includes BAs, flavonoids, phenols, nucleosides, and xanthine compounds.

DNX Prolonged the Latency and Decreased the Incidence Rate of FS

The FS rat model was successfully established, and the rectal temperature was measured at least 39.2°C, by observing the level of convulsions that was simulated to previous reports.[37,38] Furthermore, in the FS model group, various types of severe seizure-like symptoms were observed, like neck muscle rigidity and rhythmic twitching. The seizure latency (p < 0.01) was lower, and seizure duration was higher (p < 0.01, Figure 2). Interestingly, after treatment with DNX (2.8 g/kg) and positive drug of diazepam, the seizure latency was significantly increased (p < 0.01), while the seizure duration decreased (p < 0.01). Moreover, one rat died of the model group, which may be caused by repeated modeling. However, no rats died in other groups, which may be a beneficial result of continuous administration referring to the performance of diazepam drug group rat.

DNX Alleviated the Hippocampal Neuronal Damage Induced by FS

As shown in Figure 2B, the hippocampal neurons cells were irregularly arranged, with a vertebral body morphology and smaller nuclei in the control group. After hot water bath modeling, the increased intercellular space, nuclear atrophy, and inflammatory infiltration were observed. Rats treated with DNX (2.8 g/kg) and diazepam showed less cell shrinkage and less macrophage in the hippocampus compared with model group. Additionally, it was worth noting that the number of Nissl bodies decreased in the model group (Figure 2C, p < 0.05), which was reversed by treatment with high dose of DNX and positive drug of diazepam (p < 0.05). These findings suggested that the FS rat might be attributed to the increased hippocampal neuron damage and decreased Nissl bodies, whereas DNX treatment appears to alleviate this damage and increase Nissl bodies number, thus leading to an improvement of the anti-FS effects.

DNX Decreased the Level of Pro-Inflammatory Cytokines in the Hippocampus and Serum of FS Rat

Compared to the control group, in this study, the levels of TNF-α, IL-1β, IL-6, and HMGB1 in the hippocampus and serum were significantly increased in the brain tissues of the FS rat ($p < 0.01$, Figure 2D and E). In contrast, after treatment with high dose of DNX, these inflammatory factors were reduced ($p < 0.05$, Figure 2D and E). These findings revealed that DNX improved the hippocampal neuronal damage in FS rat through inhibiting the release of inflammatory factors in the hippocampus and serum.

Network Pharmacology Analysis of DNX on FS

A total of 2467 FS-related genes were collected from Gene Cards data, in which 194 intersection targets were collected, known as candidate targets (Figure 3A). The components-targets-FS network was established, including 256 nodes (62 compounds, 194 targets) and 1334 edges, in which the purple triangles represent compounds, while orange roundness represents the targets (Figure 3B). Furthermore, the 194 candidate targets were connected to establishing PPI network, which contained 121 nodes and 3648 edges after removing the isolated targets (Figure 3C). A total of 15 key targets were selected, including IL-6 (degree 214, betweenness 0.0646, closeness 0.6762), TNF-α (degree 212, betweenness 0.0600, closeness 0.6762) and AKT1 (degree 202, betweenness 0.0820, closeness 0.6189). For KEGG pathway, the TNF signaling pathway (hsa04668), GABAergic synapse (has04727), and bile secretion (hsa04976) were enriched, as shown in Figure 3D.

Furthermore, the molecular docking was employed to study the mode of action between the active components chenodeoxycholic acid (CDCA), ursodeoxycholic acid (UDCA), docosahexanoic acid, acetylleucine and key targets involved in the inflammatory pathways (IL-6, TNF-α, IL-1β) and BAs metabolic pathway (NR1H4 (FXR)) (Figure 3E). As LibDock Scores displayed (Figure 3E), the strong binding force between the CDCA and NR1H4 (−9.0 kcal/moL), UDCA and NR1H4 (−8.4 kcal/moL), docosahexanoic acid and TNF-α (−6.4 kcal/moL), docosahexanoic acid and IL-1β (−4.3 kcal/moL) were found. As for molecular docking, van der Waals, carbon hydrogen bond, alkyl, pi-alkyl bonding, and conventional hydrogen bond draw the important role. Such as, van der Waals was formed between CDCA and amino acid residues including TYRA: 369, META: 365, SERA: 332, ILEA: 335 and GLYA: 343 of NR1H4 protein, as well alkyl was formed between CDCA and META: 328, LEUA: 287, and ARGA: 331 amino acid residues of NR1H4 protein. There was formed conventional hydrogen bond between UDCA and HISA: 294 and TYRA: 361 amino acid residues of NR1H4 protein. Additionally, the alkyl was formed between acetylleucine and LYSB: 98, PROB: 100 amino acid residues of TNF-α protein. These results suggested that the inflammatory pathway and FXR pathway may have contributed to the effect of DNX on FS.

DNX Regulated Intestinal Gut Microbiota Balance and Colon Trauma in FS Rat

Moreover, the inflammatory infiltration to lead intestinal mucosal injury of FS model rats occurred, whereas this injury was reversed by treatment with high dose of DNX (Figure 4A). Therefore, we speculated that DNX may improve the intestinal damage by regulating gut microbiota balance and ultimately achieve the anti-FS effect.

Three colon content samples from each group were obtained for 16S RNA analysis. A total of 17200 operational taxonomic units (OTUs) were obtained from the three groups, of which 954, 4239, and 4186 were in the control group, the model group, and the DNX group, respectively. The gut microbiota profile in the control, model, and DNX groups exhibited distinct separations by using Principal coordinate analysis (PCoA) (Figure 4D) that indicated the protective effect of DNX for treatment of FS. The diversities in the FS and DNX groups were higher than the control group (Figure 4B). At the phylum level (Figure 4C), the relative abundance of *Firmicutes* was significantly decreased ($p < 0.05$), the *Bacteroides and Tenericutes* were significantly increased ($p < 0.05$) in the FS rat model, which was consistent with previous findings and showed as Figure 4F.[13] At the genus level (Figure 4E), the FS rat model showed a lower abundance of *Lactobacillus* ($p < 0.01$) and *Lachnospiraceae* ($p < 0.05$). However, DNX group showed an increasing abundance of *Firmicutes* ($p < 0.01$), and decreasing abundance of *Bacteroidetes*, and *Tenericutes* ($p < 0.05$) of phylum level. Meanwhile, the abundance in the genus of *Lactobacillus* and *Lachnospiraceae* increased ($p < 0.05$, Figure 4G) of

