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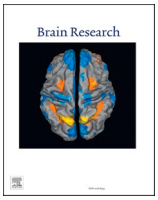

Antidepressant-like effects of Jieyu Chufan capsules in the olfactory bulbectomy rat model

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ABSTRACT

The olfactory bulbectomy (OBX) animal model of depression reproduces the behavioral and neurochemical changes observed in depressed patients. We assessed the therapeutic effects of the Jieyu Chufan (JYCF) capsule on OBX rats. JYCF ameliorated the hedonic and anxiety-like behavior of OBX rats and attenuated the cortical and hippocampal damage. JYCF enhanced the expression of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), fibroblast growth factor 2 (FGF2), and adiponectin (ADPN) in the cortex and hippocampus of OBX rats. JYCF also reduced cortisol levels and restored the levels of excitatory neurotransmitters, such as 5-hydroxytryptamine (5-HT), acetylcholine (ACH), and glutamic acid (Glu), in the brain tissue of OBX rats. Our results suggest that JYCF preserves the synaptic structure by increasing the levels of synaptophysin (SYN) and postsynaptic density protein 95 (PSD95) and alleviates the histological alterations of brain tissue by activating AKT/PKA-CREB-BDNF pathways, and by upregulating ADPN and FGF2 expression in OBX rats. JYCF exerts multiple therapeutic effects on depression, including modulating neurotransmitters, repairing neuronal damage, and maintaining synaptic integrity. These findings support the potential of JYCF as a novel antidepressant agent with therapeutic effects on depression and related neurological disorders.

1. Introduction

Depression is a serious mental disorder that affects millions of people worldwide. People with depression may experience persistent sadness, hopelessness, guilt, loss of interest, insomnia, fatigue, appetite changes, and suicidal thoughts (Park and Zarate, 2019). Depression can affect anyone, regardless of age, gender, culture, or background. Anti-depression medicines are one of the main treatments for depression, which aim to restore the balance of chemicals in the brain that regulate mood and emotions.

There are many types of anti-depression medicines available, and the most common ones are selective serotonin reuptake inhibitors (SSRIs) (Katzman et al., 2014, Stahl, 1998), serotonin and norepinephrine reuptake inhibitors (SNRIs), atypical antidepressants, tricyclic antidepressants (TCAs) (Strawn et al., 2018), and monoamine oxidase inhibitors (MAOIs). However, these medications have potential side effects, interactions, or contraindications for some people. Moreover, in some regions and countries, there is a shortage of anti-depressant medications, which poses a significant challenge. According to a report by the World Health Organization (WHO), more than 75 % of people with depression in low- and middle-income countries receive no treatment. These countries often lack the resources to provide adequate access to anti-depression medicines. Therefore, more research is needed to gain insights into the pathophysiological processes of depression and to develop new and better anti-depression medicines that target specific mechanisms or subtypes of depression.

Depression can have various causes, such as faulty mood regulation in the brain, genetic vulnerability, stressful life events, and medical

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conditions or medications. A large amount of evidence shows that the dysfunction of neurotransmitters might be the fundamental basis of depression (Abdallah et al., 2014, Fee et al., 2017, Sanacora et al., 2012, Tye et al., 2013, Yohn et al., 2017). Neurotransmitters are chemical messengers that enable communication between nerve cells in the brain and other parts of the body. They regulate mood, cognition, emotion, sleep, appetite, and other functions (Payne and Maguire, 2019). Imbalance or disruption of neurotransmitters can lead to depression symptoms. Some neurotransmitters involved in depression are serotonin, acetylcholine, norepinephrine, dopamine, glutamate, and gamma-aminobutyric acid (GABA). Different types of antidepressant medications work by restoring the normal levels or activity of these neurotransmitters in the brain. Studies have shown that alterations in neurotransmitter levels and function, as well as dysfunction of the hypothalamic–pituitary–adrenal (HPA) axis (McAllister-Williams et al., 1998, Young and Tong, 2021), are common in patients with mood disorders. The HPA axis is a neuroendocrine system that consists of the hypothalamus, the pituitary gland, and the adrenal cortex, and controls the production and release of glucocorticoids. For instance, patients with depression often have elevated levels of cortisol and reduced negative feedback sensitivity of the HPA axis. They also have reduced levels of serotonin and norepinephrine in the brain, which may impair their mood regulation and coping ability (Keller et al., 2017, Menke, 2019, Valentino and Commons, 2005). Conversely, hyperfunction of the HPA axis promotes the release of corticotrophin releasing factor (CRF), which further controls the secretion of adrenocorticotropic hormone (ACTH) in the anterior pituitary, promoting the increase of cortisol in circulation (Peng et al., 2015). Patients with increased sensitivity of the HPA axis and exaggerated cortisol response may be prone to depression (Holsboer, 2000). Therefore, understanding the dysfunction of the HPA axis is crucial for comprehending the neurobiology of depression. By clarifying the intrinsic regulation of the HPA axis, new insights and therapeutic strategies may be developed for the treatment of depression.

There are different animal models that are used to study depression and its treatment. Besides gene-edited animals, there are two most used methods of molding depression in rodents, including chronic unpredictable mild stress (CUMS) and olfactory bulbectomy (OBX) (Hao et al., 2019). CUMS means animals are exposed to various unpredictable stressors, such as food and water deprivation, cage tilt, social isolation, and predator odor. OBX model involves surgically removing the olfactory bulbs of animals, which are responsible for processing olfactory information. Both models can anhedonia, despair, anxiety, and cognitive impairment in animals(Hao, Ge et al. 2019, Katz, 1982, Song and Leonard, 2005). Although each model has its own advantages and disadvantages depending on the type and purpose of the research, they are still qualified to mimic some aspects of human depression, such as behavioral, neurochemical, and neurobiological changes. Therefore, both models are widely used to evaluate the antidepressant effects of drugs and their underlying mechanisms.

