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Original Research

Antidepressant-like mechanism of honokiol in a rodent model of corticosterone-induced depression

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Depression is closely linked to hypothalamus-pituitary-adrenal axis hyperactivity. Honokiol, a biphenolic lignan compound obtained from the traditional Chinese medicine *Magnolia officinalis*, can reduce the activity of the hypothalamus-pituitary-adrenal axis and improve depression-like behavior caused by hypothalamus-pituitary-adrenal axis hyperactivity. The current study investigated the specific mechanism of action of this effect. A depression model was established by repeated injections of corticosterone to study the antidepressant-like effect of honokiol and its potential mechanism. Honokiol prevented the elevated activity of the hypothalamus-pituitary-adrenal axis and the depression-like behavior induced by corticosterone. Treatment with honokiol resulted in greater glucocorticoid receptor mRNA expression, greater glucocorticoid receptor-positive expression, and a greater ratio of glucocorticoid receptor to the mineralocorticoid receptor in the hippocampus. Moreover, honokiol treatment led to lower levels of interleukin-1β in serum and the positive expression of the interleukin-1β receptor in the hippocampus. These results demonstrate that the antidepressant-like mechanism of honokiol, which has effects on inflammatory factors, may act through restoring the typical activity of the hypothalamus-pituitary-adrenal axis by regulating the glucocorticoid receptor-mediated negative feedback mechanism and the balance between glucocorticoid and mineralocorticoid receptors.

Keywords

Honokiol; antidepressant effect; anti-inflammatory; glucocorticoid receptor; hypothalamus-pituitary-adrenal axis

1. Introduction

Depression is a mood disorder that is manifested as anhedonia, despair, and pessimism, which has high morbidity, recurrence, and mortality rates (LeMoult and Gotlib, 2019). Many studies have reported that the hypothalamus-pituitary-adrenal (HPA) axis hyperactivity is closely linked to depression, indicated the need for it to be considered in studies of depression etiology (Galts et al., 2019; Karstens et al., 2019). Clinical studies have found that the HPA axis of many patients with depression are hyperactive and return to a normal state following antidepressant treatment (Høifødt et al., 2019). Also, depression causes an increase in the adrenal index and the volume of the pituitary and adrenal glands, and a decrease in the volume of the hippocampus (Gong et al., 2016). Related animal experiments also indicate that a high dose of glucocorticoids (GC) induces depression-like behavior in rodents (Raone et al., 2007).

As the regulatory center of the HPA axis, the hippocampus mainly maintains the normal activity of the HPA axis through the adjustment of mineralocorticoid receptor (MR) and the negative feedback of the glucocorticoid receptor (GR) (Mifsud and Reul, 2018). Chronic stress may damage this process and cause HPA axis hyperactivity. This HPA axis hyperactivity can also cause damage to the hippocampus, inducing a maladaptive cycle. This may represent the key underlying mechanism of depression (Kim et al., 2015). Also, depression is often accompanied by inflammatory symptoms, notably increased levels of interleukin-1β (IL-1β), which is likely to be associated with the inactivation of GR (Pariante, 2017). Autopsies reveal that the expression of the GR gene and GR protein decrease by 48% and 42%, respectively, in the brains of suicidal patients with depression. In rodent experiments, GR deficiency in the prefrontal cortex of mice can cause depression-like behavior. These findings indicate that GR may be an important target of depression and antidepressant therapy (de Kloet et al., 1998; Dean and Keshavan, 2017). The imbalance between GR and MR may also be a key mechanism of depression, where the hippocampus enters a state prone to damage (de Kloet et al., 2018; Lucassen et al., 2001).

Honokiol (3,5-di-2-propenyl-1,1-biphenyl-2,4-diol), a biphenolic lignan compound, is the main bioactive component of *Magnolia officinalis* (Talarek et al., 2017). Previous research has demonstrated that honokiol may play an important role in improving depression-like behavior in rats, such as shortening the immobility time in the forced swimming test (FST) and tail suspension test (TST) (Zhang et al., 2019). Recent studies conclude that honokiol has antidepressant effects on chronic unpredictable mild stress exposed rats by regulating HPA axis activity (Li et al., 2020;

Pitta et al., 2013). We further investigated the potential mechanism of honokiol in a rodent model of depression induced by repeated corticosterone (CORT) injections, which disrupts the HPA axis.