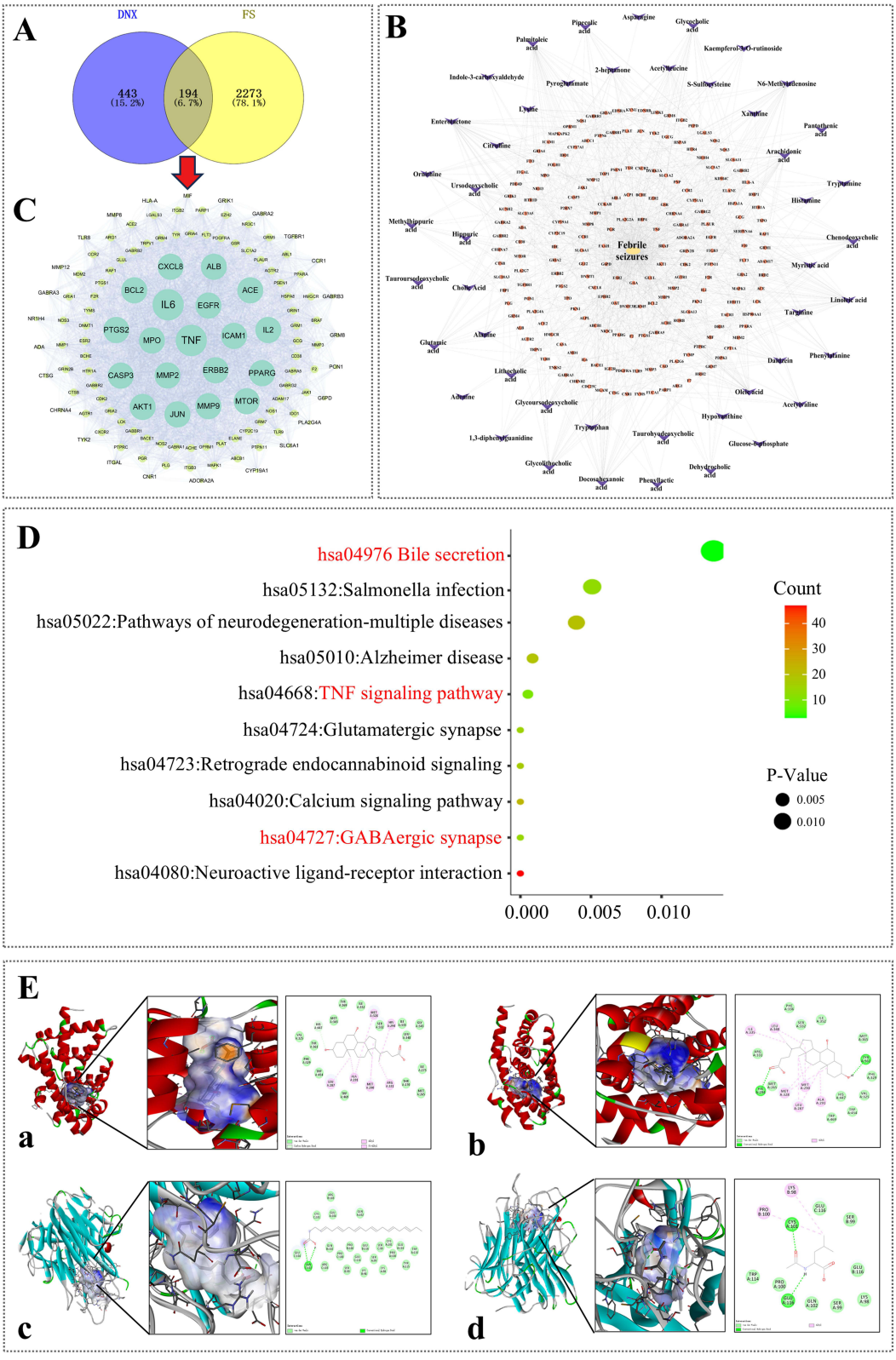

Figure 3 Network pharmacology analysis of DNX in treatment of FS. Venn diagram (**A**); Components-targets network (**B**); PPI network (**C**); KEGG pathway (**D**); molecular docking (**E**); CDCA (CAS: 474-25-9) and NR1H4 (PDB ID: 3GD2) (a); UDCA (CAS: 128-13-2) and NR1H4 (PDB ID: 3GD2) (b); docosahexanoic acid (CAS: 6217-54-5) and TNF-α (PDB ID: 1A8M) (c); acetylleucine (CAS: 99-15-0) and TNF-α (PDB ID: 1A8M) (d).

https://doi.org/10.2147/JIR.S508690

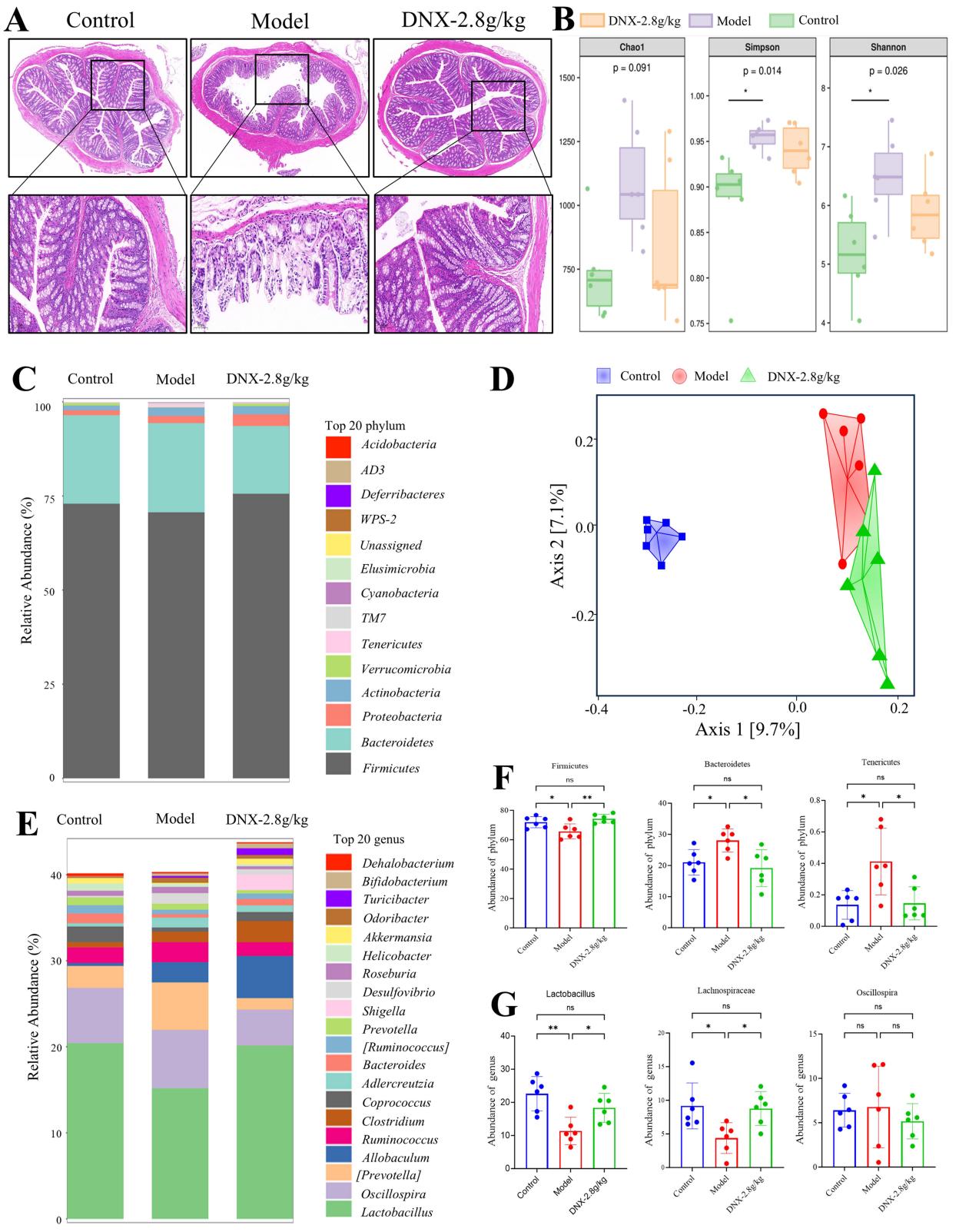

Figure 4 Effect of DNX on the alteration of fecal gut microbiota in FS rat. Hematoxylin and eosin (H&E) (100×, 400×) (**A**), n=3; alpha diversity, compared with control group, *$p < 0.05$, (**B**); Relative abundance of the gut microbiota at the phylum level (**C**); PCoA analysis of the OTUs (**D**); Relative abundance of the gut microbiota at the genus levels (**E**); Relative abundance of the significantly altered bacteria at the phylum (**F**) and genus levels (**G**), n=6. *$p < 0.05$, **$p < 0.01$, ns means no statistical difference.

genus level. In consideration of the above points, although DNX treatment did not restore the abundance of *Lactobacillus* to control level, it showed a positive effect and revealed that hot water bath induced FS model was related to the disorder of the gut microbiota, and DNX treatment promotes the recovery of the beneficial bacteria.