Traditional Chinese Medicine (TCM) has been used for centuries to address a variety of health issues, including depression. Certain herbal remedies, such as St. John's wort, Rhodiola, and licorice root, are believed to have mood-enhancing properties and may help alleviate feelings of sadness, anxiety, and stress (Liu et al., 2015). Additionally, TCM formulas can offer advantages over single botanical medicines, as they often contain a combination of herbs that work synergistically to achieve a specific therapeutic goal. One such formula is the Jieyu Chufan Capsule, which is originated and modified from Banxia Houpu Decoction and Zhizi Houpu Decoction, containing gardenia, Magnolia officinalis cortex, Pinellia, forsythia, Poria cocos, Perilla frutescens, Aurantii Fructus, and licorice. It is effective in treating qi stagnation, phlegm obstruction, and internal disturbance of stagnation of fire, and can alleviate a variety of depression symptoms, including anxiety, nervousness, fear, insomnia, and dreaminess. It also addresses physical symptoms, such as throat discomfort, chest and abdomen fullness, dizziness, tinnitus, loss of appetite, bitter mouth and dry throat, and constipation. Previous data suggested that Jieyu Chufan capsule (JYCF) improves depression-like behavior in CUMS model mice (Ding et al., 2015, Zhao et al., 2017). Despite the clinical effectiveness of the Jieyu Chufan Capsule, the molecular mechanisms underlying its antidepressant effect are not yet fully understood, which limits its clinical application. Further research is needed to uncover the specific mechanisms of action of this TCM formula and other herbal remedies for depression.

In this study, we aimed to investigate the potential antidepressant effects of Jieyu Chufan (JYCF) in rats with olfactory bulbectomy (OBX)-induced depression. Our findings demonstrated that JYCF could protect the neurons and alleviate depressive-like behaviors in the rats. The beneficial effects of JYCF were attributed to its ability to regulate the hypothalamic–pituitary–adrenal axis and synaptic plasticity.

2. Materials and methods

2.1. Drugs and dose setting

JYCF capsules were obtained from Shijiazhuang Yiling Pharmaceutical Co., Ltd. (Shijiazhuang, China, lot number A1811001). The JYCF capsule's contents were combined with pure water to create a liquid medicine with the appropriate concentration, and then the medications were injected intragastrically.

The specification of JYCF capsules: 0.4 g/capsule, 4 capsules each time, 3 times daily, the adult clinical use dose was 4.8 g/day. The proposed clinical dosage was 19 g of crude drugs per person per day, converted to the equivalent dose for model rats according to the body surface area of humans and animals: 4.8 g *0.018/0.2 kg = 0.432 g/kg. Medium and high doses were set according to 2 or 4 times the clinically equivalent dose as 0.864 g/kg, 1.728 g/kg. Fluoxetine hydrochloride clinical dosage of 40 mg/day was converted to rat dose based on animal and human clinical doses as 40 mg*0.018/0.2 kg = 3.6 mg/kg.

2.2. Animals

One hundred and twenty male WKY rats (180 g – 220 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Certificate No. SCXK [Jing] 2016–0006, Beijing, China). Rats were divided into normal control group and model group according to weight (n = 60 per group). The experimental procedures and animal welfare followed the Ethics Review Committee for Animal Experimentation of New Drug Evaluation Center of Hebei Yiling Medical Research Institute. (protocol number: N2019180).

2.3. Establishment of rats olfactory bulbectomy injury model and drugs treatment

The Zoletil®50 (50 mg/kg) (Virbac, France) was used to anesthetize the model rats. Rats were fixed in stereotactic apparatus (ALC-H, ALC-BIO, Shanghai, China) and the coronal, sagittal, lambdoidal, and cranial fontanelles of the rats were exposed, and two holes were drilled into the skull of each animal using an electric bone drill (ALC-CED, ALCBIO, Shanghai, China) that were 8 mm rostral to the bregma and 2 mm lateral to the midline. In order to stop bleeding, a hemostatic sponge was placed within the cavity after suctioning out both olfactory bulbs (Elsayed et al., 2012, Morales-Medina et al., 2017). Following anti-infection treatment, the skull was sealed with bone wax and the wound was stitched. Following 14 days of restorative single cage feeding, model rats were randomly assigned to each of the following groups (n = 15 per group): normal group (Normal), model control group (Model), positive drug fluoxetine hydrochloride group (FLX), low (JYCF-L), medium (JYCF-M) and high (JYCF-H) dose groups of Jieyu Chufan capsules. The animals in each group were given the corresponding drugs by gavage once a day for 45 days. Relevant behavioral tests were performed after the last administration, and brain tissues were taken for relevant tests.

2.4. Sucrose preference test

A testing time and a training period were set aside for the experiment. Animals were adapted to the sucrose solution during the training course. For the first 24 h, they received 1 % sucrose solution, and for the next 24 h, they received one bottle of the solution along with pure water. Animals were not starved but were denied water for 8 h before testing. During testing, one bottle of 1 % sucrose and pure water were given to the animals for 24 h. The two bottle positions were switched at the twelfth hour of the testing process to eliminate the impact of position preference. After the experiment, the preference for sucrose was determined using the equation below: sucrose preference (%) = sucrose intake / (sucrose intake + water intake) × 100 %.

2.5. Novelty Suppressed feeding test

An adaption phase and a detection period, totaling 2 days, were set out for the experiment. The animals spent 10 min in the Novelty Suppressed Feeding test chamber, followed by a 24-hour fast during which they were only allowed to drink water. Feed was placed in the middle of the box during the detection period, and the animals entered the detection area at the box's corner with their backs to the feed. JL Behv animal behavior video analysis system (Shanghai Jiliang Software Science & Technology Co., Ltd., Shanghai, China) captured the rat's first feeding behavior, which occurred within 5 min and involved the rat nibbling on food.

2.6. Open field test (OFT)

To ensure the cleanliness of the testing box, spray it with 70 % ethanol and wipe it with a paper towel before conducting the test. Allow the designated rat to acclimate to the testing environment by placing it in the testing box for 3 min prior to the test. Use the JL Behv animal behavior video analysis system to record the rat's spontaneous activity for 10 min. Clean the testing box thoroughly after each test before proceeding to the next batch of animals.

2.7. Contents of ACTH and CORT in cortex and contents of ACH, 5-HT, Glu, MAP2, MBP, and FGF2 in hippocampus

After collecting the blood, the skulls of animals were sliced apart by using bone scissors, and the brain tissues were removed with ophthalmic tweezers and placed on the chilled ice tray. After homogenizing the tissue with lysate and separating the cortex and hippocampus, the tissues were centrifuged at 3000 rpm for 10 min, and then the supernatant was collected. The contents of ACTH and cortisol (CORT) in the cortex and acetylcholine (ACH), 5-hydroxytryptamine (5-HT), glutamic acid (Glu), microtubule-associated protein 2 (MAP2), myelin basic protein (MBP), and fibroblast growth factor 2 (FGF2) in the hippocampus were detected at 450 nm according to the instructions of Rat ACTH ELISA kit (MM-0565R2), Rat CORT ELISA kit (MM-0574R2), Rat ACH ELISA kit (MM-0517R2), Rat 5-HT ELISA kit (MM-71127R2), Rat Glu ELISA kit (MM-0601R1), Rat MAP2 ELISA kit (MM-0720R1), Rat MBP ELISA kit (MM-0463R1), and Rat FGF2 ELISA kit (MM-20220R2) which were purchased from Jiangsu Meimian Industrial Co., Ltd. (Jiangsu, China).