2. Materials and methods

2.1 Animals and ethical approval

The animals used in the experiment were adult male Sprague-Dawley rats, all of which were specific pathogen-free (SPF) grade. The animal license number is SCXK (Beijing) 2014-0004, purchased from Beijing HuaFukang Biotechnology Co., Ltd. Animals were kept under standard conditions of ambient temperature (20-22 °C), and humidity (50-60%) and a 12-hour light/dark cycle (light on at 8:00 am) were used. The rats could freely obtain standard food and water. The Experimental Animal Ethics Committee of the Academic Committee of Beijing University of Chinese Medicine approved all the steps involving animals in the experiment. (project identification code: BUCM-4-2019021502-1012).

2.2 Animal study design

The forty rats were randomly divided into four groups after acclimating for one week, with ten rats in each group. There was a control group, CORT group, honokiol-treated group (10 mg/kg honokiol + CORT) and a fluoxetine-treated group (10 mg/kg fluoxetine + CORT). We established the fluoxetine-treated group as the positive control group. Except for the control group, the other three groups of rats received subcutaneously CORT injection (40 mg/kg, suspended in saline containing 0.1% dimethyl sulfoxide and 0.1% Tween-80) once daily for 21 days. At the same time, an equal volume of vehicle was subcutaneously injected in the control group. Honokiol and fluoxetine were suspended in 0.5% sodium carboxymethyl cellulose (CMC-Na) 30 minutes before the injection of corticosterone. In the drug-treated groups, rats were intragastrically administrated with 10 ml/kg volume of honokiol or fluoxetine once a day. In the normal control group and the CORT groups, rats received 0.5% CMC-Na solution in equal volume.

Based on our previous studies and related published reports, we chose established doses of CORT, honokiol, and fluoxetine (Gregus et al., 2005; Wang et al., 2018, 2017). In our previous study (Wang et al., 2017), we used three dosages (2.5, 5, and 10 mg/kg) to study the antidepressant effects of honokiol and its mechanism. Our results indicate that honokiol can improve the depression-like behavior of mice and has a specific antidepressant effect. We found that high-dose honokiol (10 mg/kg) had the most significant effect. Based on this, we conduct the current experiment. Behavioral tests were completed after the last dose on day 21. The rats were given 1% pentobarbital intraperitoneally for anesthesia after the behavioral tests, and then blood samples were collected. After collecting the blood, we removed the brain of each rat and stored it on ice for analysis.

The flow of experimental design is shown in (Fig. 1). The figure clearly describes the experimental design, including grouping, drug administration, behavioral testing, and biochemical parameters detected.

2.3 Drugs and reagents

Fluoxetine (Patheon, France), honokiol (Shanghai Titan Scientific Co., Ltd., Shanghai, P. R. China), corticosterone (Chengdu Chroma-Biotechnology Co., Ltd., Chengdu, P. R. China), interleukin-1β (IL-1β) ELISA kits (Proteintech Group, Inc., Chicago, IL. USA), rat 5-hydroxytryptamine (5-HT) ELISA Kit (Shanghai BlueGene Biotech CO., LTD, Shanghai, P. R. China), adrenocorticotropic hormone (ACTH), corticotropin-releasing hormone (CRH) and CORT radioimmunoassay kits (Beijing Sino-UK Institute of Biological Technology, Beijing, P. R. China), Hipure Total RNA mini kit (Magen Bio, Guangzhou, P. R. China), Reveraid First Strand cDNA Synthesis kit (Thermo Scientific, San Diego, CA, USA), SYBR PCR master mix (Invitrogen, San Diego, CA, USA), IL-1β antibody, goat anti-rabbit antibody, and the DAB Immunohistochemistry Color Development Kit (Wuhan servicebio technology Co., Ltd., Wuhan, P. R. China), GR antibody (Beijing Bioss Biotechnology Co., Ltd., Beijing, P. R. China), PBS (KeyGen BioTech Corp., Ltd., Nanjing, P. R. China).