Effect of DNX on the Fecal Metabolic Disorder in FS Rat

As shown in Figure 5, a clear separation trend of three groups was obtained. Obviously, the rats FS model was successfully replicated from the metabolic perspective, as indicated by the clear separation between the control and model group. Whereas the metabolic profiling in the rats treated with DNX was much separated from the model group, indicating the DNX treatment played a positive effect in rats with FS model.

Furthermore, the OPLS-DA model was obtained, of which R^2Y and Q^2Y values were 0.987 and 0.839 between control and model, and 0.898 and 0.797 between model and DNX group (Figure 5A). Based on the parameters of Variable importance in the projection (VIP) > 1 and $P < 0.05$, a total of 63 metabolites differences were identified, of which 20 metabolites were significantly reversed as metabolic markers including GABA, CDCA, UDCA, and DCA ($P < 0.05$, VIP >1) via DNX regulation (Figure 5D). Among these metabolites, GABA, CDCA, and UDCA have been reported to be associated with neuroprotective effects in FS treatment.[39,40]

Moreover, the metabolic pathway analysis was performed, including alanine, aspartate and glutamate metabolism, primary bile acid biosynthesis, and arginine and proline metabolism as significantly different ones (Figure 5B and C). These findings revealed that the fecal metabolic disorders in the FS rat model can be changed via treatment with DNX, which may be related with the altered gut microbiota.

DNX Decreased the Level of Glu and Elevated GABA in the Hippocampus and Serum of FS Rat

To further verify the regulatory effect of DNX on fecal metabolic disorder in the FS rat, the levels of GABA and Glu in the serum and hippocampus tissue were evaluated using ELISA method (Figure 5F and G). The results showed that the GABA content was decreased ($p < 0.01$ and $p < 0.05$) and the Glu content was increased ($p < 0.05$) in the FS model rats, compared with the control group. In contrast, this trend was reversed in DNX treatment. The result suggested that FS-related fecal metabolic disorder can be regulated by DNX treatment.

DNX Increased the Expression of $GABA_AR$ and FXR in the Hippocampus and Colon of FS Rat

The immunohistochemistry and RT-qPCR were carried out to verify the possible metabolic pathway of aspartate and glutamate metabolism and primary bile acid biosynthesis. As shown in Figure 6A and B, the RT-qPCR assay exhibited that the mRNA level of FXR and $GABA_AR$ in the hippocampus and colon were remarkedly decreased in the FS model rats compared with that in control group, however, these reductions were back-regulated by DNX treatment.

Furthermore, the results of the RT-qPCR assay were confirmed by subsequent immunohistochemical assays. As shown in Figures 7 and 8, the hot water bathing significantly decreased the protein expression of FXR and $GABA_AR$ in the hippocampus and colon, and these reducing effects were almost completely reversed by DNX (2.8 g/kg) treatment. These findings illustrated that the increased protein and mRNA expression of FXR and $GABA_AR$ in the hippocampus and colon might also be closely related to the ameliorative effect of DNX on FS model rats.

DNX Decreased the Expression of IL-1β, HMGB1, IL-6 and TNF-α in the Hippocampus of FS Rat

To further verify the interaction between brain inflammatory factors in DNX anti-FS. The expressions of IL-1β, IL-6, TNF-α and HMGB1 were measured in the hippocampus by RT-qPCR (Figure 6C–F). Compared with the control group, the expression of inflammatory factors was significantly increased ($p < 0.05$ and $p < 0.01$) in the hippocampus of FS rat. On the contrary, the hippocampus tissue showed the reverse trend of FS rat treated with DNX, in which the IL-6, TNF-α were notably decreased ($p < 0.05$).

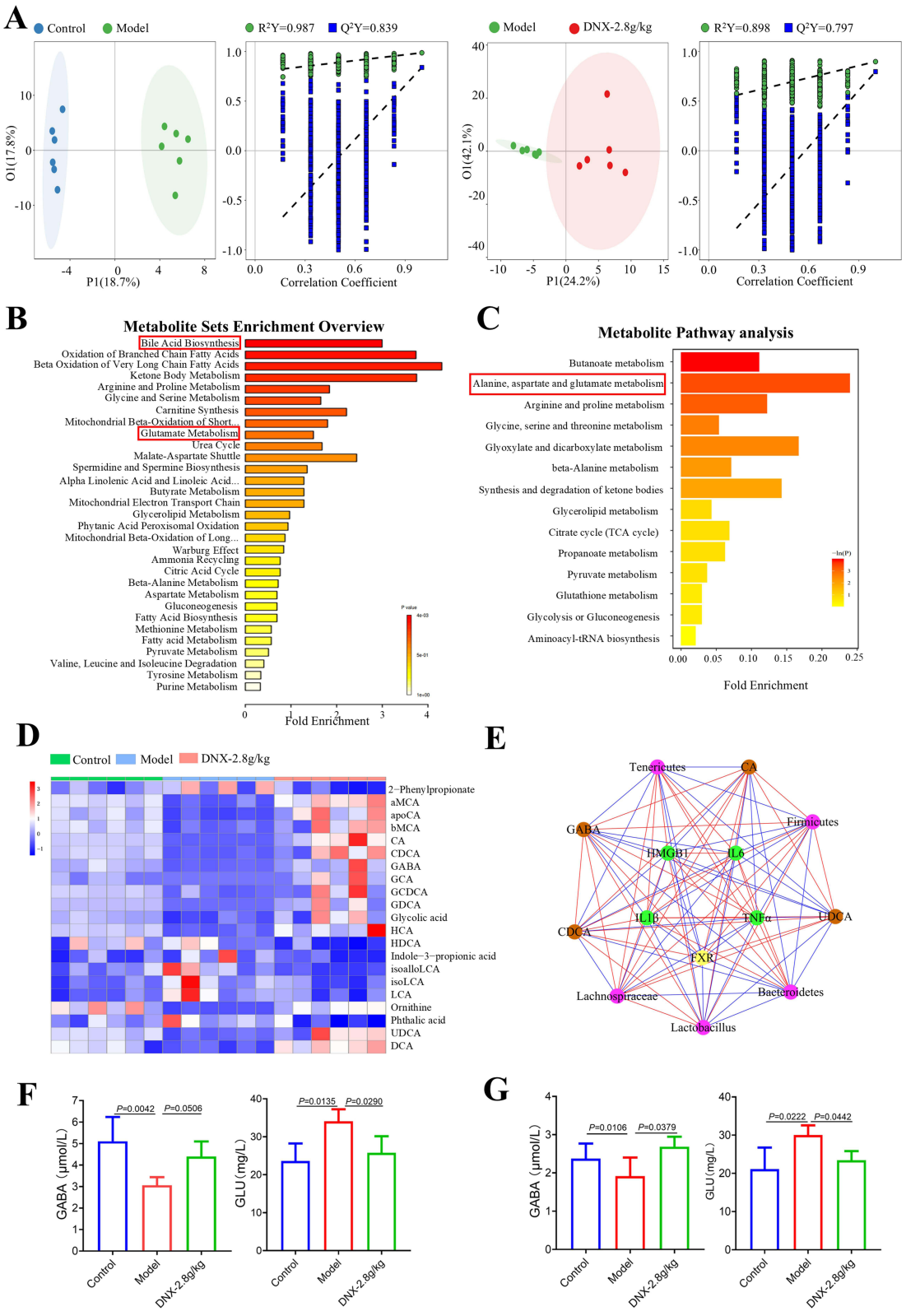

Figure 5 Effects of DNX on the fecal metabolites in FS rat. OPLS-DA analysis (**A**); Metabolic pathway with significant difference (**B** and **C**); Heatmap analysis of the potential biomarkers (**D**), *$p < 0.05$, **$p < 0.01$; Inflammatory factor-microbiota-metabolite correlation network diagram, Red represents positive correlation and blue represents negative correlation. (**E**); neurotransmitter in serum (**F**) and hippocampus (**G**), (n = 6).

