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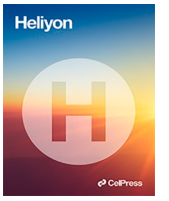

Research article

Effect of Shenqi Jieyu formula on inflammatory response pathway in hippocampus of postpartum depression rats

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ABSTRACT

Aim: To investigate whether SJF functions in similar manner as the key substance in the inflammatory process, soluble epoxide hydrolase (sEH) inhibitor, to inhibit the arachidonic acid metabolic pathway and nuclear factor kappa-B(NF-κB) signal path in the hippocampi of postpartum depression rats.

Methods: The rats were subcutaneous injected estradiol benzoate and progesterone to build PPD rat model. SJF, paroxetine hydrochloride and sEH inhibitor (AUDA) were used to treat PPD rats for 3 weeks. Then the morphological changes of hippocampi and various proteins were observed after that behavioral test were conducted in all 36 SD rats in six group: SJF, paroxetine, AUDA, PPD, sham and normal group.

Results: Weight, results of sucrose preference, upright times, total and center squares crossing decreased significantly ($P < 0.01$), whereas immobility time increased ($P < 0.01$). Results above were reversed in animals that in the SJF, paroxetine and AUDA groups. Hippocampal neurons in PPD rats partially degenerated with narrowed nuclei, increased autophagy and mitochondria bound to lysosomes were visible while the autophagy of hippocampal neurons in the paroxetine and AUDA group decreased, with a small amount of lysosomes. sEH, COX-2, 5-LOX, TNF-α, IL-1, IL-6, NF-κB p65, and Cor increased in hippocampi of PPD rats while EETs and 5-HT decreased. Protein expressions of Ibal, GFAP, p-IκBα, p65, and p-p65(S536)increased in PPD animals. Those changes were reversed by SJF, paroxetine and AUDA. Gene expressions of TNF-α, IL-1β, IL-6, 5-LOX, COX-2 and p65 increased in PPD rats and the changes of expression in these genes were reversed by paroxetine and AUDA. SJF reversed the gene expression changes of COX-2, TNF-α, and IL-1β.

Conclusion: SJF may have an analogous effect as sEH inhibitor to relieve depressive symptoms by suppressing inflammatory signaling pathways in hippocampi of PPD rats, which involves AA metabolic pathway and NF-κB signal pathway.

1. Introduction

Postpartum depression (PPD), as a global psychiatric disorder in the puerperium, seriously endangers the women, their babies and

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the society. PPD is likely caused by the interaction of multiple genetic and external factors [1] and its pathomechanism is complex and remains unclear.

An increasing number of investigations are showing that inflammatory activation appears to be associated with the onset of depression and involves the arachidonic acid (AA) cascade [2–4]. Inhibition of the expression of 5-LOX, one of the three metabolic pathways of AA, can attenuate depressive symptoms and restore the impaired memory and learning [5]. Soluble epoxide hydrolase (sEH) is a core protein in AA metabolic pathway to promote inflammatory response. Epoxyeicosatrienoic acids (EETs) is an important substance to prevent inflammatory response, which were hydrolyzed by sEH into dihydroxyeicosatrienoic acid (DHET). sEH inhibitors can reduce inflammatory reaction by raising EETs, thus to rapidly ameliorate depressive state in mice [6]..

The NF-κB pathway is closely associated with the pathogenesis of depression [7,8]. It was activated in depressed mice' hippocampi with the phosphorylation of NF-κB and overexpression of inflammatory cytokines [9]. Extracts of compounds in Chinese herbs have been found to not only alleviate depression-like behavior in mice, but also to reduce the activity of NF-κB [7,10,11]. We conjecture that overexpression of AA/NF-κB pathway can increase the inflammatory reaction and lead to depression.

The traditional Chinese herbal prescription, Shenqi Jieyu Formula (SJF), has long been applied to effectively treat women with PPD [12]. Our previous randomized, controlled clinical trial showed that SJF significantly improved depressive symptoms and quality of life in women diagnosed with PPD [12]. SJF has been shown to affect multiple cellular targets that cause depression, including hypothalamic–pituitary–adrenal (HPA) activation, Hypothalamic-pituitary-gonadal(HPG) activation and neurodegenerative processes [13]. Our recent studies found that SJF can balance the immune response in the brain [14–16] especially inhibiting the expression of IL-1 and IL-6 in the hippocampus of PPD rats [17]. Considering the key role of sEH in the inflammatory reaction process, we hypothesize that SJF acts as an inhibitor of sEH.

Therefore we speculate SJF inhibits the activity of sEH, increases the content of EETs, down-regulates the NF-κB pathway, thus reducing expression of inflammatory cytokines and ultimately inhibiting HPA axis dysfunction and inducing an antidepressant effect.

This study investigated whether SJF acts in similar manner as sEH inhibitor in regulating the AA/NF-κB pathway and explored its mechanism of suppressing the inflammatory response of postpartum depression-like behavior in rats.

2. Methods

2.1. Animals and interventional drugs

Two months old female SD rats (SPF, No. scxk2017-0005 of Shanghai Slake Animal Co., Ltd.) were raised in standard environments with temperature range from 20 °C to 22 °C, humidity range from 60 % to 70 % and 12:12 light-dark cycle. Total 36 rats were housed into 12 cages (3 per cage) with unrestricted standard food and water. Animal humanitarianism were always adhered according to relevant animal experiment standard [18].

Paroxetine hydrochloride (cat. 22005122) were come from Beijing Fuyuan Co.LTD. AUDA[12-(3-adamantan-1-yl-ureido)-dodecanoic acid] (cat.HY-108570), as the sEH inhibitor, was bought from MedChemexpress (Shanghai, China).

2.2. Animal model preparation and intervention

Total 24 rats were randomly selected to be arranged bilateral ovariectomy. While other 6 rats were randomly selected to be removed some adipose tissue from the abdominal cavity, and were marked as sham group and another 6 rats had not any operate marked as normal group. All animals were not treated with antibiotics.

After surgery, the 24 castrated rats were subjected to continuous vaginal smears for 5 days to confirm that the castration surgery were successful. Subsequently, they were modeled for postpartum depression using the classic method of simulating pregnancy with hormone injection and acute withdrawal [19]. From day 1 to day 16, rats were subcutaneous injected with estradiol benzoate (2.5 μg/d, no.190504, Quanyu Biotechnology (Shanghai) Co., Ltd) and progesterone (4 mg/d, no.190509, Quanyu Biotechnology (Shanghai) Co., Ltd), and then only estrogen (50 μg/d). On day 24, the estrogen injection was stopped to simulate hormone withdrawal status after childbirth. Served as the control one for the PPD group, the sham group were only subcutaneously injected with glycerol (0.1 mL/day) from day 1 to day 23.

The 24 modeled rats were evaluated by OFT open field experiments to make sure of success. And 18 in the 24 rats were unorderly selected into SJF group (n = 6), AUDA group(n = 6) and paroxetine group(n = 6) and then were respectively treated with SJF by gavage (1 mL/100 g with 1.25 g/mL dissolved in water), AUDA by intraperitoneal injection (7 mg/kg with 2 mg/mL dissolved in 10 % DMSO + 90 % corn oil), and paroxetine hydrochloride by gavage (1 mL/100 g with 1 mg/mL dissolved in water) for 3 weeks. The left 6 in the 24 rats with depressive symptoms were only given 2 mL water by gavage marked as ones in PPD model group. Rats in sham, and normal groups were also given 2 mL water by gavage. …

Based on established standards for animal studies using Chinese herbal medicinals [20], the dosage of herbal medicines for rats is 7 times that of humans, and the daily dosage of herbal medicine per 100 g (0.1 kg) of rat is 107 g/human/day ÷ 60 kg × 7 × 0.1 kg = 1.25 g. Our previous research used a high dose (2.5 g/L), medium dose (1.25 g/L), and a low dose (0.5 g/L) in pre-experiments to measure the impact of SJF on depressive-like behavior in rats. It was found that the effect of medium-dose was obviously better compared to those of the high and low doses. Therefore, we selected this dose based on our previous research [16,17].

2.3. Behavioral assessments

The immobility time in forced swim test (FST), upright times, total and center squares crossing in open field test (OFT) and sucrose preference swere conducted after treatment finished. Specific procedures of these assessments were described in our previous article [17]. After the completion of behavioral testing, all animals were sacrificed by decapitation and all rats' hippocampi were collected for next assessment.

2.4. IHC experiment to detect p65, p-p65 P-IκBα, and GFAP in the hippocampus

Three hippocampi in each group were used to be observed. Procedures for immunohistochemical staining (IHC) including fixing, embedding, slicing, dewaxing, grilling, dewaxing, dehydration of anhydrous ethanol, antigen repairing with high-pressure, and blocking with 5 % BSA. The slides were cultured for 12 h with the NF-κB p65(1: 50, no. AF5006, Affinity Biosciences, Jiangsu, China), phospho-NF-κB p65(1: 50, no. AF2006), phospho-IKBα (1: 50, no. AF2002, all from Affinity Biosciences, Jiangsu, China) , Ibal (diluted 1: 50, no. DF7552, Affinity Biosciences, Jiangsu, China) , and glial fibrillary acidic protein (GFAP) antibody (1: 50, no. AF6166, Affinity Biosciences, Jiangsu, China) in a water bath at 4 °C.After incubated with goat anti-rabbit IgG) stained with 3,3′-diaminobenzidine (DAB),re-dyed by hematoxylin, finally sealed by neutral gum, three hippocampi sections with two visual fields were randomly selected for observation. Specific procedures of IHC were described in our previous article [21].

2.5. ELISA detection of proteins

The tissue protein and nucleoprotein of hippocampus were extracted according to instructions. The ELISA kits were come from Jiangsu Meimian Industrial Co., Ltd(Nanjing, China) and their detailed information can be seen in Table 2. Specific procedures of Elisa were described in our previous article [21].