2.4 Behavior testing

2.4.1 Sucrose preference test

The sucrose preference test (SPT) is an effective method to measure anhedonia in rodents (Eagle et al., 2016). We modified the SPT based on the previous reference. In brief, we trained all rats to consume sucrose solution in the following ways before the formal test: all rats were given a bottle of 1% (w/v) sucrose water and a bottle of drinking water to acclimate them to the choice of two bottles in the test. 24 hours before the start of the formal test, rats were deprived of water and food. Subsequently, 100 mL 1% sucrose solution and 100 mL drinking water were placed in each cage, and individual rats could enter the cage freely. 3 hours later, the consumption levels of the sucrose solution and water were recorded, respectively. The sucrose preference was calculated as follows: the sucrose preference rate (%) = sucrose consumption/(sucrose consumption + water consumption).

2.4.2 Open field test

The open-field test (OFT) is a method of measuring the general locomotor and exploratory behaviors of rats (Ramos and Mormède, 1997). Following the previously used protocols, a quiet, light-stable environment was selected, and the rats were placed in a four-sided black wooden box with dimensions of 100 cm × 100 cm × 40 cm (length × width × height) (Cheng et al., 2018). White lines were used to divide the bottom plate into 25 equal squares, and nine squares were denoted in the center of the bottom as the central area. A single rat was placed in the middle of the central area at the start of the test. The rats' horizontal walking score (one point), vertical standing score (one point), and the total score (the sum of the horizontal and vertical movement scores) within 5 minutes were recorded by the small animal behavior analysis system (Etho-Vision XT9, Noldus, The Netherlands). After each rat was tested, the open field device was cleaned and dried to eliminate the effects of odor and feces on the next rat.

2.5 Detection of ACTH, CORT, CRH levels in serum

On the 22nd day, blood samples were collected by the abdominal aortic method at 9:00-11:00 a.m., cooled on ice as soon as possible, and centrifuged at 4 °C. We then took 300 μL of the supernatant sample for radioimmunoassay. Radioimmunoassay kits were used to determine the concentrations of adrenocorticotropic hormone (ACTH), CORT, and corticotropin-releasing hormone (CRH) in serum samples according to the manufacturer's instructions. A gamma radioimmuno counter and a computer RIA data