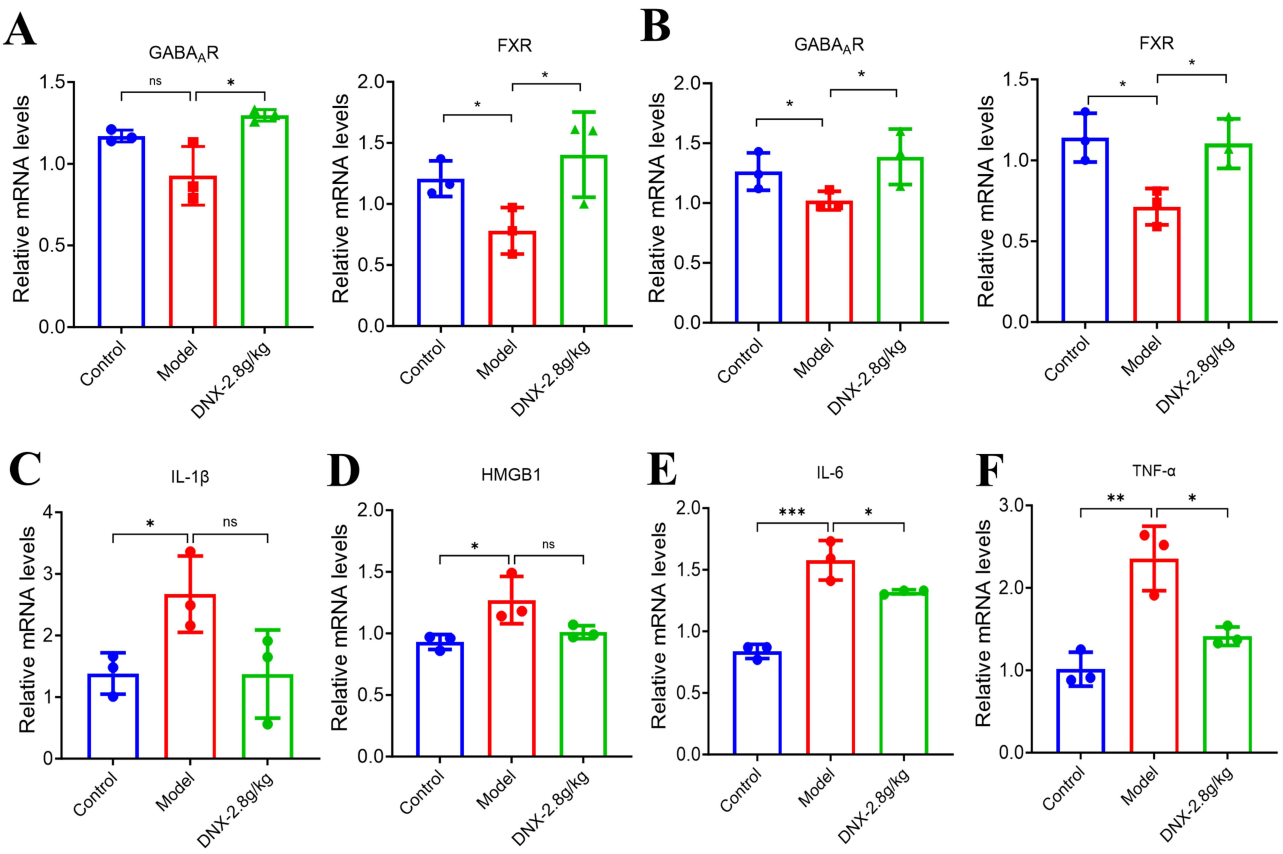

Figure 6 DNX increased the mRNA expression of GABA$_A$R and FXR in the hippocampus (**A**) and colon (**B**), decreased the mRNA expression of IL-1β (**C**), HMGB1 (**D**), IL-6 (**E**) and TNF-α (**F**) in the hippocampus. n=3, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns means no statistical difference.

Integrated Network of the Mechanism of DNX for FS

To investigate the molecular mechanism of DNX anti-FS by regulating gut microbiota-mediated GABA and FXR pathways while alleviating neuroinflammation. The correlation network diagram was established to analyze the relationship between the TNF-α, IL-1β, IL-6, HMGB1 and *Firmicutes, Lactobacillus, Bacteroidetes, Tenericutes*, GABA, CA, CDCA. As shown in Figure 5E, The *Firmicutes* and *Lactobacillus*, FXR were negatively correlated with TNF-α, IL-1β, and IL-6, respectively. Whereas the *Tenericutes* were positively correlated with these inflammatory factors. Additionally, FXR, GABA and UDCA were negatively correlated with HMGB1, and GABA were negatively correlated with IL-1β, and IL-6, as well the CA were negatively correlated with IL-6. The network provides a mechanism for DNX to treat FS. Hence, the mechanism action of DNX in the treatment FS is shown as Figure 9. By and large, DNX mainly inhibits harmful bacteria and back-regulate beneficial intestinalbacteria to further promote the metabolism of BAs, GABA and then interfering with FXR and GABA signaling pathways. At the same time, it alleviates intestinal and neuroinflammation to combine action anti-FS.

Discussion

FS was one of the neurological findings and commonly occurred in the evening, which was evaluated complex FS of children in clinic.[41,42] Currently, the increasing amounts of pathological studies have demonstrated that the imbalance of immune system could produce inflammatory cytokines including IL-1β and HMGB that was one of the risks to enhance FS and seizures and brain damage.[29] Moreover, research in the seizures demonstrated that IL-1β and HMGB1 were highly released in activated astrocytes and microglia of brain.[43] The IL-1β, IL-6 and TNF-α level were significantly raised in hippocampus of FS rat.[44] Therefore, relieving the inflammatory response in the brain was taken into consideration to investigate effects and mechanism of DNX anti-FS in this work. Pharmaceutical processing methods