2.6. Electron microscopy

Hippocampus samples less than 1 mm thick were fixed i for 4 h, picked from the solution with a clean toothpick and rinsed thrice for 15 min each time with 0.1 M pH 7.0 PBS. Then the hippocampus samples were fixed in 1 % osmic acid solution for 2 h and rinsed again as previously. Next, they were dehyrated for 15 min with gradient concentration ethanol solution (50 %, 70 %, 80 %, 90 %, and 95 %), subsequently treated with pure ethanol for 20 min and then with pure acetone for 20 min. The samples were immersed in a mixture of epoxy resin-acetone (V/V = 1/1) for 1 h, then the mixture (V/V = 3/1) for 3 h, and pure epoxy resin 12 h for penetration of the samples. The samples were transferred to dry centrifuge tubes for overnight permeation at room temperature. The permeated samples were removed by a clean toothpick and transferred to a 0.5 mL dry EP tube (prefilled with 300 μL of embedding agent) and then incubated overnight at 70 °C. Next, the samples were trimmed and cut into 50–70 nm sections using an ultramicrotome (EM UC7, Leica Microsystems, Wetzler, Germany) then stained with 100 μL saturated solution of 50 % ethanol containing uranium acetate for 30 min, washed with double distilled water, and finally stained with 100 μL of lead citrate for 15 min. The samples were photographed via a TEM (H-7650; Hitachi, Japan).

2.7. Western blot analysis

The 50 mg of hippocampal tissue were crushed by grinding rod, added into 1 mL mixture of RIPA(cat. P0013D, Beyotime Biotechnology, shanghai, China) and protease inhibitor(cat. 60237, Cowin Biotech, Jiangsu, China) (V/V = 99:1) and homogenized in a tissue homogenizer. After lyse in ice for 15–30 min, the homogenate were centrifuged at 4 °C for 5 min with 12000 g and the supernatant fluid were obtained. Measure the concentration using a BCA reagent kit. (cat.pc0020; Solarbio Life Sciences, Beijing,

Table 1
The composition of Shenqi Jieyu Formula (SJF).

latin name	Common names	Weight (g)
Astragalus membranaceus (Fisch.) Bunge	astragalus root	20 g
Codonopsis pilosula (Franch.) Nannf.	codonopsis root	12 g
Ziziphus jujuba var. *spinosa (Bunge) Hu ex H.F.Chow*	fried sour jujube seed	15 g
Cornus officinalis Sieb. et Zucc.	cornus fruit	15 g
Curcuma zedoaria (Christm.) Roscoe	curcuma rhizome	15 g
Citrus reticulata Blanco	aged tangerine peel	10 g
Citrus medica L.	citron fruit	10 g
Angelica sinensis (Oliv.) Diels	tangkuei	10 g

Shenqi Jieyu Formula (SJF, Table 1) were sourced from *China Resources Sanjiu Corporation*(Shenzhen, China) and authenticated by First Affiliated Hospital of Zhejiang University of Chinese Medicine. The granules are manufactured through a series of modern pharmaceutical processes, including extraction, concentration, separation, drying, granulation, and packaging [22]. The concentration of SJF was 1.25 g/mL with a dose of SJF (107 g of crude herbs) dissolved in 85.6 mL water. The quality of granules met the national drug standards for traditional Chinese medicine formula granules issued by the *State Food and Drug Administration.*

Table 2
The list of ELISA kits.

Testing index	ELISA kits	Lot No.
NF-κB p65	Rat nuclear transcription factor p65 ELISA kit	MM-0699R1
TNF-α	Rat TNF-α ELISA kit	MM-0180R1
IL-1 β	Rat IL-1 β ELISA kit	MM-0047R1
IL-6	Rat IL-6 ELISA kit	MM-0190R1
sEH	Rat sEH ELISA kit	MM-70265R1
EET	Rat eicosatrienoicacid (EET) ELISA kit	MM-70218R1
5-LOX	Rat 5-lipoxygenase (5-LOX) ELISA kit	MM-70267R1
COX-2	Rat PTGS2/COX-2 ELISA kit	MM-70227R1
5-HT	Rat serotonin (5-HT) ELISA kit	MM-0442RI
Cor	Rat cortisol ELISA Kit	MM-0574R1

China))

Proteins of each group were added to the electrophoresis gel with constant 120 V electrophoresis and transferred to a PVDF membrane (no. 10600023; GE Healthcare Life Sciences, Pittsburgh, PA) at a steady 200 mA for 120 min using a Tanon-VE 186 Electrophoresis Tank (Koram Biotech, Seoul, Korea). The PVDF membranes were washed three triple by TBST for 10 min and then shaken for 2 h immersing in TBST with 5 % defatted milk in an incubation chamber. Then the PVDF membranes were incubated with antibodies (1:2000 anti-NF-kB p65 [ab16502, no. 6R3206623-1; Abcam, MA, USA], 1:1000 anti-NF-kB p65 (phospho S536) [ab76302, 6R3324524-7; Abcam], 1:1000 IκBα (L35A5) mouse mAb [no. 4814s and no. 15; Cell Signaling Technology, Danvers, MA, USA]) and β-actin (8H10D10) mouse mAb, (1:1000; no. 3700s; Cell Signaling) for 12 hat 4 °C. After washed by PBS, the PVDF were cultured with the HRP-labeled secondary antibody for 1 h and detected by High-quality ECL Chemiluminescence Development Kit. Immunoreactive bands were scanned using a chemiluminescence apparatus (610020-9Q; Clinx Science Instruments, Shanghai, China) and analyzed with Chemi capture software. The density of each protein band was normalized against its β-actin. Each indicator has 3 hippocampal protein bands analyzed and each analysis was repeated three times.

2.8. The RT-PCR experiment

Three weeks after initiation of SJF intervention, the RNA was extracted from all rats'hippocampus using Trizol reagent (no. B511311; Sangon Bioech, Shanghai, China). RT-PCR was set using a Dynamo Flash SYBR Green qPCR kit (no. CW2601; Cowin Biotech, Jiangsu, China) based on the corresponding instructions. The conditions: 42 °C for 15min, 85 °C for 5min. RT-PCR was performed with the Quick Reverse Transcription Kit (no. CW2569; Cowin Biotech). Forward and reverse primers were synthesized (Table 3).

The cycling conditions were set as follows: initial denaturation at 95 °C for 10 min, 40 cycles at 95 °C for 15s, and annealing at 60 °C for 1 min. The melting-curve analyses was used to validate specificity of the PCR products. Each indicator has three hippocampus specimens for RT-PCR analyzed and each analysis was repeated three times. Comparative CT was applied to obtain The quantitative data of gene expressions was shown by Comparative CT. The expression of each gene was normalized against its β-actin.

2.9. Statistical treatment

Data were analyzed by SPSS 19.0 statistical software. One-way ANOVA was used to measure whether there exists a statistically difference among the six independent groups, and SNK method was applied to compare the difference between two groups. All data were described as mean ± standard deviation $\bar{}(\chi \pm s)$, $P < 0.05$ was considered statistically significant.

2.10. UPLC-MS/MS of SJF

Preparation of SJF: One dose of SJF granules (10 g) was dissolved in 150 mL water, ultrasonically extracted for 30 min, subsequently centrifuged at 12000 rpm for 20 min To retain The supernatant was retained.

UPLC-MS/MS operate: The LC-MS system include the Thermo Fisher Scientific Vanquish UHPLC and the Q Exactive mass

Table 3
Primers applied in the current study.

Gene	Forward Primer	Reverse Primer
TNF-α	ATCCGAGATGTGGAACTGGC	CGATCACCCCGAAGTTCAGT
IL-1β	CCTTGTCGAGAATGGGCAGT	CAGGGAGGGAAACACACGTT
IL-6	AAAGTCAACTCCATCTGCCCT	TGTGGGTGGTATCCTCTGTG
5-LOX	CGAGAAGCGCAAATACAGGC	CTCAGGACAATCTCGCCCTC;
COX-2	ATCAGAACCGCATTGCCTCT	GCCAGCAATCTGTCTGGTGA
p65	CACCCCTTTTGTGTTGCAGG,	CCAGGGCAAAGTCTTCCGAT
β-actin	ATGATTCTACCCACGGCAAG	CTGGAAGATGGTGATGGGTT.

spectrometer. The Waters ACQUITY Premier HSS T3 Column (1.8 μm, 2.1 × 100 mm) was applied. The mobile phase A was 0.1 % (V/V) formic acid–water and the mobile phase B was 0.1 % (V/V) formic acid-acetonitrile-isopropanol. The flow rate was 0.3 mL/min at 40 °C. The injection volume was 2 μL. And the elution procedure: 0.0–2.0 min A/B (90:10 V/V), 6.0–15.0 min A/B (40:60 V/V), 15.1–17.0 min water/acetonitrile (90:10 V/V).

Mass spectrometry conditions: ESI source was negative and positive ion detection mode; sheath gas volume flow was 40 arb; spray voltage was 3000 V/-2800 V at 350 °C. The tube temperature was 320 °C; primary and secondary scanning range were *m/z* 70–1050 Da and *m/z* 200–2000 Da, respectively.

Data processing: The Progenesis QI software (Milford, USA) was applied to treat the experimental data. Secondary mass spectrometric fragments were matched with mz Cloud database (http://www.hmdb.ca/, https://metlin.scripps.edu) and a self-built traditional Chinese medicine component database.

Numerous common compounds in the SJF were selected , whose data can been saw in Table 4 and Fig. 1. All compound information can be found in the supplementary materials.

3. Results

3.1. Animal weights and behaviors

There were no differences between the normal and sham groups in the weights, results of sucrose preference, upright times, total and center squares crossing and immobility time. In the PPD group, weights, results of sucrose preference, upright times, total and center squares crossing diminished significantly ($P < 0.01$), whereas immobility time increased ($P < 0.01$). Compared to the PPD group, weights, results of sucrose preference, upright times, total and center squares crossing in the SJF, AUDA, and paroxetine groups enhanced ($P < 0.01$), while the immobility time diminished ($P < 0.01$). These results above in SJF, AUDA, and paroxetine groups had no difference($P > 0.05$). [Fig. 2 (A-F)].

3.2. Morphologic changes in the hippocampus

Electron microscopy revealed that the hippocampal neurons of the normal and sham rats had normal morphology with integrated organelles such as nuclei, mitochondria, endoplasmic reticulum, and ribosomes. In the PPD group, the hippocampal neurons degenerated partially, the nuclei narrowed, autophagy increased, and mitochondria bound to lysosomes were visible. Compared to

Table 4
Common compounds of SJF detected by UPLC-MS/MS.