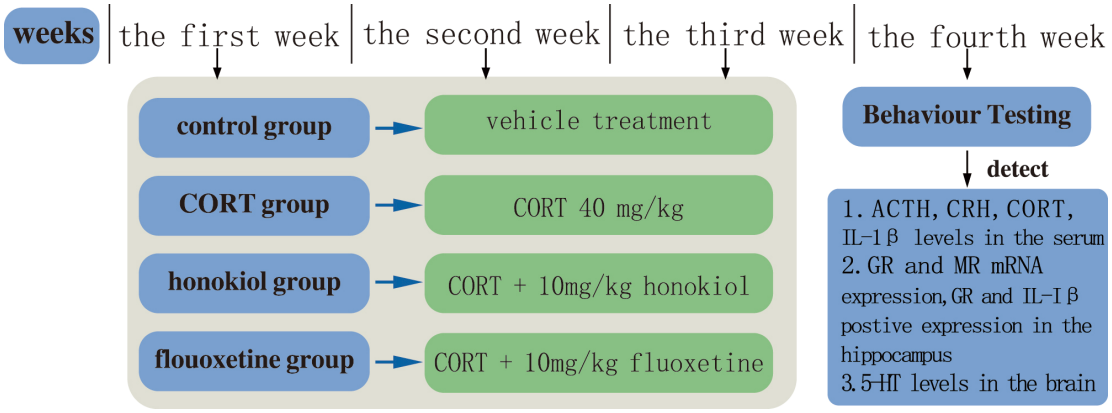

Fig. 1. A detailed schedule of the experimental design. The CORT group, honokiol-treated group, and fluoxetine-treated group received a subcutaneous injection of CORT every day for 21 days. The control group was injected with an equal volume of vehicle subcutaneously. In the honokiol-treated group, rats were intragastrically administrated with honokiol once a day. In the fluoxetine-treated group, rats were intragastrically administrated with fluoxetine. The behavioral tests were after the last dose on day 21. The rats were then sacrificed to evaluate the relevant biochemical parameters. Abbreviations: ACTH, adrenocorticotropic hormone; CRH, corticotrophin-releasing hormone; CORT, corticosterone; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; IL-1β, interleukin-1β; 5-HT, 5-hydroxytryptamine.

analysis program were used to obtain the radioactivity count of each tube precipitation, and the standard curve and sample concentration were obtained.

2.6 Analysis of pro-inflammatory cytokine (IL-β) and 5-HT levels

2.6.1 Detection of IL-β in serum

Following the behavioral testing, blood samples were collected by an abdominal aortic method, immediately cooled on ice, and centrifuged at 4 °C. 100 μL of the supernatant sample was taken, and the ELISA kit was used to determine the concentration of IL-1β in the serum sample according to the manufacturer's instructions.

2.6.2 Detection of 5-HT in the brain

The rats were decapitated, and their brains were quickly taken out and placed on ice. Then the cerebral cortex and hippocampus were dissected. After separation, the cerebral cortex was put in a centrifuge tube containing 4 °C saline at four times the volume of tissue, and an electric homogenizer was used to thoroughly homogenize the tissues. The homogenized tissue was then rapidly put on ice. Following the ELISA kit instructions, we determined the 5-hydroxytryptamine (5-HT) content in the supernatant.

2.7 RT-PCR detection of hippocampal GR and MR gene expression

The hippocampus of five rats from each group was dissected from the brains. Based on the Hipure Total RNA mini kit instructions, the total RNA was extracted. The ultraviolet spectrophotometer (UV-2000, Unico, Shanghai, P. R. China) was used to detect the RNA concentration. We used the RevertAid first-strand cDNA synthesis kit to synthesize cDNA. According to the manufacturers' instructions, we used the cDNA and the SYBR PCR master mix to conduct the reverse transcription on the T100 Thermal Cycler PCR machine (Bio-Rad, USA). Amplification and quantitative detection were performed in a Real-Time PCR machine (Bio-Rad, USA). The RT-PCR protocol was as follows: initial denaturation for 10 min at 95 °C, then 45 cycles for 10 s at 95 °C, and finally 59 °C for 60 s. The following primers sequences for target genes were used:

MR F: 5' CAAGGAGCCATCGGTGAACT 3'

MR R: 5' AGAGGAGTTGGCTGTTCGTG 3' (341bp);

GR F: 5' GGCTTCTGTCTAGAATATGCCTG 3'

GR R: 5' GATTTGTCCCCATTATATAGCCTT 3' (156bp);

β-actin F: 5' GCACCATGAAGATCAAGATCATT 3'

β-actin R: 5' TAACAGTCCGCCTAGAAGCATT 3' (172bp).

The $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) was used for relative quantitative analysis of the results.