https://doi.org/10.2147/JIR.S508690

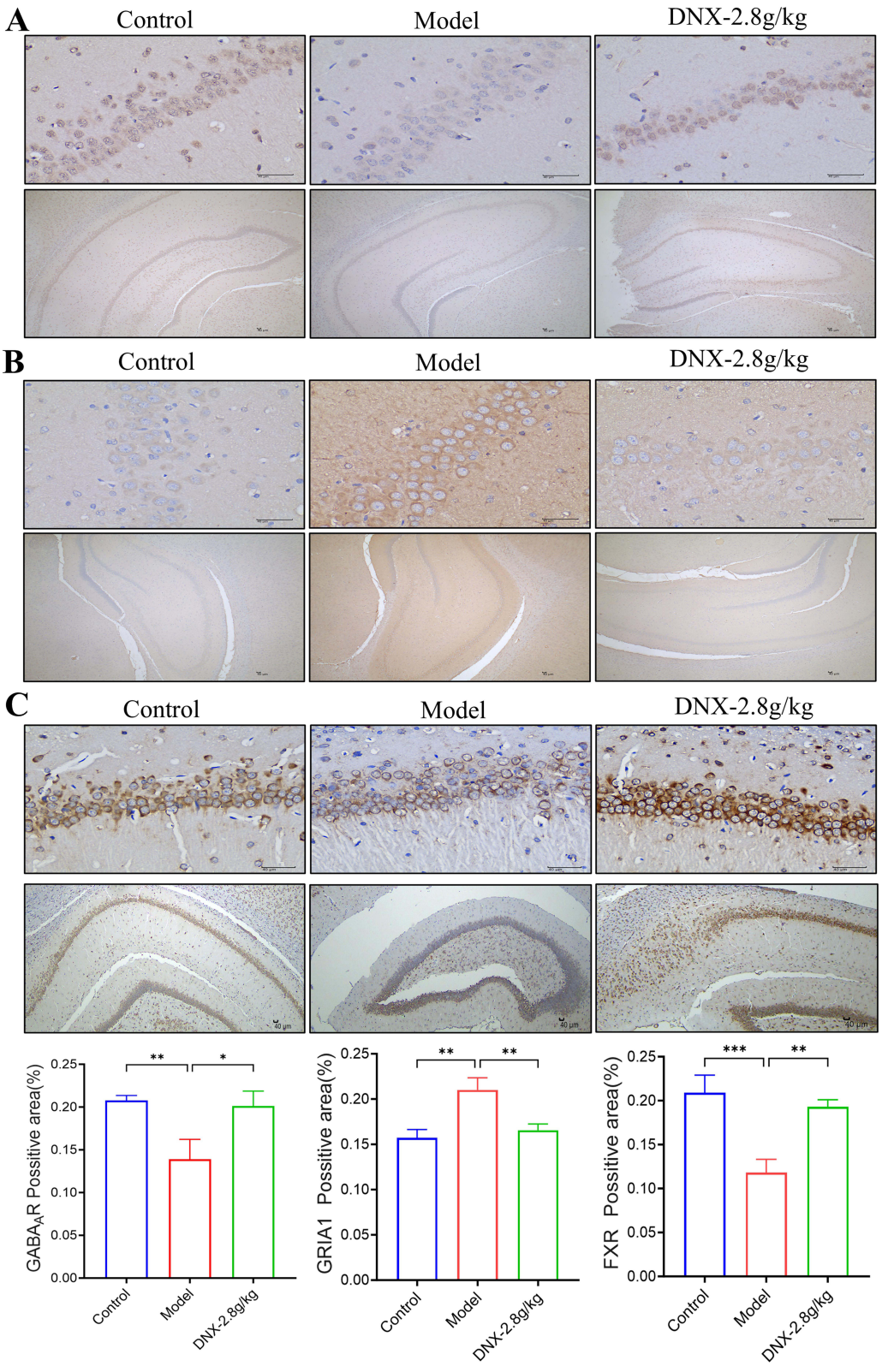

Figure 7 Effect of DNX on the expression of GABA$_A$R (**A**), GRIA1 (**B**) and FXR (**C**) in the hippocampus of FS rat model. All data were compared using one-way ANOVA, and *P*-values reflect differences between experimental groups (n=8 or n=7).

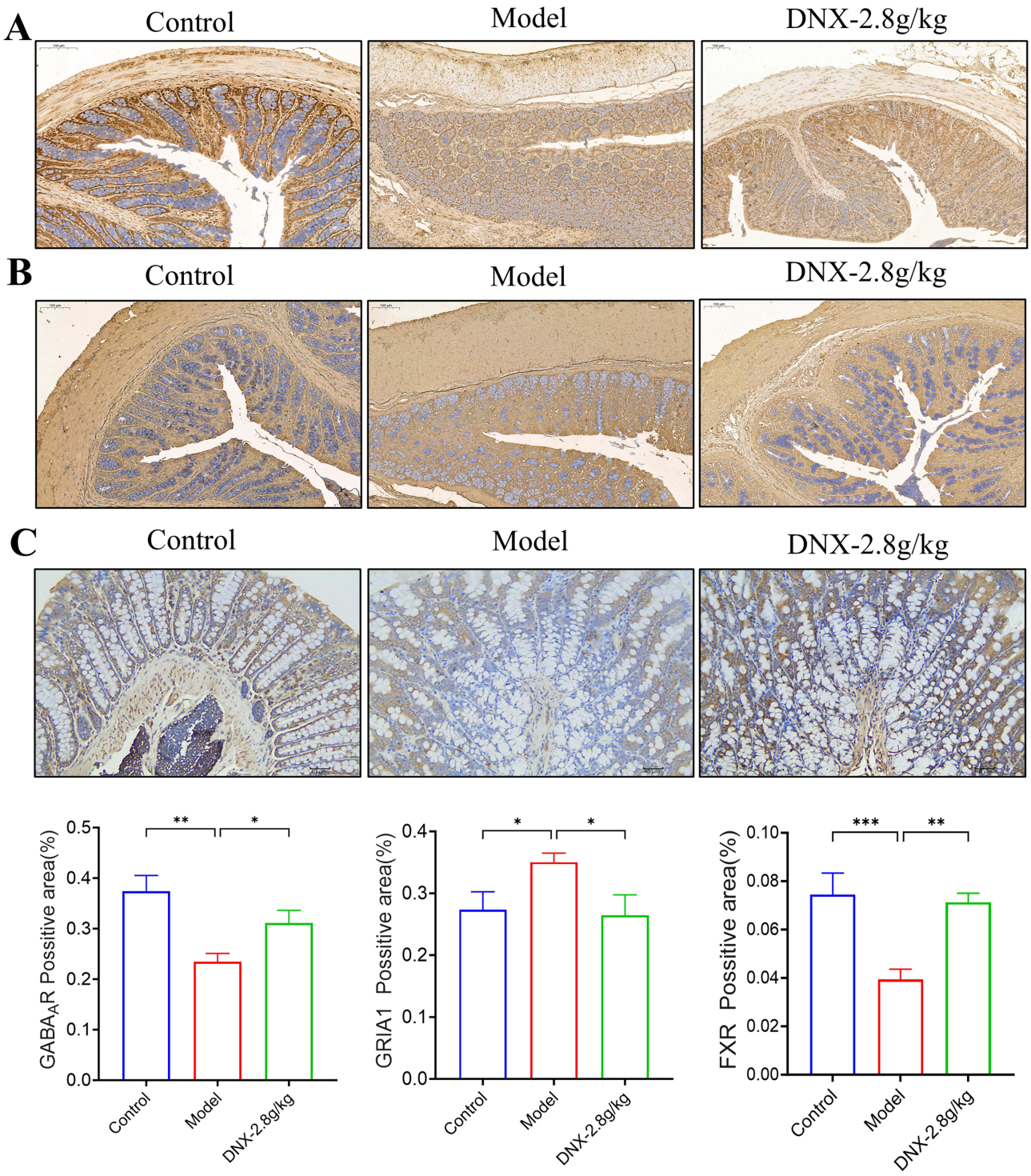

Figure 8 Effect of DNX on the expression of $GABA_AR$ (**A**), GRIA1 (**B**) and FXR (**C**) in the colon of FS rat model. All data were compared using one-way ANOVA, and *P*-values reflect differences between experimental groups (n=8 or n=7).