Mode	Metabolite	Formula	Fragmentation Score	Retention time(min)	Adducts	*m/z*	Mass Error (ppm)	relative content
pos	5-Hydroxy-3,6,7,8,3′,4′-Hexamethoxyflavone	C21H22O9	85.1	8.354	M + H, M + Na	419.13315	−1.215996	2570032.23
pos	Tetramethylscutellarein	C19H18O6	90.9	7.957	M + H	343.117358	−0.7502639	1017033.44
pos	Isoastragaloside I	C45H72O16	67.1	8.302	M + H–2H2O, M + Na, M + H–H2O	851.478467	−0.3236356	792029.17
pos	Gardenin A	C21H22O9	75.5	8.953	M + H	419.133278	−0.9106871	671758.608
pos	Astragaloside IV	C41H68O14	84.7	6.455	M + H	785.466843	−1.7091235	368922.462
pos	Alisol B	C30H48O4	69.7	8.660	M + H	473.362074	−0.979609	98629.0109
pos	Glycitin	C22H22O10	95.8	6.429	M + H	447.128101	−1.05851	85517.8956
pos	3,4,5-Trimethoxycinnamic acid	C12H14O5	72.3	6.403	M + H–H2O, M + H	239.091102	−1.2519727	82145.4031
pos	Pilocarpic acid	C11H18N2O3	55.8	4.525	M + H	227.138811	−0.9186859	18752.1993
pos	R-(−)-Mandelic acid	C8H8O3	52.1	3.588	M + H	153.05456	−0.3970034	17826.1477
pos	asarylaldehyde	C10H12O4	77.6	6.482	M + H	197.080618	−1.106761	14628.3544
pos	R- [6]-Gingerol	C17H26O4	78.4	7.918	M + H–H2O	277.179463	−1.2161152	10336.5642
pos	α-Asarone	C12H16O3	92.4	8.470	M + H	209.11708	−0.67005139	4713.512986
neg	Citric acid	C6H8O7	93.2	1.033	M-H	191.020081	1.84976114	182603799
neg	Hesperidin	C28H34O15	91.2	5.588	M − H, M + Cl	609.188322	9.55116294	12905631.4
neg	Caffeic acid	C9H8O4	91.1	4.858	M − H, 2M − H, M + Cl	179.035513	2.94892237	3986306.64
neg	Gentisic acid	C7H6O4	86.9	3.891	M − H, 2M − H, M + Cl	153.019721	2.52462164	3166538.98
neg	Baicalin	C21H18O11	98.1	5.887	M − H, 2M-H	445.078611	2.18718936	730637.702
neg	Kaempferol-3-O-rutinoside	C27H30O15	95.2	5.374	M-H	593.153202	3.37920634	698790.729
neg	Forsythoside E	C20H30O12	87.4	4.237	M-H	461.167182	1.58353903	529427.179
neg	Geniposidic acid	C16H22O10	85.1	3.947	M-H	373.115202	3.15810477	298241.044
neg	Gallic acid	C7H6O5	87.9	1.862	M + Cl	204.991403	2.81481492	271156.692
neg	Ferulic acid	C10H10O4	87.1	7.516	M-H	193.051123	2.52975154	48334.426
neg	Quercitrin	C21H20O11	97	5.599	M-H	447.09432	2.30988926	45927.3424

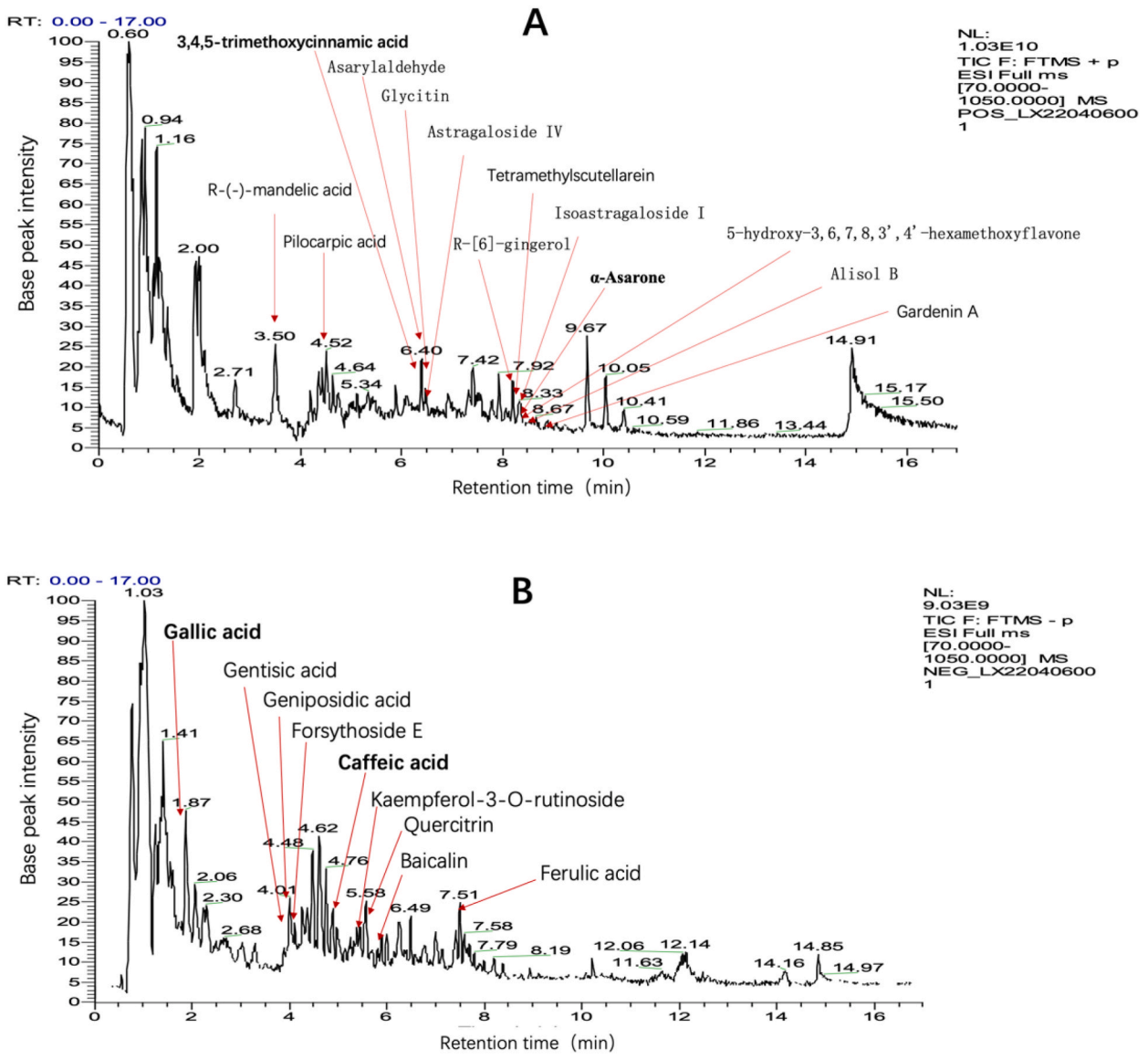

Fig. 1. Ion chromatograms of SJF in the positive (**A**) and negative (**B**) ion modes.

PPD group, the autophagy of hippocampal neurons in the AUDA, paroxetine group decreased, with a small amount of lysosomes. [Fig. 3(A-F), Fig. 4(A-F)).

3.3. Expressions of sEH, EETs, 5-LOX, COX-2, IL-1, IL-6, TNF-α, NF-κB p65, 5-HT and cor in hippocampi of each rat group

There were no differences between the normal and sham groups in sEH, EETs, COX-2, 5-LOX, TNF-α, IL-1, IL-6, NF-κB p65, 5-HT and Cor in the hippocampi of rats. In the PPD group, these proteins level enhanced ($P < .01$), whereas 5-HT and EETs diminished ($P < .01$). In the SJF, AUDA, paroxetine groups, these proteinsdiminished ($P < 0.05$), whereas 5-HT and EETs increased ($P < 0.05$) (Fig. 5A–J).

3.4. Gene expressions of p65, 5-LOX, COX-2, TNF-α, IL-1β and IL-6 in hippocampus of rats

There were no differences between the normal and sham groups in the gene expressions of TNF-α, IL-6, IL-1β, COX-2, 5-LOX and p65 in the hippocampi of rats($P > 0.05$). Gene expressions of TNF-α, IL-6, IL-1β, COX-2, 5-LOX and p65 in the PPD group enhanced obviously ($P < 0.01$). Compared to PPD group, gene level of COX-2, TNF-α, and IL-1β decreased in the SJF group and p65, 5-LOX, COX-2, TNF-α, IL-1β, IL-6 in AUDA and paroxetine groups decreased ($P < 0.05$ or $P < 0.01$) (Fig. 6A–F).

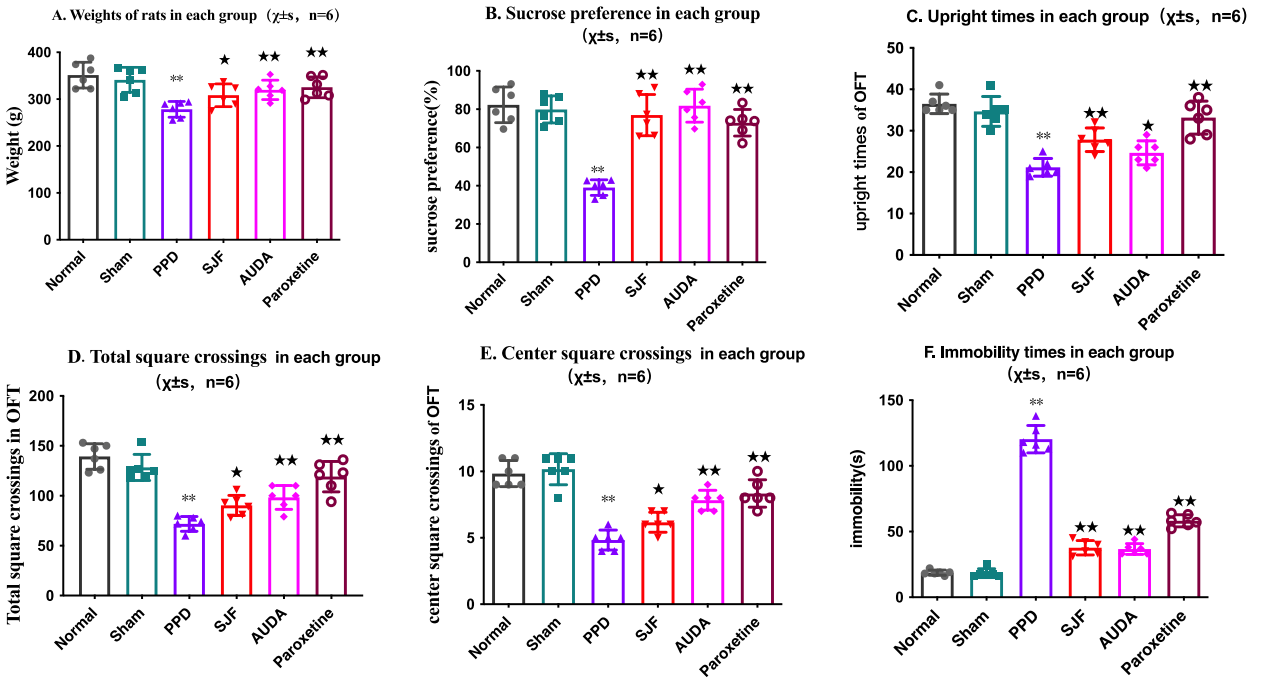

Fig. 2. Weights and behaviors of rats in each group ¯(χ ±s, n = 6). Weights (A), sucrose preference (B), total grid crossings (D), center grid crossings (E), and upright times (F) of rats decreased in PPD animals while immobility time (F) increased. These results were reversed by SJF, AUDA, and paroxetine hydrochloride. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. normal rats; $^{\star\star}P < 0.01$, $^{\star}P < 0.05$ vs PPD rats.