2.8. Histopathological analysis

After the behavioral test, the cortex was fixed by 10 % formaldehyde and hippocampus was embedded in paraffin and later sectioned onto the glass slides (4 μm). The tissue sections of cortex and hippocampus were stained with Hematoxylin&Eosin (H&E) to observe the morphological changes of the cortex and hippocampus.

2.9. Immunohistochemistry (IHC)

IHC was used to assess the microtubule-associated protein 2 and myelin basic protein (MBP) expression levels in the cortex and hippocampus tissue. Primary antibodies against MAP2 (1:100, Abclonal, Wuhan, China) and MBP (1:100, Abclonal, Wuhan, China) were incubated with tissue slices for one night at 4 °C. The samples were then treated for 30 min at room temperature with a secondary antibody, HRP Goat Anti-Rabbit IgG (H + L) (1:100, Abclonal, Wuhan, China). After incubating in 3,3′-diaminobenzidine tetrahydrochloride with 0.05 % H_2O_2 for 5 min, the nucleus was counterstained with hematoxylin. At a magnification of × 100, pictures of the sections were taken.

2.10. Electron microscopic examination

The right brain's hippocampus was promptly dissociated from it and fixed for two hours in a solution containing 2 % glutaraldehyde. The CA3 hippocampal tiny tissue, which has a volume of less than 1 mm^3, was removed and fixed for two hours in the fixative solution. After the tissue had been cleaned with PBS, a sample that had been combined with 2 % osmic acid for two hours was dehydrated, embedded, and then examined and captured using an electron microscope.

2.11. Western blot

Western blotting was used to identify the synaptophysin (SYN), postsynaptic density protein-95 (PSD95), protein kinase B (AKT), brain-derived neurotrophic factor (BDNF), cyclic adenosine monophosphate response element binding protein(CREB), p-cyclic adenosine monophosphate response element binding protein (p-CREB), protein kinase A (PKA), adiponectin receptor 1 (ADIPOR1), fibroblast growth factor receptor 2 (FGFR2), and fibroblast growth factor receptor 3 (FGFR3). Briefly, the samples were mixed with loading buffer, heated at 95 °C for 5 min, separated by SDS-PAGE, and transferred onto polyvinylidene fluoride (PVDF) membranes (Merck Millipore, USA). The membranes were blocked with Rapid Blocking Buffer (SEVER, Wuhan, China) for 30 min and then incubated with SYN, PSD95, AKT, BDNF, CREB, p-CREB, PKA, ADIPOR1, FGFR2, FGFR3 (1:1000; all from Abclonal, Wuhan, China), and β-actin (1: 100000; Abclonal, Wuhan, China) at 4 °C overnight. The membranes were washed thrice with Tris Buffered Saline containing 0.1 % Tween 20 (TBST) and incubated with a secondary antibody HRP-Goat Anti-Mouse IgG(H + L) (1: 5000; Abcam, USA) for one hour at room temperature (RT). Signals were detected by the Odyssey imaging system and quantified by image analysis software (LI-COR, Biosciences, USA).

2.12. Statistical analysis

SPSS16.0 was used for statistical analysis. The level of statistical significance was set as $P < 0.05$. The measurement data were expressed as mean ± standard deviation ($\overline{x} \pm s$). Leven's test was used to test normality and homogeneity of variance. If normality and homogeneity of variance were met ($P > 0.05$), analysis of variance (ANOVA) and Tukey's post-hoc test were used for statistical analysis; if it did not conform to normality and homogeneity of variance ($P \leq 0.05$), the Kruskal-Waillis test shall be used. If the Kruskal-Wallis test was statistically significant ($P \leq 0.05$), Dunnett's Test shall be used for comparative analysis, and the statistical difference and biological significance shall be considered in the evaluation.

3. Results

3.1. JYCF improved behavioral deficits in OBX rats

In Fig. 1A, the results demonstrated that the sucrose preference index in the Model group was significantly lower than that in the Normal