were classical techniques in the development of TCM that aimed to reduce toxicity and increase the therapeutic effectiveness.[45] One of the reason animals' bile was used for preparation of DNX, and the introduction of bile in DNX was more advantageous in the treatment FS. In addition, research investigated that the FXR pathway was regulated via BAs in treatment of many diseases including FS. At the same time, the FXR cloud inhibits neuroinflammation to protect against FS especially.[25] In our findings, the TUDCA, THDA, Glycocholic acid, UDCA, Cholic acid (CA), and

https://doi.org/10.2147/JIR.S508690

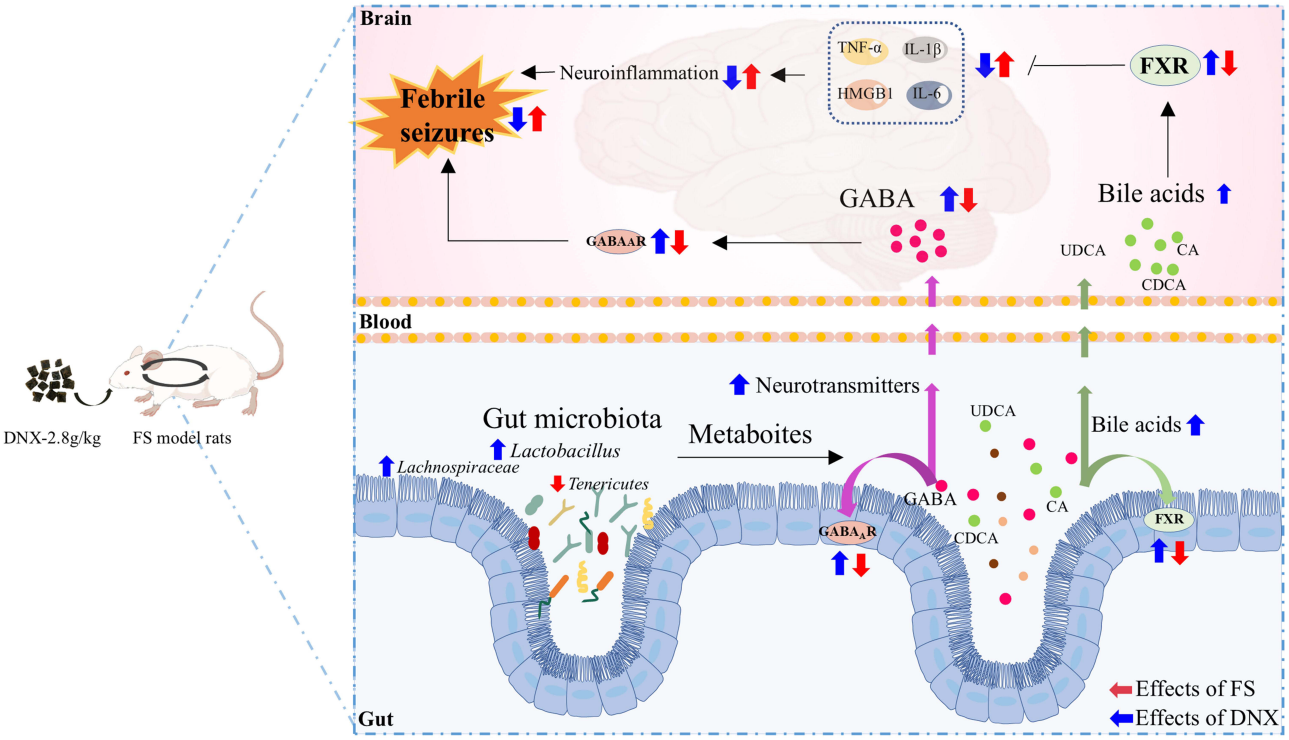

Figure 9 Possible mechanism of DNX in the treatment of FS by inhibiting neuroinflammation via regulating FXR and GABA pathway and gut microbiota.
Abbreviations: DNX, Arisaema Cum Bile; FS, febrile seizure; GABA, Gamma-aminobutyric acid; GABAAR, gamma-aminobutyric acid types A receptor; HMGB1, High mobility group box 1; IL-1β, Interleukin-1β; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor alpha; UDCA, Ursodeoxycholic acid; FXR, Farnesoid X receptor; BAs, Bile acids; CA, Cholic acid; CDCA, Chenodeoxycholic acid.

CDCA of DNX were verified to use UPLC-Q-TOF-MS/MS, in which CDCA can prevent progressive neurological dysfunction in patients, TUDCA has neuroprotective effects, and UDCA can inhibit apoptosis in neural cells. As well as CA, UDCA and CDCA promoted FXR expression to alleviate the neuronal apoptosis.[27,46,47] Overall, these compounds may be key of DNX for FS. Based on H&E and Nissl staining results that the hippocampal tissues alleviated FS via DNX (2.8 g/kg) in treatment.

Further research found that the upregulated FXR pathway can decrease the inflammatory factor levels (IL-1β, IL-6, TNF-α, and HMGB1). Besides, as present evidence was reported that the GABA, a neurotransmitter for relieving seizure, was also adjusted by FXR regulation.[25] Importantly, GABA exerts postsynaptic inhibitory action mainly via $GABA_AR$ to alleviate FS.[48,49] In this study, the level of GABA and $GABA_AR$ were markedly raised in the brain of DNX (2.8 g/kg) group. Furthermore, the Glu, an excitatory neurotransmitter for seizures in the brain, was significantly down-regulated in DNX (2.8 g/kg) group. Meanwhile, The GRIA1 protein expression have the same tendency with Glu.

Moreover, the "gut-brain" axis has aroused increasing study, which were a complex bidirectional way to link the direct neuronal, neuroendocrine, and immunological pathways and enables communication between the gut microbiota and the brain,[50,51] such as the BAs could cross the blood brain barrier (BBB) by circulating that was reached the central nerve system via passive diffusion or BA transporters. The primary BAs was synthesized by the liver and the gut microbiota was specified secondary BAs both to consist circulating BAs pool, and the brain derived BAs also contributed to the BAs pool.[52] For instance, some neurotransmitters are created by gut microbiota in treatment disease, such as GABA was produced via the *Bifidobacteria* family, and *Lactobacillus*.[53–55] Above these points, the gut microbiota and fecal metabolism were analyzed to explore the mechanism on anti-FS of DNX by regulation of the "gut-brain" axis. In our study, at the phylum, the *Firmicutes* were significantly back-regulated, and the *Bacteroidetes* and *Tenericutes* were obviously down-regulated of FS in DNX (2.8 g/kg) group. The *Tenericutes* was reported to be associated with the development of disease (pneumonia and depression).[56,57] Its suggest that the abundance of harmful bacteria can be inhibited by DNX. Furthermore, at the genus level, the abundance of *Lactobacillus* and *Lachnospiraceae* were

significantly increased by DNX in treatment. And the FXR pathway and GABA pathway were recognized as key metabolic pathways were verified by RT-qPCR and immunohistochemistry experiments. In addition, network pharmacology was used to combine analysis of the mechanisms of DNX for FS in this research. We find that the inflammatory (IL-6 and TNF-α) were core target in PPI analysis, and further GO and KEGG results were shown that the GABA-A receptor activity (GO: 0004890), neurotransmitter receptor activity (GO: 0030594), TNF signaling pathway (hsa04668), and Bile secretion (hsa04976) were mainly represented. Thereby, these results point towards adjustment of the inflammatory response and BAs metabolism could alleviate FS. As well as molecular docking was employed to examine the action mode between key components (UDCA, CDCA, docosahexanoic acid) and fatal proteins (FXR, TNF-α). In summarization, these results suggested that DNX mainly regulated gut microbiota that increased probiotics (*Lactobacillus* and *Lachnospiraceae*) and decreased pathogenic bacteria (*Tenericutes*), as well further to inhibited neuroinflammation, regulated FXR and GABA pathways to alleviating hot water bathing induced FS.

Conclusion

Collectively, DNX showed protective effects on neuroinflammation of FS rat, possibly through regulating FXR pathway and GABA pathway via restoring the gut microbiota. Our findings provide a clinical basis for the DNX treatment of FS and its related metabolic syndromes.