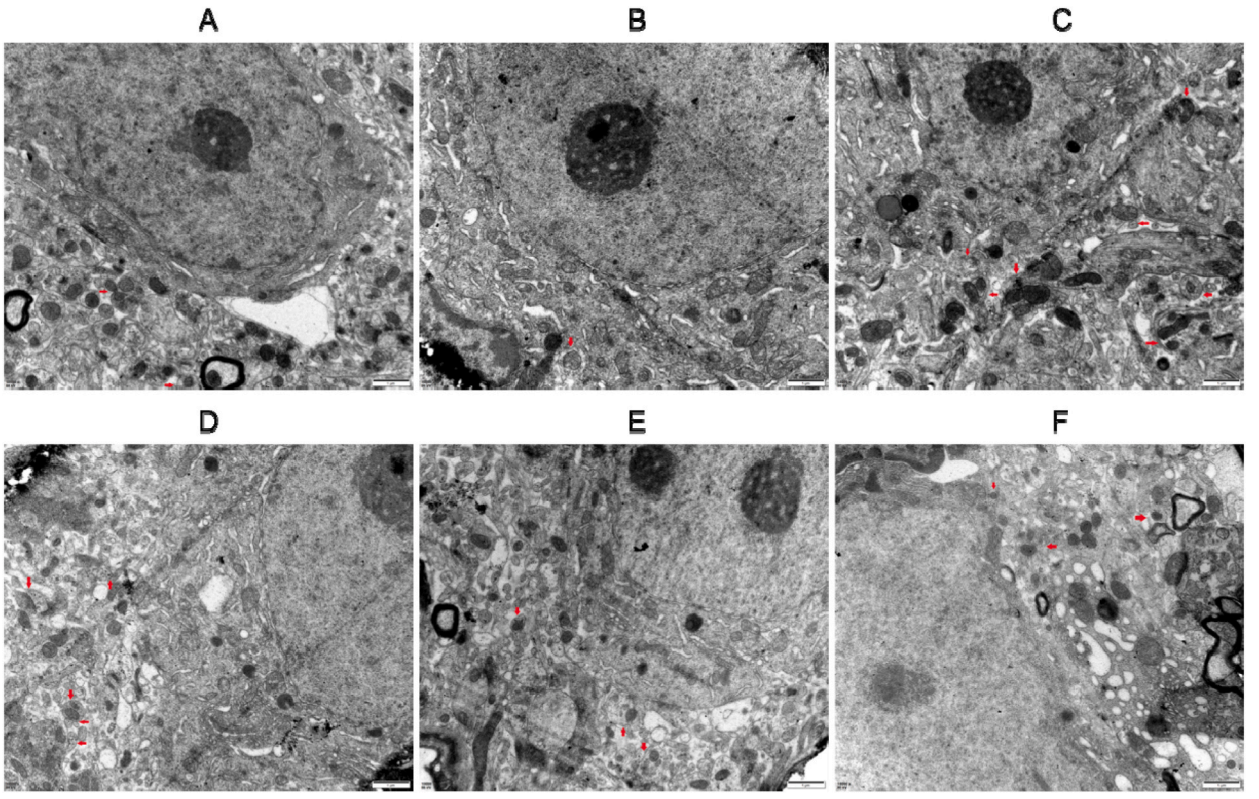

Fig. 3. Transmission electron microscopy (15000 ×) showing morphologic and ultrastructural changes in hippocampi. (A: normal; B: sham; C: PPD; D: SJF; E: AUDA; F: paroxetine. Red arrows represent autophagosomes. Scale bar = 15000 × .

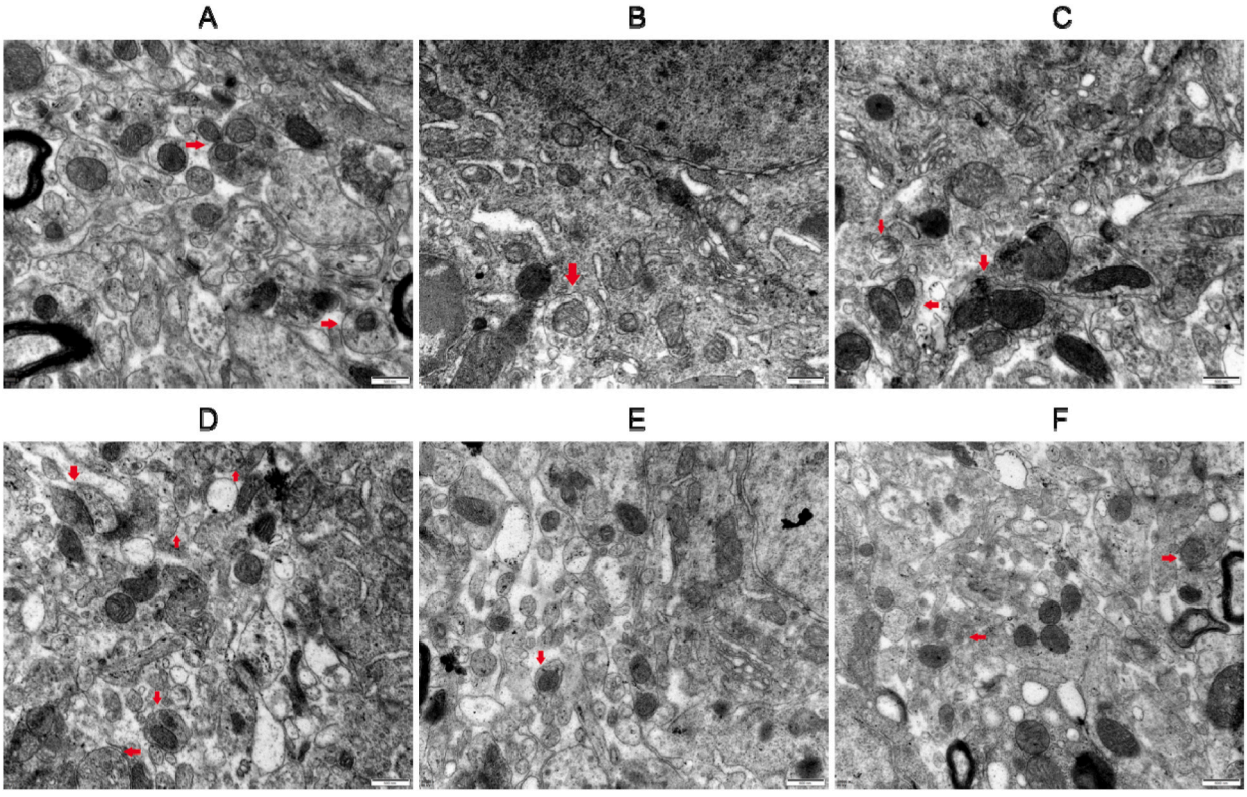

Fig. 4. Transmission electron microscopy (30000 ×) showing morphologic and ultrastructural changes in hippocampi. (A: normal; B: sham; C: PPD; D: SJF; E: AUDA; F: paroxetine. Red arrows represent autophagosomes. Scale bar = 30000 × .

3.5. Protein levels of p65, p-p65 (S536), p-IκBα in hippocampi of each rat group

There were no differences between the normal and sham groups in the protein levels of p-p65 (S536), p-IκBα, p65 in the hippocampi of rats in sham group ($P > 0.05$), whereas protein levels of p65, p-p65 (S536), p-IκBα in PPD group obviously enhanced ($P < 0.01$). Compared to PPD group, protein levels of p65, p-p65 (S536), p-IκBα in SJF, paroxetine and AUDA groups decreased ($P < 0.05$ or $P < 0.01$) (Figs. 7–10).

3.6. Expressions of GFAP and Iba1 antibody, a marker antibody of astrocytes and microglia in hippocampi of rats in each group

There were no differences between the normal and sham groups in the expressions of Iba1and GFAP antibody in hippocampi ($P > 0.05$) while expressions of Iba1and GFAP antibody in the PPD group increased and decreased in the SJF, AUDA, paroxetine groups ($P < 0.05$ or $P < 0.01$) (Figs. 11 and 12).

4. Discussion

In this study, we found PPD rats generally have declined sucrose preference, upright times, center and total squares crossings and extended immobility time. These depression-like behaviors can be attenuated by SJF, paroxetine and AUDA, suggesting that SJF and AUDA can mitigate PPD symptoms as effectively as paroxetine hydrochloride.

We found that hippocampal neurons in PPD rats partially degenerated with narrowed nuclei, increased autophagy and mitochondria bound to lysosomes were visible while the autophagy of hippocampal neurons in the paroxetine and AUDA group decreased, with small amounts of lysosomes. A previous study found atrophic changes with narrowed nuclei in hippocampus induced by chronic unpredictable mild stress [23]. Autophagy in hippocampal neurons was also found in rats with long-term depression [24]. Results of our study were consistent with those in previous studies.