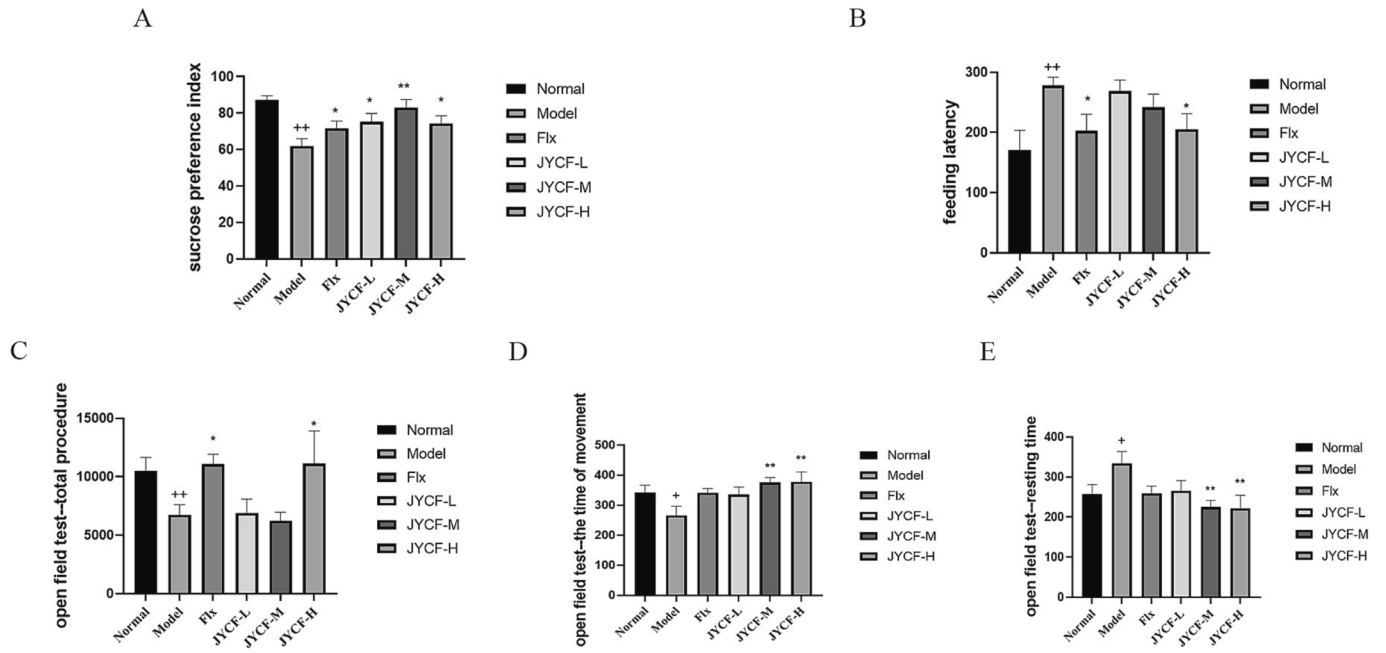

Fig. 1. Effect of JYCF on behavior of OBX rats. (A). Sucrose preference. (B). Feeding latency. (C). Open field test-the time of movement. (D).Open field test-resting time. (E). Open field test-tota procedure. Data were expressed as the mean ± SD (n = 14), $^{++}P < 0.01$, $^{+}P < 0.05$ vs. Normal. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. Model.

group ($P < 0.01$; $F_{1,A} = 5.009$). However, regular treatment with JYCF increased the sucrose intake rates in OBX rats ($P < 0.05$ or $P < 0.01$) compared to the Model group. Fig. 1B revealed that feeding latency rates in the Model group were significantly higher than that in the Normal group ($P < 0.01$; $F_{1,B} = 3.030$). JYCF treatment significantly decreased the feeding latency rates in OBX rats ($P < 0.05$) compared to the Model group. In addition, as shown in Fig. 1C and E, the time of movement and total procedure in the Model group was significantly decreased compared to the Normal group. However, treatment with middle or high doses of JYCF significantly inhibited these reductions ($P < 0.05$ or $P < 0.01$; $F_{1,C} = 2.686$ and $F_{1,E} = 2.610$). Similarly, in Fig. 1D, the resting time in the Model group was significantly increased compared to the Normal group, and JYCF treatment also inhibited this increase ($P < 0.01$; $F_{1,D} = 2.699$).

3.2. JYCF improved brain tissue lesions in OBX rats

In Fig. 2, the results showed that there were no abnormal conditions in the visible neuronal morphology, glial cells, and hippocampal pyramidal cells of the hippocampus in the Normal group. However, in the Model group, pyramidal cells were sparsely and disorderly arranged, and some were necrotic or showed interstitial edema. The JYCF-L group showed improvement in pyramidal cell necrosis. In the JYCF-M, JYCF-H, and FLX groups, some hippocampal pyramidal cells were closely arranged, and the number of hippocampal pyramidal cells with interstitial edema decreased significantly.

3.3. JYCF increased the level of neurotransmitters in the brain of OBX rats

In Fig. 3, the data indicated that the levels of ACH, Glu, and 5-HT in the hippocampus of the Model group were lower than those in the Normal group ($P < 0.05$; $F_{3,A} = 13.965$, $F_{3,B} = 3.393$ and $F_{3,C} = 2.899$). However, after treatment with JYCF and FLX, there were significant increases in the levels of these neurotransmitters. Specifically, the JYCF-H and FLX groups showed significant increases in ACH levels ($P < 0.01$), while the JYCF-M and JYCF-H groups showed significant increases in 5-HT levels ($P < 0.05$, $P < 0.01$). Moreover, the levels of Glu were significantly increased in the JYCF-L, JYCF-M, JYCF-H, and FLX groups ($P < 0.05$ or $P < 0.01$) compared to the Model group.

3.4. JYCF regulated hypothalamus pituitary adrenal (HPA) axis

To visualize the effect of JYCF on CORT levels, we measured the contents of ACTH and CORT in cortical tissue. As shown in Fig. 4, the contents of ACTH and CORT in the cerebral cortex of the Model group were significantly higher than those in the Normal group ($P < 0.05$; $F_{4,A} = 7.667$ and $F_{4,B} = 6.585$). However, treatment with JYCF-L, JYCF-M, JYCF-H, and FLX significantly decreased the contents of ACTH and CORT in the cerebral cortex compared with the Model group ($P < 0.05$, $P < 0.01$). These results suggested that high doses of JYCF might reduce CORT levels in the cortex by suppressing ACTH release.

3.5. JYCF improved the pathological changes of pyramidal cell arrangement and interstitial edema

In Fig. 5A and B, the levels of MAP2 and MBP in the Model group were significantly decreased ($P < 0.05$ or $P < 0.01$; $F_{5,A} = 1.824$ and $F_{5,B} = 7.511$), and treatment with a high dose of JYCF significantly inhibited this decrease ($P < 0.05$ or $P < 0.01$). Under the light microscope, some pyramidal cells were sparsely arranged and disordered, some were necrotic, and interstitial edema was observed in the Model group, and the positive expression of MBP and MAP2 was significantly reduced. Under the transmission electron microscope, the synaptic gap of neurons was significantly widened, and the rate of the synaptic interface was significantly increased. Treatment with the JYCF capsule was found to improve the pathological changes in pyramidal cell arrangement and interstitial edema in the hippocampus of rats.