Abbreviations

BAs, bile acids; BBB, blood brain barrier; CA, Cholic acid; CDCA, Chenodeoxycholic Acid; DNX, *Ding Nan Xing*; FXR, Farnesoid X receptor; FS, Febrile seizures; GABA, Gamma-aminobutyric acid; $GABA_AR$, gamma-aminobutyric acid types A receptor; Glu, Glutamate; GO, Gene Ontology; GRIA1, Glutamate lonotropic Receptor AMPA Type Subunit; H&E, hematoxylin-eosin; HMGB1, High mobility group box 1; IL-1β, Interleukin-1β; IL-6, Interleukin-6; KEGG, Kyoto Encyclopedia of Genes and Genomes; NF-κB, nuclear factor kappa-B; OTUs, operational taxonomic units; OPLS-DA, Orthogonal partial least squares discriminant analysis; PCoA, Principal coordinate analysis; PPI, protein-protein interaction; RT-qPCR, Real-Time quantitative reverse transcription polymerase chain reaction; TCHM, traditional Chinese herbal medicine; TNF-α, Tumor necrosis factor alpha; UDCA, Ursodeoxycholic acid; UPLC-MS/MS, Ultra performance liquid chromatography tandem mass spectrometry; VIP, Variable importance in the projection.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work is financially supported by the National Natural Science Foundation of China (No.81803732); Shaanxi Province high level of traditional Chinese medicine key disciplines of traditional Chinese medicine processing science of China, Shaanxi Provincial Key R&D Program (2024SF-ZDCYL-03-11); The key research and development plan of Xian yang city in Shaanxi province of China (L2023-ZDYF-SF-019); Natural Science Basic Research Program of Shaanxi Provincial Department of Science and Technology (2025JC-YBMS-923); Shaanxi Provincial Department of Education Key Scientific Research Programme Project (24JR055); Qinchuangyuan Traditional Chinese Medicine Industry Innovation Aggregation Zone Project (L2024-QCY-ZYYJJQ-X34); Key Research & Development Program of Shaanxi Provincial Department of Science and Technology (2024CY-JJQ-36).

Disclosure

The authors confirm that there are no conflicts of interest in this work.

References

1. Bozon B, Kelly A, Josselyn SA, Silva AJ, Davis S, Laroche S. MAPK, CREB and zif268 are all required for the consolidation of recognition memory. *Philos Trans R Soc London Ser B*. 2003;358(1432):805–814. doi:10.1098/rstb.2002.1224
2. Paul SP, Eaton M. At a glance: febrile convulsion in children. *J Fam Health Care*. 2013;23(1):34–37.
3. MacDonald BK, Johnson AL, Sander JW, Shorvon SD. Febrile convulsions in 220 children--neurological sequelae at 12 years follow-up. *Eur Neurol*. 1999;41(4):179–186. doi:10.1159/000008048
4. Neligan A, Bell GS, Giavasi C, et al. Long-term risk of developing epilepsy after febrile seizures: a prospective cohort study. *Neurology*. 2012;78 (15):1166–1170. doi:10.1212/WNL.0b013e31824f807a
5. Vezzani A, Maroso M, Balosso S, Sanchez MA, Bartfai T. IL-1 receptor/Toll-like receptor signaling in infection, inflammation, stress and neurodegeneration couples hyperexcitability and seizures. *Brain Behav Immun*. 2011;25(7):1281–1289. doi:10.1016/j.bbi.2011.03.018
6. Xu Y, Wang Q, Wu Z, et al. The effect of lithium chloride on the attenuation of cognitive impairment in experimental hypoglycemic rats. *Brain Res Bull*. 2019;149:168–174. doi:10.1016/j.brainresbull.2019.04.019
7. Chen Y, Chen X, Luo Z, et al. Exercise-induced reduction of IGF1R sumoylation attenuates neuroinflammation in APP/PS1 transgenic mice. *J Adv Res*. 2024;69:279–297. doi:10.1016/j.jare.2024.03.025
8. Kauer JA, Gibson HE. Hot flash: TRPV channels in the brain. *Trends Neurosci*. 2009;32(4):215–224. doi:10.1016/j.tins.2008.12.006
9. Rose-John S. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. *Int J Bio Sci*. 2012;8 (9):1237–1247. doi:10.7150/ijbs.4989
10. Chen C, Liao J, Xia Y, et al. Gut microbiota regulate Alzheimer's disease pathologies and cognitive disorders via PUFA-associated neuroinflammation. *Gut*. 2022;71(11):2233–2252. doi:10.1136/gutjnl-2021-326269
11. Qian X, Hai W, Chen S, Zhang M, Jiang X, Tang H. Multi-omics data reveals aberrant gut microbiota-host glycerophospholipid metabolism in association with neuroinflammation in APP/PS1 mice. *Gut Microbes*. 2023;15(2):2282790. doi:10.1080/19490976.2023.2282790
12. Yao H, Zhang D, Yu H, et al. Gut microbiota regulates chronic ethanol exposure-induced depressive-like behavior through hippocampal NLRP3-mediated neuroinflammation. *Mol Psychiatry*. 2023;28(2):919–930. doi:10.1038/s41380-022-01841-y
13. Hu B, Zhu X, Zheng J, et al. Changes of serum metabolomics and gut microbiota reveal specific characteristics of children with febrile seizures. *Eur J Neurol*. 2023;30(11):3516–3528. doi:10.1111/ene.15275
14. Zhang MM, Wu X, Wang J, et al. Revealing the optimal traditional processing methods and its protective effects against febrile seizures of Arisaema cum bile. *Biomed Chromatogr*. 2024;38(11):e5977. doi:10.1002/bmc.5977
15. Waruiru C, Appleton R. Febrile seizures: an update. *Arch Dischildhood*. 2004;89(8):751–756. doi:10.1136/adc.2003.028449
16. Gerges NZ, Aleisa AM, Schwarz LA, Alkadhi KA. Chronic psychosocial stress decreases calcineurin in the dentate gyrus: a possible mechanism for preservation of early ltp. *Neuroscience*. 2003;117(4):869–874. doi:10.1016/S0306-4522(02)00766-2
17. Peng J, Kim MJ, Cheng D, Duong DM, Gygi SP, Sheng M. Semiquantitative proteomic analysis of rat forebrain postsynaptic density fractions by mass spectrometry. *J Biol Chem*. 2004;279(20):21003–21011. doi:10.1074/jbc.M400103200
18. Xu XK, Wang SY, Chen Y, et al. Fangjing decoction relieves febrile seizures-induced hippocampal neuron apoptosis in rats via regulating the Akt/mTOR pathway. *Biosci Rep*. 2018;38(5):BSR20181206. doi:10.1042/BSR20181206
19. Atabaki R, Roohbakhsh A, Moghimi A, Mehri S. Protective effects of maternal administration of curcumin and hesperidin in the rat offspring following repeated febrile seizure: role of inflammation and TLR4. *Int Immunopharmacol*. 2020;86:106720. doi:10.1016/j.intimp.2020.106720
20. Yu Y, Yang Z, Jin B, et al. Cannabidiol inhibits febrile seizure by modulating AMPA receptor kinetics through its interaction with the N-terminal domain of GluA1/GluA2. *Pharmacol Res*. 2020;161:105128. doi:10.1016/j.phrs.2020.105128
21. Wu Z, Liu Y, Lin L. Historical evolution and modern research of Dannanxing processing. *Mod Chin Med*. 2020;22:1938–1943. (In Chinese).
22. Zeng P, Yan YM, Yu HL, et al. Effect of Arisaema Cum bile on brain tissue injury and inflammation in febrile seizures model mice. *J Nanjing Univ Tradit Chin Med*. 2022;38:520–526. (In Chinese).
23. Shan LQ, Liu XF, Cui YC, Liu Y, Gao H. Detoxification and modification effect of processing *Tiannanxing* into *Dannanxing*. *J Chin Pat Med*. 2021;43:1608–1612. (In Chinese).
24. Wu X, Wang J, Zou T, et al. Based on data mining, the compatibility law and application characteristics discussed Arisaema cum bile medicine. *Central South Pharm*. 2023;21:526–530. (In Chinese).
25. Sun X, Xue H, Zan B, et al. Anti-convulsant effects of cultures bear bile powder in febrile seizure via regulation of neurotransmission and inhibition of neuroinflammation. *J Ethnopharmacol*. 2020;262:112998. doi:10.1016/j.jep.2020.112998
26. Huang CR, Lin CH, Hsiao SC, et al. Drug interaction between valproic acid and carbapenems in patients with epileptic seizures. *Kaohsiung J Med Sci*. 2017;33(3):130–136. doi:10.1016/j.kjms.2016.12.001
27. Su FZ, Bai CX, Luo Y, et al. Cattle bile Arisaema aqueous extracts protect against febrile seizures in rats through regulating neurotransmitters and suppressing neuroinflammation. *Front Pharmacol*. 2022;13:889055. doi:10.3389/fphar.2022.889055
28. Su FZ, Zhu EL, Bai CX, et al. A comparative study on the antipyretic effect and underlying mechanisms of different bile-fermented Arisaemas. *J Ethnopharmacol*. 2025;337(Pt 3):118951. doi:10.1016/j.jep.2024.118951
29. Huang WX, Yu F, Sanchez RM, et al. TRPV1 promotes repetitive febrile seizures by pro-inflammatory cytokines in immature brain. *Brain Behav Immun*. 2015;48:68–77. doi:10.1016/j.bbi.2015.01.017
30. Bender RA, Dubé C, Baram TZ. Febrile seizures and mechanisms of epileptogenesis: insights from an animal model. *Adv Exp Med Biol*. 2004;548:213–225.
31. Berg AT, Shinnar S, Darefsky AS, et al. Predictors of recurrent febrile seizures. A prospective cohort study. *Arch Pediatr Adolesc Med*. 1997;151 (4):371–378. doi:10.1001/archpedi.1997.02170410045006
32. Bai YY, Tian R, Qian Y, et al. Integrated small intestine microbiota and serum metabolomics reveal the potential mechanisms of wine steaming in alleviating rhubarb-induced diarrhea. *J Inflamm Res*. 2024;17:7851–7868. doi:10.2147/JIR.S479654
33. Zhang Q, Li RL, Tao T, et al. Antiepileptic effects of cicadae periostracum on mice and its antiapoptotic effects in H2O2-stimulated PC12 cells via regulation of PI3K/Akt/Nrf2 signaling pathways. *Oxid Med Cell Longev*. 2021;2021:5598818. doi:10.1155/2021/5598818
34. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019;47(D1):D607–D613. doi:10.1093/nar/gky1131