In this study, we found that sEH, 5-LOX, COX-2, IL-1, IL-6, TNF-α, expressions of p-IκBα, p-p65(S536), p65, GFAP, Ibal and Cor increased in hippocampi of PPD rats while EETs and 5-HT decreased. SJF, paroxetine and AUDA reversed those changes. Shenqi Jieyu Formula (SJF) is based on the ancient Chinese herbal prescription known as Restore the Spleen Decoction (*gui pi tang*). Various studies have been conducted on the anti-depressive effects of the active chemical constituents in SJF. Gallic acid [25] and 3,4,5-trimethoxycinnamic acid [26] in SJF can ameliorate depression. Alpha-asarone acts in SJF on the hippocampal neurons to attenuate

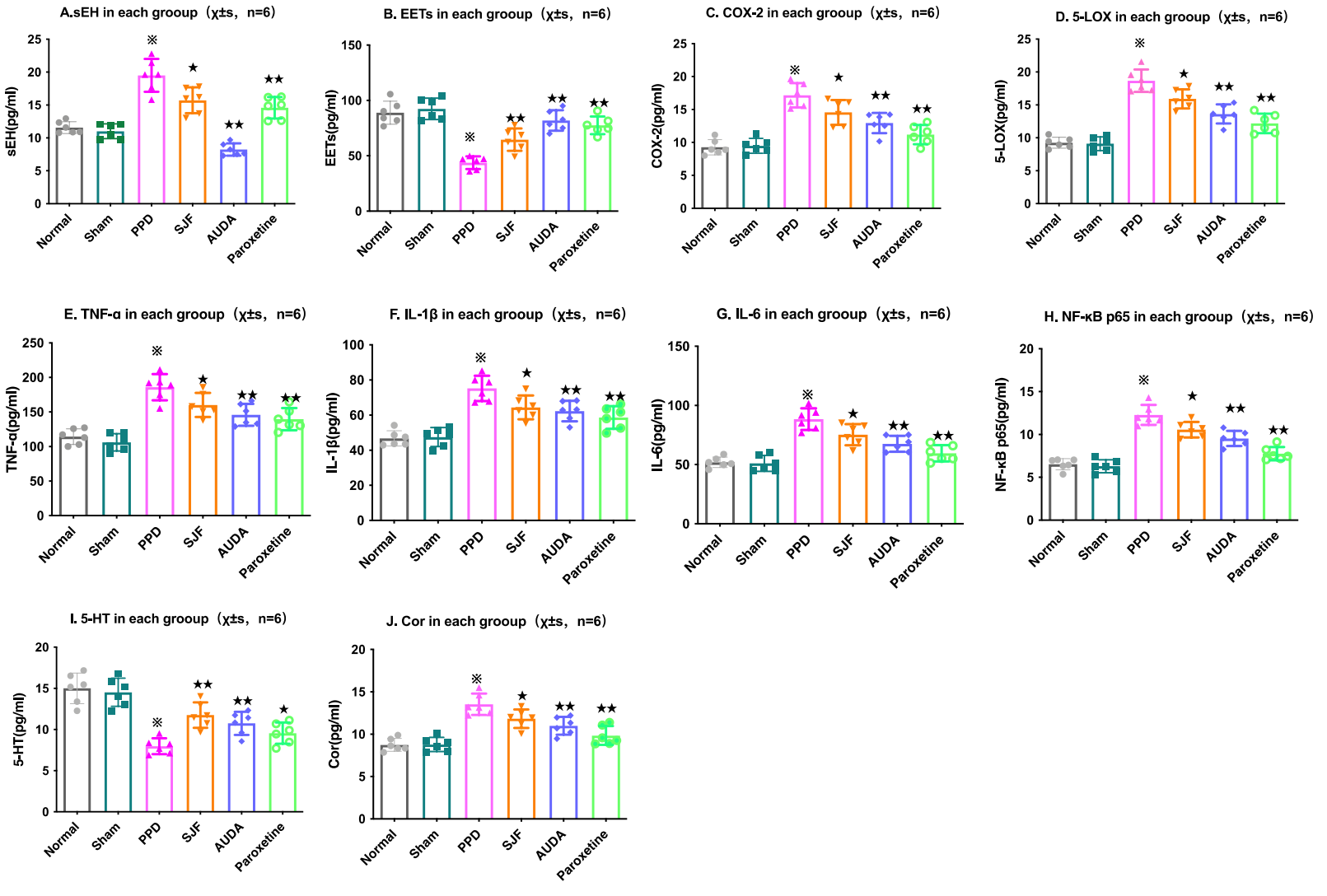

Fig. 5. Expressions of sEH(A), EETs(B), COX-2(C), 5-LOX(D), TNF-α(E), IL-1(F), IL-6(G), NF-κB p65(H), 5-HT (I) and Cor (J) $^{-}(\chi \pm s, n = 6)$ in hippocampi of rats in each group. sEH, 5-LOX, COX-2, IL-1, IL-6, TNF-α, NF-κB p65, and Cor increased in PPD animals while EETs and 5-HT diminished. The changes of these proteins were reversed by SJF, AUDA, and paroxetine hydrochloride. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. normal rats; $^{\star\star}P < 0.01$, $^{\star}P < 0.05$ vs. PPD rats.

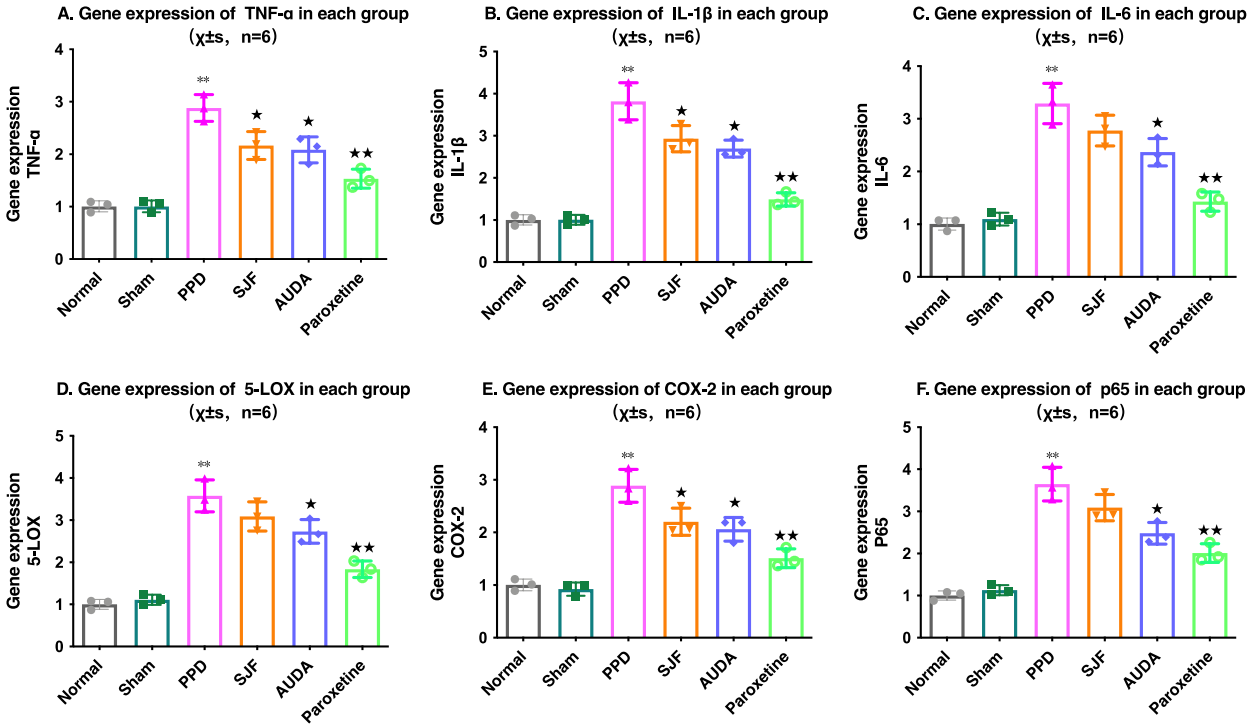

Fig. 6. Gene expressions of TNF-α(A), IL-1β(B), and IL-6(C), 5-LOX(D), COX-2(E) and p65(F) $\bar{}$(χ ±s, n = 3) in hippocampi of rats in each group. Gene expressions of TNF-α, IL-1β, IL-6, 5-LOX, COX-2 and p65 increased in PPD animals. The changes of these gene expressions were reversed by AUDA and paroxetine hydrochloride. SJF reversed the gene expression changes in COX-2, TNF-α, and IL-1β. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. normal rats; $^{\star\star}P < 0.01$, $^{\star}P < 0.05$ vs. PPD rats.

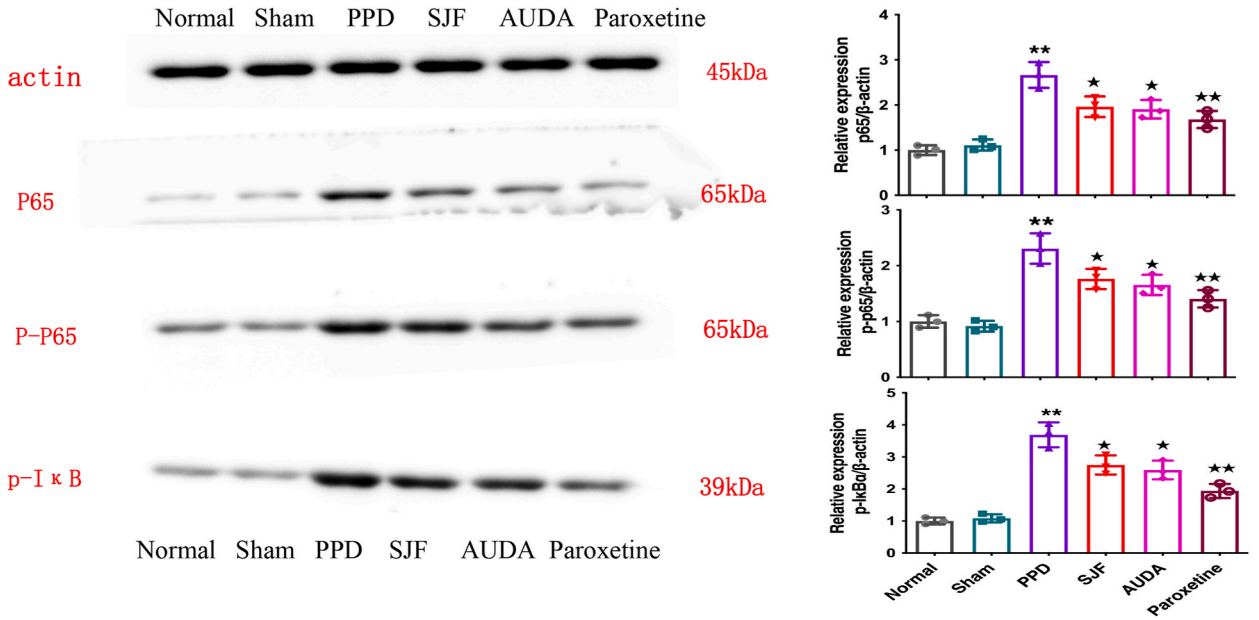

Fig. 7. Protein expressions of p65, p-p65 (S536), p-IκBα $\bar{}$(χ±s, n = 3) in hippocampi of rats in each group. Protein expressions of p65, p-p65 (S536), and p-IκBα increased in PPD animals, which reversed by SJF, AUDA, and paroxetine hydrochloride. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. normal rats; $^{\star\star}P < 0.01$, $^{\star}P < 0.05$ vs. PPD rats.