3.6. JYCF enhanced the expression of synapsis-associated proteins

In Fig. 6A-D, the results showed that the levels of AKT, PKA, p-CREB, and BDNF protein expression were significantly decreased in the Model group compared to the Normal group ($P < 0.05$; $F_{6,A} = 2.527$, $F_{6,B} = 3.310$, $F_{6,C} = 4.448$ and $F_{6,D} = 4.378$). However, in the JYCF-H group, the expression levels of these proteins were considerably increased compared to the Model group ($P < 0.05$). In Fig. 6E, the expression level of ADIPOR1 was significantly decreased in the Model group compared to

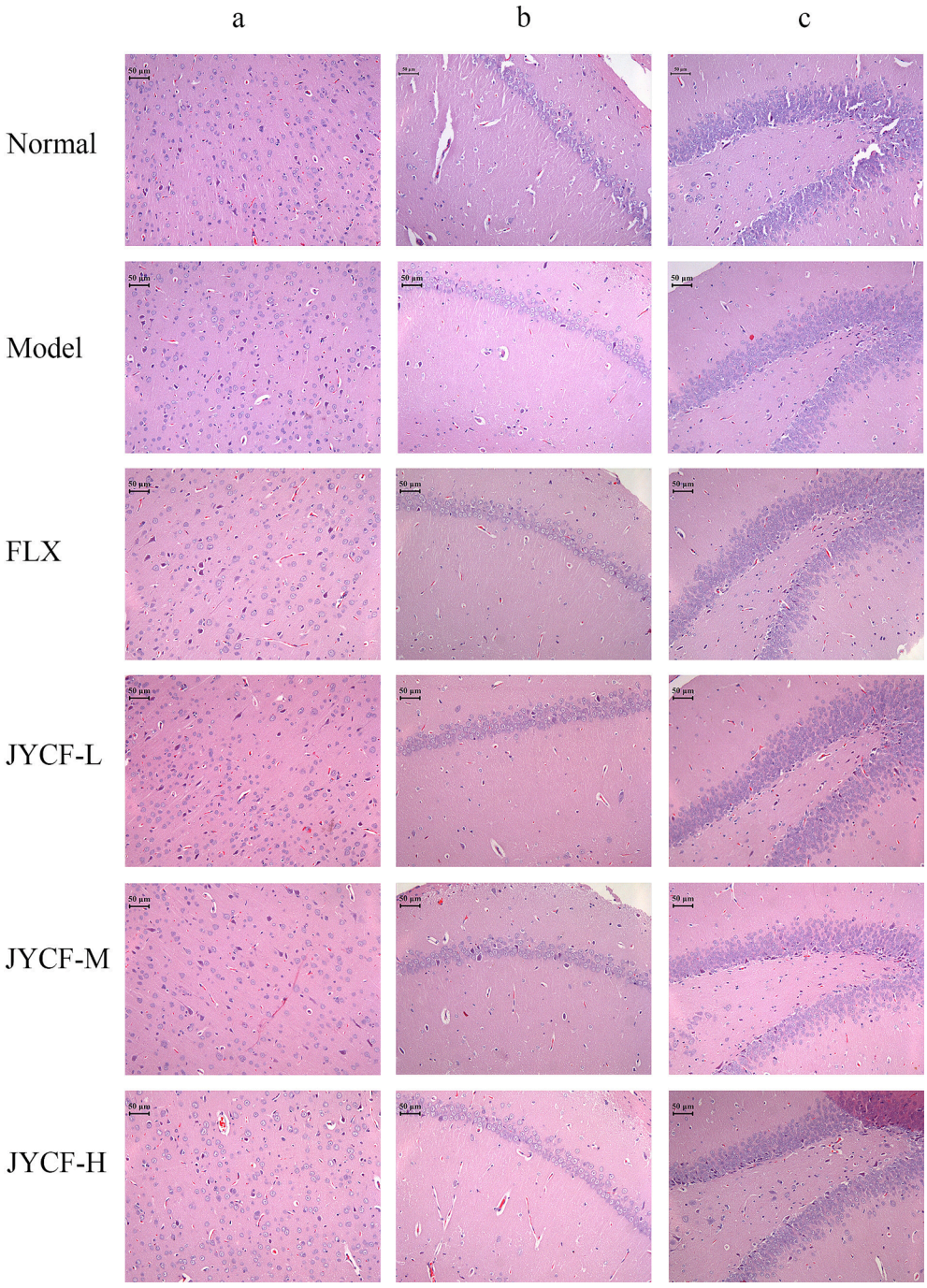

Fig. 2. Effect of JYCF on pathology of cortex and hippocampus in OBX rats. (A). H&E staining of rat cortex. (B). H&E staining of rat hippocampus. (C). H&E staining of rat dentate gyrus of hippocampus.

the Normal group, while it was significantly increased ($P < 0.001$; $F_{6,E} = 37.494$) in the JYCF-H group. Fig. 6F, G, and J demonstrated that the expression levels of FGF2, FGFR3, and FGFR2 were significantly lower in the Model group compared to the Normal group, and they were significantly increased in the JYCF-H group compared to the Model group ($P < 0.05$, $P < 0.01$, $P < 0.001$; $F_{6,F} = 9.928$, $F_{6,G} = 4.280$ and $F_{6,J} = 3.703$). In Fig. 6H and I, the expression levels of SYN and PSD95 were considerably decreased in the Model group relative to the Normal group ($P < 0.05$;$F_{6,H} = 3.526$ and $F_{6,I} = 3.996$), while they were dramatically increased in the JYCF-H group compared to the Model group. These results suggest that JYCF capsules could promote the expression of neuroprotective factors and improve synaptic function.

4. Discussion

In this study, we used the olfactory bulbectomy (OBX) rat model of depression to investigate the therapeutic potential of JYCF administered orally. Our results showed that JYCF treatment significantly improved the depressive state of the OBX rats by increasing their locomotor activity and distance in the OFT, improving the lesions in the cortex and hippocampus, maintaining the levels of excitatory neurotransmitters such as 5-HT, ACH, and Glu, and decreasing cortisol. Mechanistically, the therapeutic benefits of JYCF were attributed to its ability to increase the expression of neurotrophic factors such as BDNF, FGF2, and adiponectin (ADPN) in the cortex and hippocampus and improve synaptic plasticity in OBX rats, as indicated by the increased level of SYN and

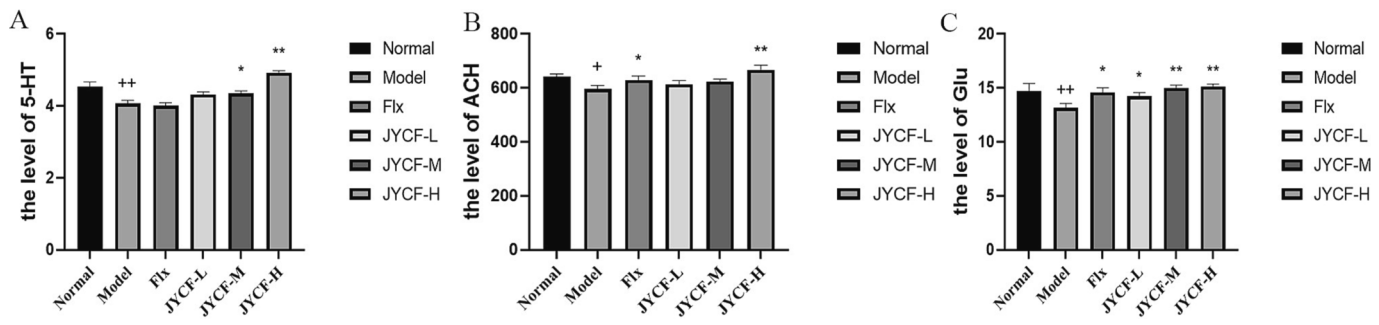

Fig. 3. Effect of JYCF on neurotransmitters in OBX rat brain. (A, B, and C). The expression level of 5-HT, ACH, and Glu in hippocampus. Data were expressed as the mean ± SD (n = 15), $^{++}P < 0.01$, $^{+}P < 0.05$ vs. Normal. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. Model.

