Materials and Methods

Chemicals

Alcohol was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Scutellarin (purity 98%) was purchased from Jiuzhi Chemical Co., Ltd. (Shanghai, China). Biochemical kits for analyzing superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) were obtained from Jiancheng Bio Engineering Institute (Nanjing, China). TRIzol was purchased from Vazyme Biological Co., Ltd. (Nanjing, China). The cDNA synthesis kit and genome removal reagent was purchased Monad Biotechnology Co., Ltd. (Wuhan, China). All primer sets were synthesized by General Biological Co., Ltd. (Anhui, China).

Animals

SPF Balb/c male mice (6 weeks of age, 18–22 g weight) were purchased from Pizhou Oriental Breed Co., Ltd. (Xuzhou, China). The laboratory mice are used in accordance with the guidelines for the care and use of laboratory animals and all animal experiments were approved by the Jiangsu Ocean University Animal Ethics Committee (Permission number: 2020220670). The experimental mice were placed under standard laboratory conditions, room temperature 25 °C, (50±10)% humidity control, free access to food and water, and natural light and dark alternately placed for 12 h. Before the formal experiment, without any treatment, the animals were kept for 2 weeks and allowed to adapt to the laboratory environment.

Experimental design

The mice were randomly divided into 5 groups with 6 mice in each group, including the negative control group, alcohol group, scutellarin treatment groups with low/middle/high doses {(10 mg, 25 mg, 50 mg/kg BW+50% ethanol, 12 mL/kg BW)}. Equal volume of distilled water or scutellarin were injected intraperitoneally (i.p.) once daily for 3 days and alcohol were administered by oral gavage after 12 h since the last injection. Mice were fasted for 12 h after ethanol treatment and then euthanized for further experiments.

Histological analysis

The fresh mouse brain tissues were washed with PBS, soaked in 4% paraformaldehyde for more than 24 h, dehydrated by ethanol, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E), and finally examined microscopically.

Biochemical indicators measurement

The mice were euthanized by spinal dislocation, and the brain tissue was immediately taken out. The fresh brain tissues were grinded in physiological saline at a ratio of 1:10 (w/v) with glass grinder, and the homogenates were centrifuged at 4000*g* for 15 min. The collected supernatants were directly used for the determination of the enzyme activity levels of SOD, CAT, MDA, iNOS, and the remaining tissues were stored at −80℃ for subsequent experiments.

Quantitative real-time PCR assay

A total of 20-50 mg brain tissues was grinded in 1 mL of TRIzol with an electric homogenizer on ice, extracted with chloroform, precipitated with alcohol, and dissolved in RNA-free water to obtain the total mRNA. The genomic DNA contamination was wiped off by DNase digestion, and cDNA was synthesized by RT-PCR synthesis kit. qPCR assay was carried out on a Stepone Plus machine (ThermoFisher, USA). Mouse β-actin was selected as an internal control to evaluate the mRNA levels by $2^{-\Delta\Delta C_T}$ method. The primers used in this study are shown in Table S1.

Statistical analysis

All data were presented as the mean±SD. Significance was analyzed by one-way ANOVA for multiple comparisons. All analysis were performed using Prism software (Graph Pad, version 8.0, San Diego, California, USA). $P<0.05$, $P<0.01$ and $P<0.001$ were considered as statistically significant, while n.s. was considered as no significant difference.

Table S1 Primer sequences for quantitative real-time PCR.

Gene name	Forward primer sequences (5'→3')	Reverse primer sequences (5'→3')
IL-1β	TGCCTTCTTGGGACTGATGC	GCAAGTGCATCATCGTTGTTC
IL-6	AGGAGAACCAAGCAACGACA	CTCTGCTTGTGAGGTGCTGA
TNF-α	GACGTGGAACTGGCAGAAGA	GGCTACAGGCTTGTCACTCG
β-actin	GCCATGTACGTAGCCATCCA	ACGCACGATTTCCCTCTCAG