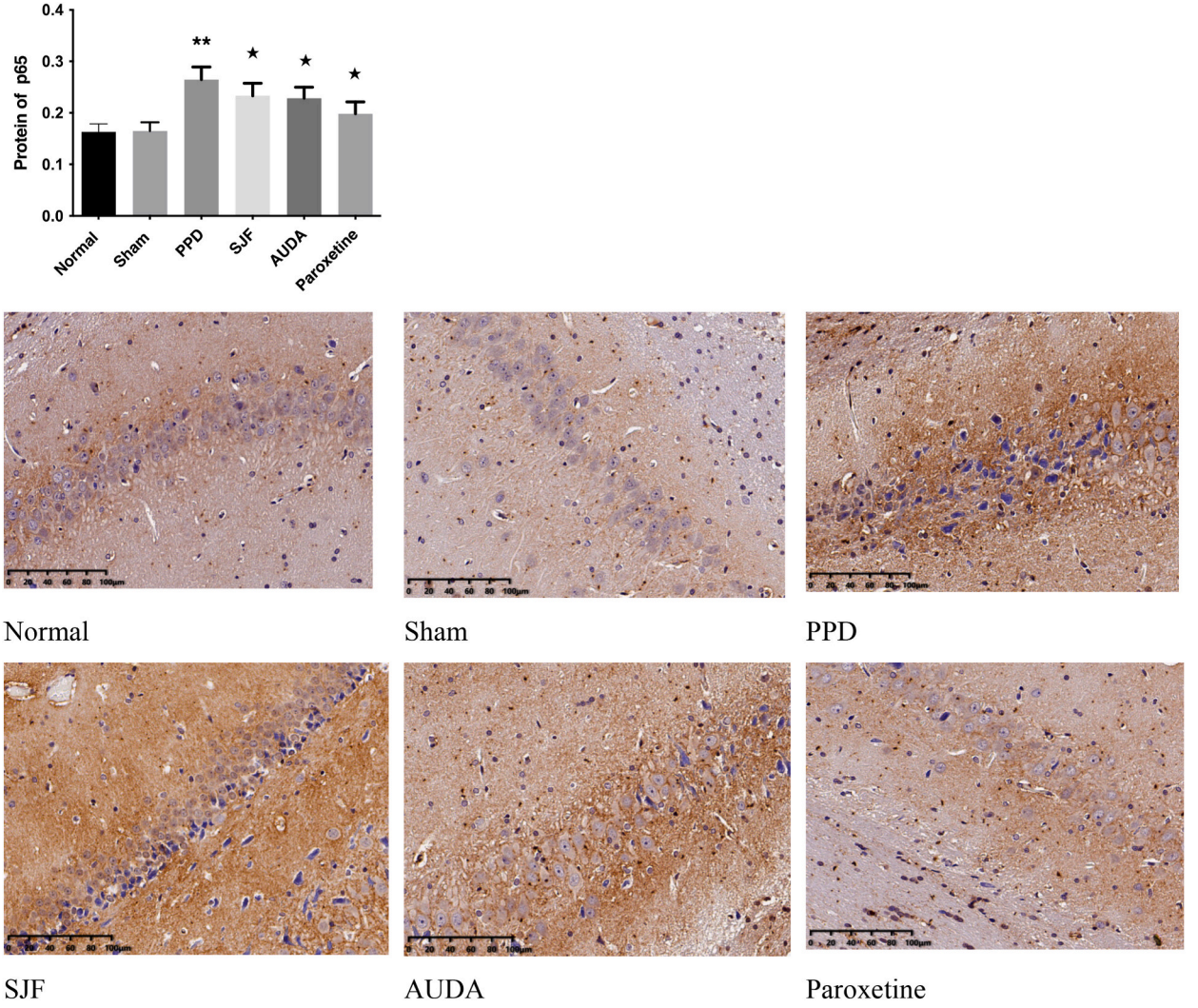

Fig. 8. Protein expressions of p65 $^{-}(\chi\pm s, n = 3)$ in hippocampi of rats in each group (200 ×). Protein expressions of p65 increased in PPD animals, which reversed by SJF, AUDA, and paroxetine hydrochloride. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. normal rats; $^{\star\star}P < 0.01$, $^{\star}P < 0.05$ vs. PPD rats. Scale bar = 100 μm.

depression-like behavior in rats [27]. Caffeic acid in SJF inhibits the decrease of Norepinephrine (NE) in rats with depressive-like behavior subjected to chronic unpredictable mild stress (CUMS) [28]. The herbs in this formula are mild, such that postpartum patients do not need to stop breastfeeding, thus it is suitable for wide clinical usage. [12];

Our previous studies have found that SJF can regulate estrogen receptor expression in the brain, as well as monoamine transmitter and hormone levels related to the HPA axis and HPG axis [13,29–34]. SJF also influences immune regulation by protecting the spleen and thymus in rats with postpartum-like depression, regulating the proportion of $CD3^{+}$, $CD4^{+}$, $CD8^{+}$, Th17/Treg T, and Th1/Th2 cells in the peripheral blood of rats with PPD-like behavior [14–16]. Furthermore, SJF has been shown to inhibit expression of IL-6 ， IL-6R, IL-1 and IL-1Rin the hippocampus of rats with PPD-like behavior [17]. In this study, we found that SJF not only prevented the expressions of cytokines TNF-α, IL-6, IL-1, but also decreased levels of COX-2, 5-LOX, sEH, p-IκBα, p65, NF-κB p65, p-p65 (S536), Ibal, GFAP, and Cor. Furthermore, SJF increased EETs and 5-HT in the hippocampi in rats with PPD-like behavior.

COX-2, 5-LOX, sEH, and EETs belong to three metabolic pathways of arachidonic acid (AA) metabolism. 5-LOX and COX-2 are mainly involved in promoting inflammation reaction and are associated with depression [35,36]. Inhibition of 5-lipoxygenase (5-LOX) expression increases sucrose consumption, shortens the latency period of the water maze test, and reduces the number of errors in the step-down test in rats subjected to ongoing stress [5]. AA can predict depression severity through mediation by serotonin transporter (5-HTT) binding potential [37]. Moreover, 5-LOX inhibitor can produce antidepressant effects based on prompting the phosphorylation of glutamatergic receptor type 1 and regulating synaptic plasticity in the depression condition [38]. In our previous study, we found COX-2, 5-LOX, sEH increased and EET decreased in the prefrontal lobe of PPD rats [21] and we focused on the sEH inhibitor–AUDA' effect on these inflammatory indicator in prefrontal lobe of PPD rats [21]. While in this study, we further found the same

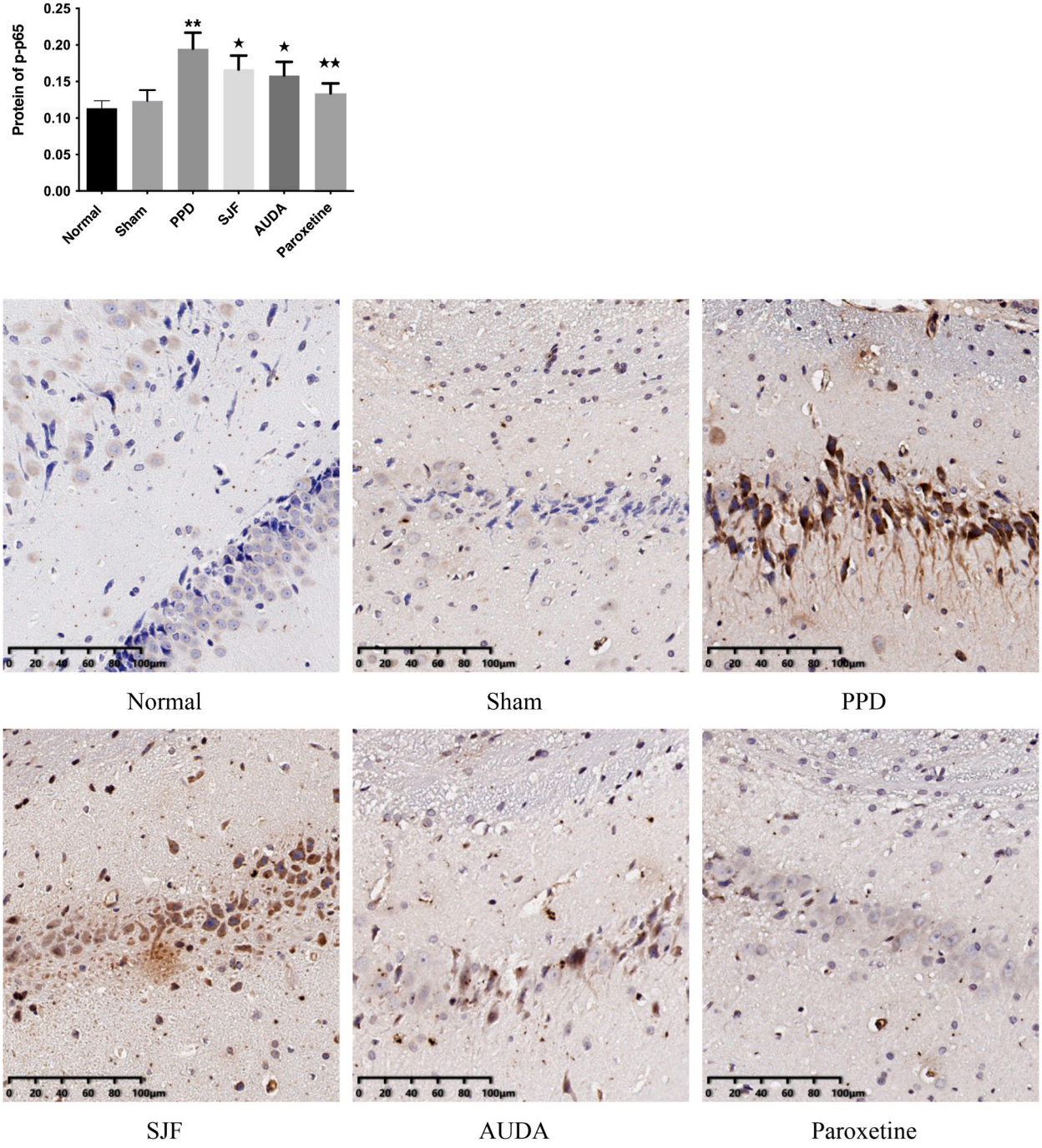

Fig. 9. Protein expression of p-p65 $\bar{}$(χ±s, n = 3) in hippocampi of rats in each group (200 ×). Protein expression of p-p65 increased in PPD animals. The changes were reversed by SJF, AUDA, and paroxetine hydrochloride. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. normal rats; $^{★★}P < 0.01$, $^{★}P < 0.05$ vs. PPD rats. Scale bar = 100 μm.

effect of SJF as AUDA in the hippocampi of PPD rats.