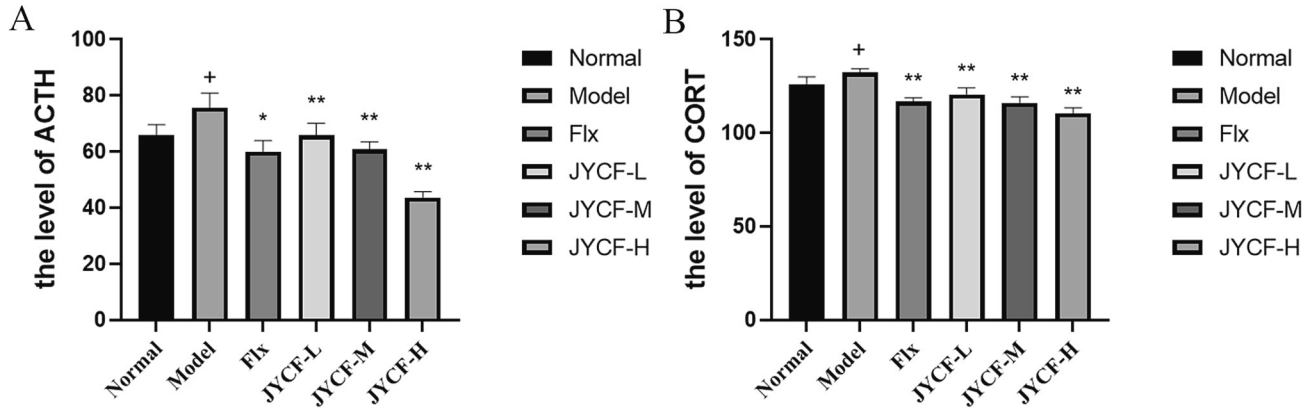

Fig. 4. Effect of JYCF on hypothalamus pituitary adrenal axis function in OBX rats. (A). The expression level of ACTH in cortex. (B). The expression level of CORT in cortex. Data were expressed as the mean ± SD (n = 11), $^{++}P < 0.01$, $^{+}P < 0.05$ vs. Normal. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. Model.

PSD95. These findings suggest that JYCF has multiple therapeutic effects, including regulating neurotransmitters, reducing neuronal damage, and maintaining synaptic integrity, making it a promising treatment for depression.

The efficacy of antidepressant agents can be evaluated by using the OBX animal model of depression, which induces behavioral and neurochemical changes similar to those seen in depressive patients. We found that JYCF improved the sucrose preference index and prolonged the feeding latency time of OBX rats, indicating an increase in hedonic behavior. JYCF also reduced anxiety-like behavior, as demonstrated by increased locomotor activity, and decreased resting time in the OFT (Fig. 1). These results indicated that the JYCF capsule has antidepressant-like properties in OBX rats. In addition to behavioral changes, OBX leads to impaired neurogenesis and synaptic loss in the hippocampus and cortex, two brain regions that play important roles in learning, memory, and emotion regulation. Damage to the hippocampus and cortex in OBX rats may contribute to the cognitive and affective symptoms of depression, such as impaired memory, anhedonia, and reduced motivation (Morales-Medina, Iannitti et al. 2017). We found that light microscopy of hippocampal tissue from olfactory bulb-removed rats showed a sparse and disorganized arrangement of some pyramidal cells, necrosis of some pyramidal cells, and interstitial edema. JYCF treatment significantly improved the pathological changes in the hippocampus of OBX rats, including cone cell arrangement and interstitial edema.

Furthermore, the HPA axis can become dysregulated and overactive under depressive disorder and result in detrimental effects on brain structure and function, manifesting the impairments of negative feedback mechanisms and the increasing levels of corticotropin releasing hormone (CRH), ACTH, and CORT. Our results indicated that these damage factors in the brain tissue of OBX rats were significantly reduced by JYCF (Fig. 4), suggesting that JYCF alleviated the overactivation of the HPA axis. Additionally, the hippocampus plays an important role in pressure regulation by releasing biochemical factors such as acetylcholine, glutamatergic systems, and other neurotransmitters (Kim and Park, 2021). In the present study, the expression of ACH, Glu, and 5-HT was decreased in OBX rats (Fig. 3), and JYCF increased these excitatory neurotransmitters to promote the hedonic behavior.

In our study, the synaptic ultrastructure obtained from transmission electron microscopy showed JYCF significantly reduced the neuronal synaptic gaps and increased the synaptic interface rate. SYN and PSD95 are presynaptic and postsynaptic vesicle proteins, respectively, that are used as biomarkers to indicate the integrity of synaptic structures (Gardoni et al., 2009, Pan et al., 2016). We found that JYCF restored the proteins expression in the OBX rats (Fig. 6I and J). JYCF enhanced the production of MBP and MAP2 (Fig. 5), which play pivotal roles in maintaining the structural and functional stability of neurons, especially in synaptic integrity, and are considered markers of neuronal health. These findings suggested that JYCF had neuroprotective effects on the hippocampus of OBX rats, improving synaptic structure and function. Notably, the proteins of SYN and PSD95 are also the biomarkers to evaluate synaptic plasticity (Liu et al., 2010, Rosenbrock et al., 2005, Wang et al., 2022). It is reported that impaired synaptic plasticity in the hippocampus and the PFC can contribute to the development and maintenance of depression, as well as its cognitive and emotional symptoms. On the other hand, restoring synaptic plasticity in these brain regions can be a potential mechanism of action for antidepressant treatments, as well as a biomarker of treatment response and outcome (Abdallah et al., 2017). Therefore, although we failed to detect the improvement effects of memory and cognitive abilities, the therapeutic effect of JYCF in the OBX model and the changes in biomarkers related to synaptic structure and function suggest the role of JYCF in