35. Bolyen E, Rideout JR, Dillon MR, et al. QIIME 2: reproducible, interactive, scalable, and extensible microbiome data science. *PeerJ Pre Prints*. 2018;6:e27295v2.
36. Xie G, Wang L, Chen T, et al. A metabolite array technology for precision medicine. *Anal Chem*. 2021;93(14):5709–5717. doi:10.1021/acs.analchem.0c04686
37. *National Nsitu Tes of Health Febrile Seizures: Consensus Development Conference Summary.*Vol. 3. Bethesda, MD: National Institutes of Health; 1980.
38. Knudsen FU. Febrile seizures—treatment and outcome. *Brain Dev*. 1996;18:438–449. doi:10.1016/S0387-7604(96)00059-9
39. Alshabi AM, Shaikh IA, Asdaq SMB. The antiepileptic potential of Vateria indica Linn in experimental animal models: effect on brain GABA levels and molecular mechanisms. *Saudi J Biol Sci*. 2022;29(5):3600–3609. doi:10.1016/j.sjbs.2022.02.059
40. Ramalho RM, Viana RJ, Low WC, Steer CJ, Rodrigues CM. Bile acids and apoptosis modulation: an emerging role in experimental Alzheimer's disease. *Trends Mol Med*. 2008;14(2):54–62. doi:10.1016/j.molmed.2007.12.001
41. Yamaguchi H, Nagase H, Ishida Y, et al. Diurnal occurrence of complex febrile seizure and their severity in pediatric patients needing hospitalization. *Epilepsy Behav*. 2018;80:280–284. doi:10.1016/j.yebeh.2018.01.015
42. Güneş A, Fidan S, Dulkadir R, Ünlü E. Evaluation of risk factors associated with first episode febrile seizure. *Eur Rev Med Pharmacol Sci*. 2021;25(22):7089–7092. doi:10.26355/eurrev_202111_27261
43. Maroso M, Balosso S, Ravizza T, et al. Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures. *Nature Med*. 2010;16(4):413–419. doi:10.1038/nm.2127
44. Ji W, Zhu H, Xing B, et al. Tetrastigma hemsleyanum suppresses neuroinflammation in febrile seizures rats via regulating PKC-δ/caspase-1 signaling pathway. *J Ethnopharmacol*. 2024;318(Pt A):116912. doi:10.1016/j.jep.2023.116912
45. Song S, Qiu R, Jin X, et al. Mechanism exploration of ancient pharmaceutic processing (Paozhi) improving the gastroprotective efficacy of Aucklandiae Radix. *J Ethnopharmacol*. 2022;287:114911. doi:10.1016/j.jep.2021.114911
46. Abdelkader NF, Safar MM, Salem HA. Ursodeoxycholic acid ameliorates apoptotic cascade in the rotenone model of Parkinson's disease: modulation of mitochondrial perturbations. *Mol Neurobiol*. 2016;53(2):810–817. doi:10.1007/s12035-014-9043-8
47. Lu X, Yang RR, Zhang JL, et al. Tauroursodeoxycholic acid produces antidepressant-like effects in a chronic unpredictable stress model of depression via attenuation of neuroinflammation, oxido-nitrosative stress, and endoplasmic reticulum stress. *Fundament Clinic Pharmacol*. 2018;32(4):363–377. doi:10.1111/fcp.12367
48. Werner FM, Coveñas R. Classical neurotransmitters and neuropeptides involved in generalized epilepsy in a multi-neurotransmitter system: how to improve the antiepileptic effect? *Epilepsy Behav*. 2017;71(Pt B):124–129. doi:10.1016/j.yebeh.2015.01.038
49. Asisipo M, Gwladys NT, Musa MV. Effect of a novel prolonged febrile seizure model on GABA associated ion channels. *Metab Brain Dis*. 2020;35(3):441–449. doi:10.1007/s11011-019-00492-3
50. Westfall S, Lomis N, Kahouli I, Dia SY, Singh SP, Prakash S. Microbiome, probiotics and neurodegenerative diseases: deciphering the gut brain axis. *Cell Mol Life Sci*. 2017;74(20):3769–3787. doi:10.1007/s00018-017-2550-9
51. Gong X, Cai Q, Liu X, et al. Gut flora and metabolism are altered in epilepsy and partially restored after ketogenic diets. *Microb Pathogenesis*. 2021;155:104899. doi:10.1016/j.micpath.2021.104899
52. Xing C, Huang X, Wang D, et al. Roles of bile acids signaling in neuromodulation under physiological and pathological conditions. *Cell Biosci*. 2023;13:106. doi:10.1186/s13578-023-01053-z
53. Derrien M, Alvarez AS, de Vos WM. The gut microbiota in the first decade of life. *Trends Microbiol*. 2019;27(12):997–1010. doi:10.1016/j.tim.2019.08.001
54. Dinan TG, Stilling RM, Stanton C, Cryan JF. Collective unconscious: how gut microbes shape human behavior. *J Psychiatr Res*. 2015;63:1–9. doi:10.1016/j.jpsychires.2015.02.021
55. Lyte M. Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. *BioEssays*. 2011;33(8):574–581.
56. Wang Y, Yu X, Liu F, et al. Respiratory microbiota imbalance in children with Mycoplasma pneumoniae pneumonia. *Emerg Microbes Infect*. 2023;12(1):2202272. doi:10.1080/22221751.2023.2202272
57. Afroz KF, Manchia M. Gut microbiome and psychiatric disorders. *BMC Psychiatry*. 2023;23(1):488. doi:10.1186/s12888-023-05003-4