Epoxyeicosatrienoic acid (EETs), as the metabolite of the P450 pathway, can inhibit inflammation through many ways. EETs in the astrocytes of mouse' brain also can help mouse to adapted to stress [39]. In young adult mice, EET can reduce depression-like behavior caused by defective promoter IV with BDNF protein induction by EET being the greatest in the hippocampus and frontal cortex [40]. However, sEH mainly acts on EETs and hydrolyzes them to dihydroxyeicosatrienoic acid, thus reducing anti-inflammatory effect of EETs, and thus attenuating depression-like behavior in rodents [41,42]. Our previous study had found that sEH inhibitor(AUDA) can improve postpartum depression symptoms in PPD rats [21], which is consistent with previous research findings [42,43]. Moreover, the

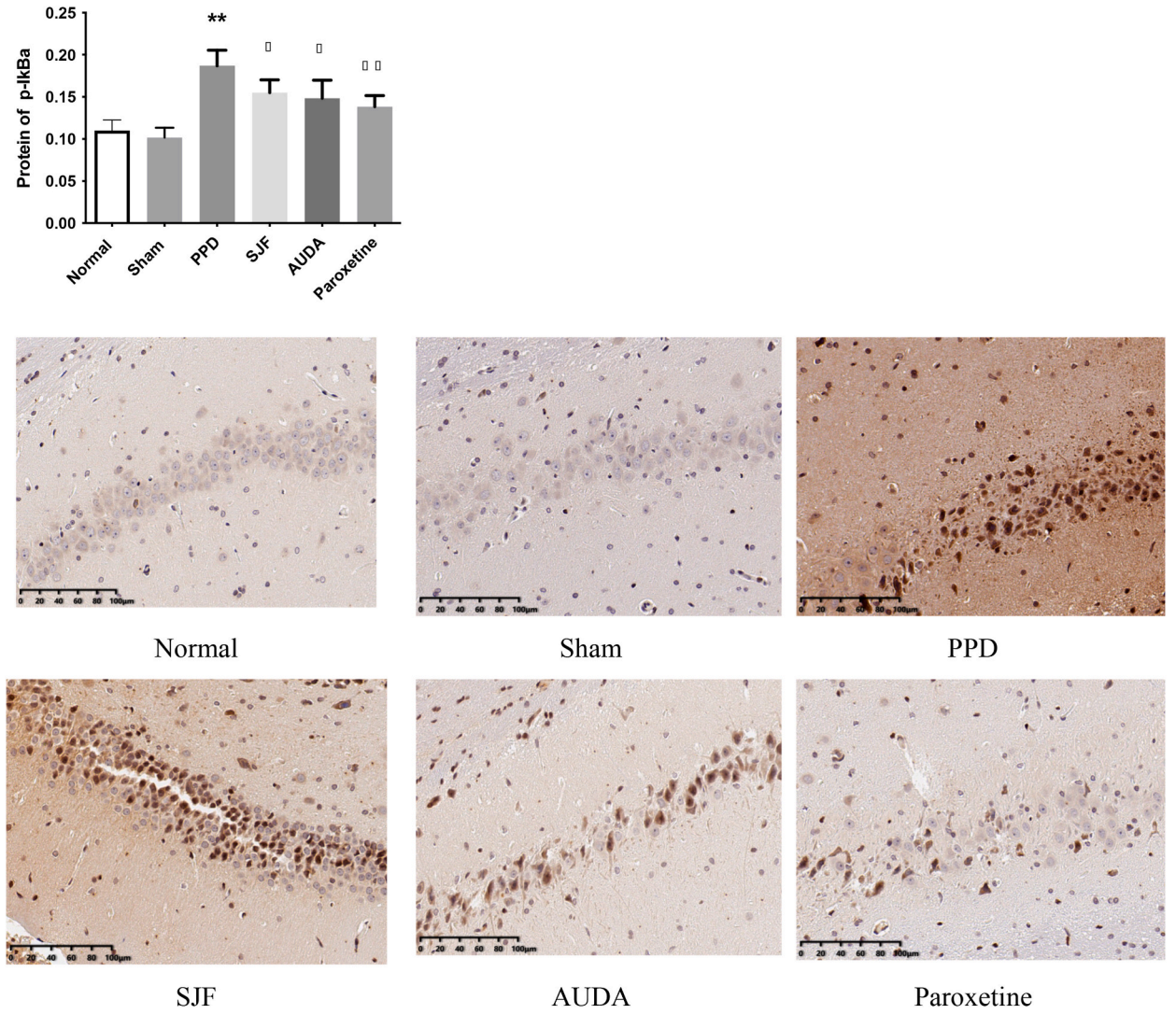

Fig. 10. Protein expression of p-IκBα ($\bar{\chi}\pm s$, n = 3) in hippocampi of rats in each group (200 ×). Protein expression of p-IκBα increased in PPD animals. SJF, AUDA, and paroxetine hydrochloride reversed the changes in protein expression of p-IκBα. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. normal rats; $^{\star\star}P < 0.01$, $^{\star}P < 0.05$ vs. PPD rats. Scale bar = 100 μm.

results in this study that SJF had the same effects on sEH, COX-2, 5-LOX, and EETs as AUDA suggests that SJF treats depression by regulating the AA metabolic pathway.

The NF-κB pathway is important for inflammatory responds and can be activated by inflammatory substances of AA metabolism [44,45]. In our previous study, the NF-κB pathway were found increased in the prefrontal lobe in the PPD rats [21]. This results also found that p-IκBα, p-p65, and NF-κB p65 in the hippocampi of PPD rats increased significantly, indicating an over-expression of the NF-κB pathway. Decreases in NF-κB p65, p-p65, and p-IκBα following SJF intervention indicated that SJF appears to down regulate the NF-κB pathway. IkBα is a 40 kDa protein that functions to prevent NF-κB translocation to the nucleus by combining with NF-κB to form a dimer in the cytosol. The interaction of IκBα with NF-κB masks the nuclear localization sequence of NF-κB, while activation of NF-κB requires that IκB be phosphorylated on specific serine residues, which results in targeted degradation of IκB [46]. Therefore, in this study, p-IκBα also indicated the activation of NF-κB signal pathway.

NF-κB p65 is a key transcription factor that is extensively expressed in the majority of cells and is associated with cellular proliferation, differentiation, carcinogenesis [9,47,48]. NF-κβ p65 can be declined by ferulic acid with substance P and caspase-3 levels in hippocampi of mice with depressive-like behavior [49]. Furthermore, the NF-κB/IκB-α pathway involves 5-O-methylvisammioside affecting LPS-induced depression, which reduces the content of inflammatory factors, improves oxidative stress, regulates neurotrophic factors in the depressed mouse brain, and ultimately inhibits excessive activation of LPS-stimulated BV-2 microglia [50]. Ginsenoside Rh2 has been found to mitigate depression-like behavior by decreasing the excessive activation of neuroinflammation via the HMGB1/TLR4/NF-kappaB pathway [51]. Isoastragaloside I and astragaloside IV in SJF can inhibit NF-κB activation and