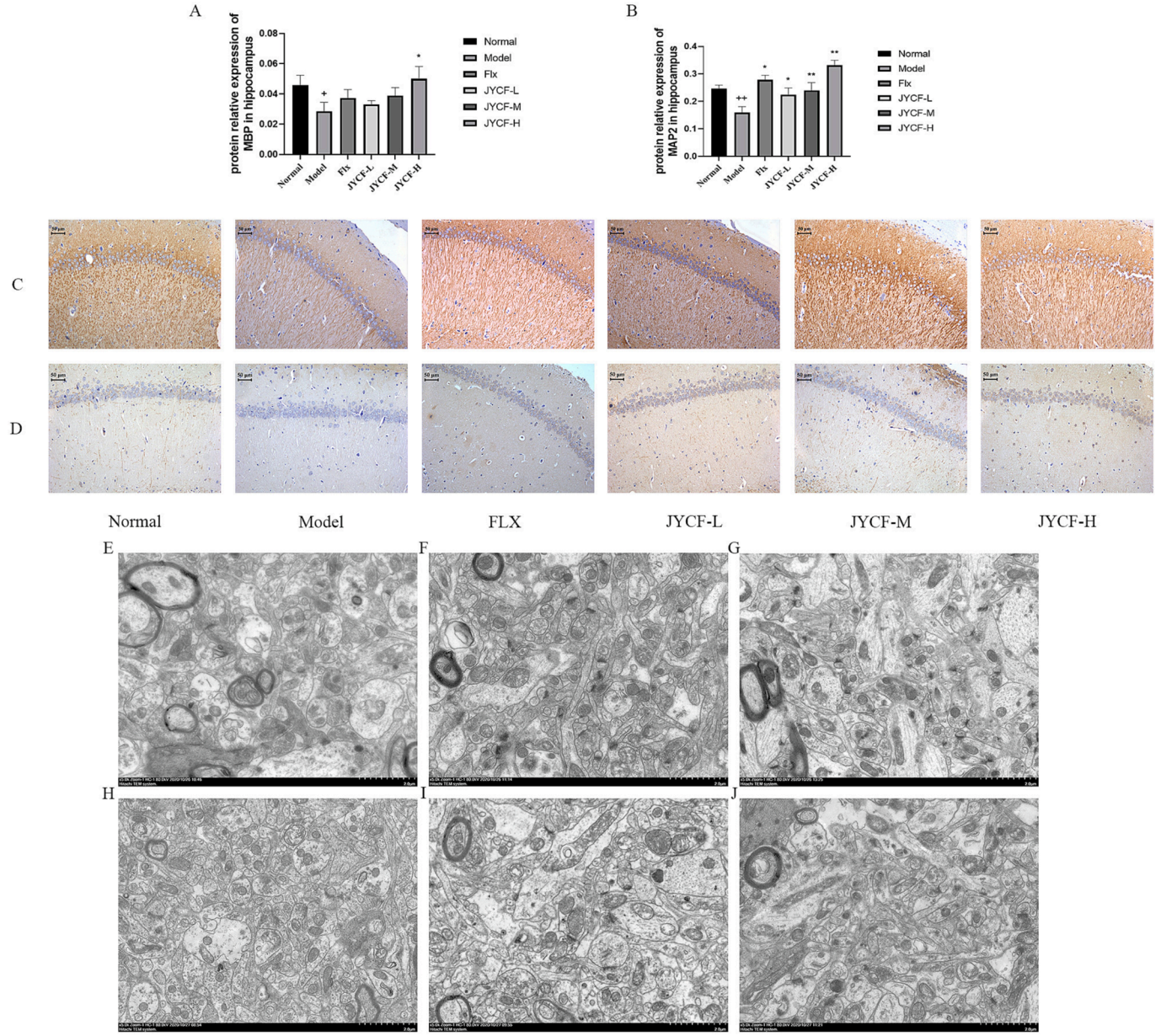

Fig. 5. Effect of JYCF on MAP2 and MBP in cortex and hippocampus tissue and synaptic morphology of hippocampus in OBX rats. (A, B). Protein relative expression of MAP2 and MBP in hippocampus. (C, D). Effect of JYCF on MAP2 protein expression and MBP protein expression in OBX rat hippocampus. (E). Morphological characteristics of hippocampal synapses in the Normal group were detected by TEM. (F). Morphological characteristics of hippocampal synapses in the Model group were detected by TEM. (G). Morphological characteristics of hippocampal synapses in the FLX group were detected by TEM. (H). Morphological characteristics of hippocampal synapses in the JYCF-L group were detected by TEM. (I). Morphological characteristics of hippocampal synapses in the JYCF-M group were detected by TEM. (J). Morphological characteristics of hippocampal synapses in the JYCF-H group were detected by TEM. (Scale bar = 8000 nm) Data were expressed as the mean ± SD (n = 10), $^{++}P < 0.01$, $^{+}P < 0.05$ vs. Normal. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. Model.

maintaining synaptic function and regulating synaptic plasticity.

Several cellular pathways and neurotrophic factors are involved in regulating synaptic plasticity. These pathways include the HPA axis, AKT/PKA-CREB-BDNF pathways, ADPN, and FGF2 (Elsayed, Banasr et al. 2012, Herman and Tasker, 2016, Pousti et al., 2018, Soman et al., 2021, Woodbury and Ikezu, 2014, Zarneshan et al., 2022). BDNF is a crucial modulator of synapse structure and neuronal function, which controls fundamental neuronal function and prevents neurons from being harmed by stress. BDNF can be generated through the activation of AKT/PKA-CREB-BDNF pathways (Soman, Tingle et al. 2021, Zarneshan, Fakhri et al. 2022). Our results suggested that the JYCF capsule could promote BDNF expression in the brain tissue of OBX rats by activating AKT/PKA-CREB-BDNF pathways (Fig. 6A-E). Moreover, JYCF could also increase the expression levels of ADPN and FGF2 (Fig. 6F and K), confirming that JYCF treatment promoted cell proliferation and survival in the central nervous system. Recent research proves that FGF2 promotes the proliferation of neural progenitor cells by binding to FGFR2/FGFR3, enhancing synaptic plasticity (Elsayed, Banasr et al. 2012, Tang et al., 2017, Zarneshan, Fakhri et al. 2022). In the present experiment, we observed a decrease in the expression levels of FGF2 and its receptors FGFR2 and FGFR3 in the OBX rats. However, JYCF treatment reversed this decrease (Fig. 6G, H, and K). Although the interaction between the neurotrophins and the overactivation of the HPA axis is still unclear, JYCF treatment might provide neurogenic niches that were conducive to restoring the normal balance of neurogenesis and synaptic plasticity.