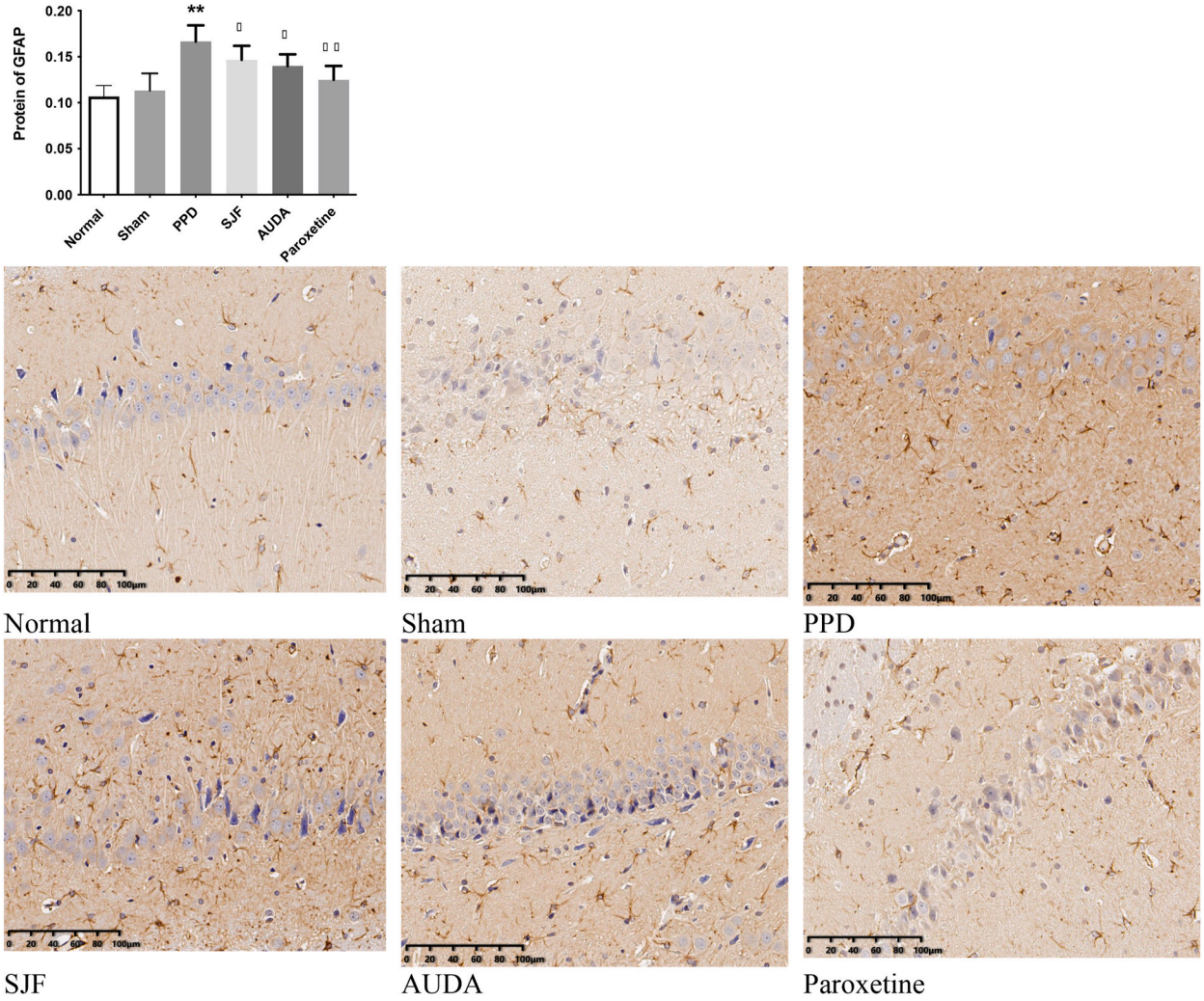

Fig. 11. Protein expression of GFAP¯(χ±s, n = 3) in hippocampi of rats in each group (200 ×). Protein expression of GFAP increased in PPD animals. SJF, AUDA, and paroxetine hydrochloride reversed the changes in protein expression of GFAP. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. normal rats; $^{\star\star}P < 0.01$, $^{\star}P < 0.05$ vs. PPD rats.

inflammatory responses, and astragaloside IV can regulate serum metabolomics in rats with CUMS-induced depressive-like behavior in rats [52–54]. 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone in SJF has anti-inflammatory effects via NF-κB inactivation in lipopolysaccharide-stimulated RAW 264.7 macrophages. [55];

Moreover, several Chinese herbal extracts and medicinal prescriptions can ameliorate depressive-like behavior in rodents by regulating the NF-κB pathway in hippocampal astrocytes and microglia [56–60]. In our study, results that SJF reduced GFAP and Ibal in PPD rats' hippocampi are consistent with the aforementioned research findings, as astrocytes and microglia were stained by GFAP and Ibal, respectively.

Paroxetine hydrochloride was selected as the control drug in this study because it is a selective 5-hydroxytryptamine (5-HT) reuptake inhibitor (SSRI). It is a widely applied drug to treat PPD. It inhibits the reuptake of the neurotransmitter 5-HT in the gap between nerve synaptic cells so as to increase the concentration of 5-HT that can be combined with postsynaptic receptors outside the cells. 5-HT, as well as 5-HT(1A), inhibits apoptotic cell death, and increases intranuclear levels of the p50 and p65 subunits of NF-κB [61]. 5-HT, when increased by aerobic exercise, can reduce inflammasome activation and alleviate stress induced mitochondrial damage by decreasing calcium overload through the way inhibiting NF-κB/NLRP3 inflammasome initiation [62] 5-HT secretion has been shown to significantly elevate NF-κB signaling during hypoxia in gut enterochromaffin cells [63]. In addition, 5-HT1AR inhibitor can decrease levels of TNF-α, GFAP and Iba1 in the hippocampus and inhibit phospho-NF-κB in the hippocampus of Aβ1-42-injected mice [64]. Thus, our study results are consistent with the above research findings in that the Iba1, GFAP, phospho-NF-κB, and inflammatory cytokines are increased while 5-HT is decreased in the hippocampi of PPD rats.In this study we further expanded the target of SJF on PPD. Together with our previous study [21], we both observed the inflammatory indicators such as IL-6, IL-1β,COX-2, 5-LOX, sEH and the NF-κB pathway in PPD model. However the two involved different brain regions for previous one focusing on the

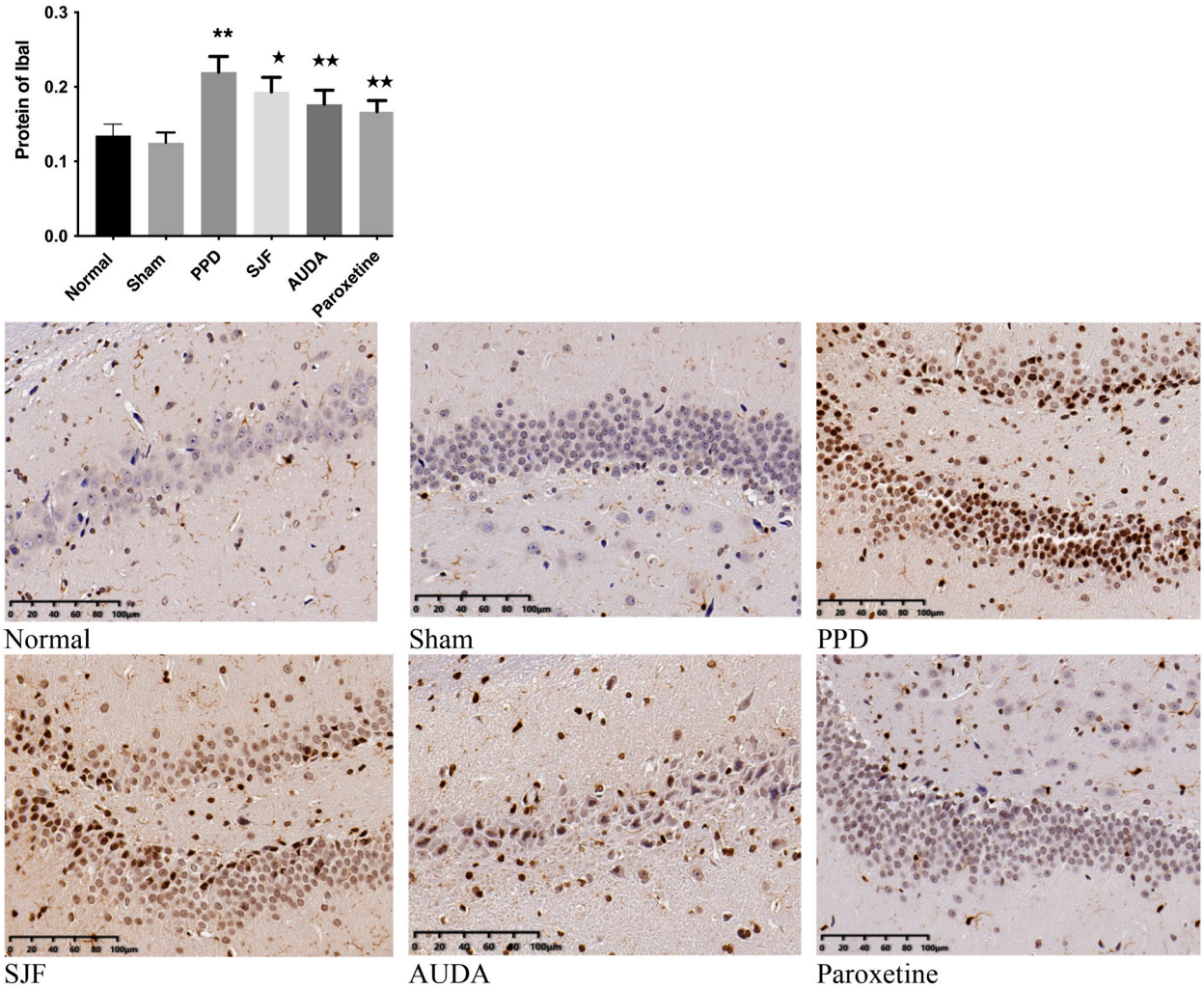

Fig. 12. Protein expression of Iba1^{-}($\chi\pm s$, n = 3) in hippocampi of rats in each group (200 ×). Protein expression of Iba1 increased in PPD animals. SJF, AUDA, and paroxetine hydrochloride reversed the changes in protein expression of Iba1. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. normal rats; $^{★★}P < 0.01$, $^{★}P < 0.05$ vs. PPD rats. Scale bar = 100 μm.

prefrontal lobe and this one focusing on hippocampus. Our researches still has many shortcomings. First, more brain regions related to depression and more research methods should be involved. Second, deeper study should be focus on the relationship between the inflammation, the HPA axis and HPG axis in PPD to understand the inflammatory pathogenesis of postpartum depression. Third, it is very necessary to investigate the network mechanism of SJF on PPD.

5. Conclusion

This study preliminarily prompt that SJF, similar to sEH inhibitor, possesses comparable effect as paroxetine hydrochloride. SJF may inhibit AA metabolism and downregulate the following NF-κB pathway in rats with PPD-like behavior, thereby inhibiting the inflammatory reaction and significantly increasing the level of 5-HT to treat PPD.

Approval of the submission

All authors read and approved the final manuscript.

Duplicate publication

This manuscript has not been published previously and is not under consideration for publication elsewhere, in full or in part.

Ethics approval and consent to participate

The animal experiment protocol was approved by Laboratory animal management and Ethics Committee of Hangzhou Yingyang Biological(EYOUNG-20201117-12). The procedures used were in accordance with the Guidelines for Animal Experimentation of Zhejiang Chinese Medical University. All effort was made to keep the number of animals used in this study to a minimum and to minimize their suffering. All animal-handling procedures were performed according to the *Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.*

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Data availability statement

Data will be made available on request and some data can be found in supplementary material.

CRediT authorship contribution statement

Li Jingya: Writing – original draft, Methodology. **Linhong Song:** Formal analysis. **Lu Lu:** Data curation. **Qing Zhang:** Project administration. **Weijun Zhang:** Supervision, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

List of abbreviations

AA	arachidonic acid
EET	epoxyeicosatrienoic acids
5-HT	5-hydroxytryptamine;
PPD	postpartum depression
sEH	soluble epoxide hydrolase
SSRI	selective serotonin reuptake inhibitor

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29978.

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