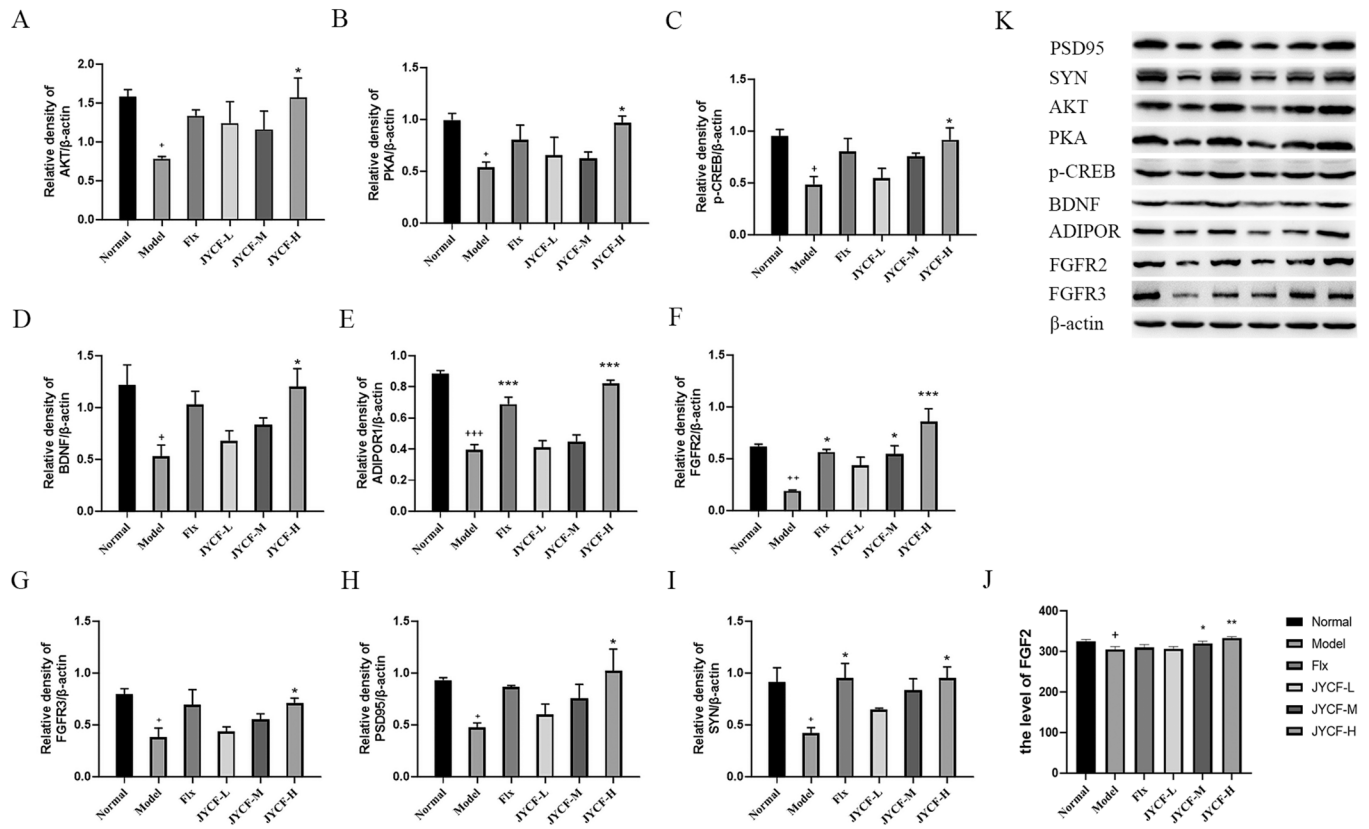

Fig. 6. Effect of JYCF on protein expression in hippocampus of OBX rats (A, B, C, D, E, F, G, H, I and J). Relative density of AKT/β-actin, PKA/β-actin, p-CREB/β-actin, p-CREB/CREB, BDNF/β-actin, ADIPOR1/β-actin, FGFR2/β-actin, FGFR3/β-actin, PSD95/β-actin and SYN/β-actin (n = 3). (J).The expression level of FGF2 in hippocampus (n = 15). Data were expressed as the mean ± SD, $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ vs. Normal. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ vs. Model.

In summary, our study demonstrates that the JYCF capsule has antidepressant-like effects in the OBX rat model of depression, by improving hedonic behavior, reducing anxiety-like behavior, and restoring synaptic plasticity in the hippocampus and cortex. The neuroprotective effects of JYCF may be mediated by the modulation of neurotransmitters, the activation of neurotrophic factors, and the regulation of the HPA axis function. These findings suggest that JYCF may be a potential therapeutic agent for the treatment of depression and other neurological disorders. However, further studies are needed to explore the underlying mechanisms of action and to validate these findings in clinical trials.

Ethics approval and consent to participate

The animal study protocol was approved by the Ethics Review Committee for Animal Experimentation of New Drug Evaluation Center of Hebei Yiling Medical Research Institute (protocol number: N2019180).

Consent for publication

Not applicable.

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6. Authors' contributions

CZ: Validation, Investigation, Writing - Original Draft, Visualization; **MYW and TTL**: Validation, Investigation, Visualization; **TS, WWC, and QYZ**: Data analysis, Writing - Review and Editing; **YLH**: Conceptualization, Methodology, Supervision, Project administration. All of the authors listed have revised and approved the manuscript.

CRediT authorship contribution statement

Chi Zhao: Validation, Investigation, Writing – original draft, Visualization. **Mingye Wang:** Validation, Investigation, Visualization. **Tongtong Li:** Validation, Investigation, Visualization. **Tao Song:** Writing – review & editing. **Wenwen Cui:** Writing – review & editing. **Qiuyan Zhang:** Writing – review & editing. **Yunlong Hou:** Conceptualization, Methodology, Supervision, Project administration.

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.

Data availability

Data will be made available on request.

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