2.8 Immunohistochemical assay for GR and IL-1βR in the hippocampus

The other five rats per group were perfused with icy phosphate buffered-saline (PBS) transcardially, and their brains were fixed in 4% paraformaldehyde at 4 °C for 24 hours. Conventional coronal paraffin sections were then prepared. Paraffin sections were placed in xylene for dewaxing, and then the sections were placed in citric acid antigen retrieval buffer (pH 6.0) for antigen retrieval. At room temperature, we used 3% hydrogen peroxide to treat the sections for 15 minutes. The treated sections were then incubated in 5% goat serum (30 minutes, 37 °C). Then sections were incubated overnight at 4 °C with primary antibodies against GR (1 : 400) and interleukin-1β receptor (IL-1βR) (1 : 200) and then incubated with horseradish peroxidase-labeled goat anti-rabbit secondary antibody (1 : 200) for 50 minutes at room temperature. The sections were visualized with DAB coloring solution, counterstained with hematoxylin, dehydrated, and fixed with neutral gum. The microscopic examination showed that the nucleus of hematoxylin was stained blue, and the positive expression of DAB was brownish-yellow. Photographs were collected using a B034 fluorescence inverted biomicroscope (Nikon Eclipse Ti-SR, Japan) and a panoramic scanner (Pannoramic MIDI, 3D HISTECH, Hungary). We quantitatively analyzed the integrated optical density (IOD) of positive GR and IL-1βR of the CA1 region using Image-J software (The National Institutes of Health, Bethesda, MD, USA).

2.9 Statistical analysis

Data were expressed as mean ± SEM and analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. Statistical analysis was performed using SAS 8.2 (IBM, Armonk, NY, USA) and GraphPad Prism 6.01 (GraphPad Software Inc, San Diego, CA, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1 Effect of honokiol on the sucrose preference test

SPT is one of the most commonly used behavioral tests for depression. We applied the SPT to assess the antidepressant effect of honokiol on corticosterone-induced rats exhibiting depressive behavior. Compared with the control group, the sucrose preference of the CORT group was significantly lower ($P < 0.01$) (Fig. 2), while significantly greater after treatment with honokiol (10 mg/kg) and fluoxetine (10 mg/kg) ($P < 0.01$, $P < 0.05$) (Fig. 2).

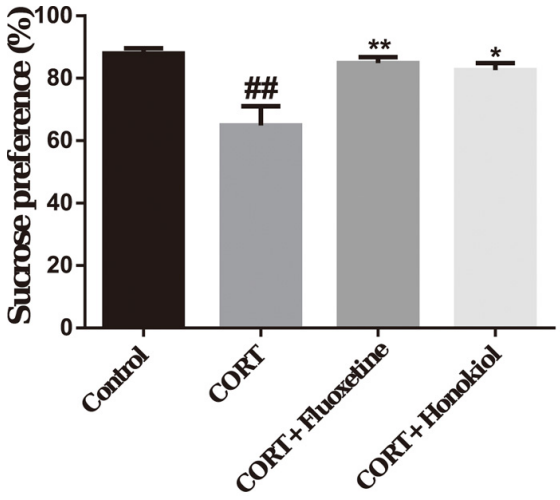

Fig. 2. The effects of honokiol on the sucrose preference test. Honokiol can increase the sucrose preference rate of corticosterone-induced depressive rats. Data are expressed as mean ± SEM (n = 10/group). $^{\#\#}P < 0.01$ vs. the control group; $^{**}P < 0.01$, $^{*}P < 0.05$ vs. the CORT group.

3.2 Effects of honokiol on open field test

OFT is another commonly used behavioral test for depressive behavior. Compared with the control group, the horizontal movement score, vertical movement score, and total movement score of the CORT group were significantly lower ($P < 0.01$, $P < 0.001$) (Fig. 3). Treatment with honokiol (10 mg/kg) and fluoxetine (10 mg/kg) resulted in significantly greater horizontal movement scores, vertical movement scores, and total movement scores ($P < 0.05$, $P < 0.01$) (Fig. 3).

3.3 Effect of honokiol on CORT, ACTH, CRH levels in serum

To measure the regulatory effects of honokiol on the HPA axis, the concentrations of ACTH, CORT, and CRH in serum were determined by radioimmunoassay. Compared with the control group, the serum levels of ACTH, CORT, and CRH were significantly greater in the CORT group (CORT and CRH: $P < 0.05$; ACTH: $P < 0.01$) (Fig. 4). On the contrary, the levels of CORT and ACTH in honokiol group and fluoxetine group were significantly lower than those in CORT group ($P < 0.05$, $P < 0.01$) (Fig. 4), the levels of CRH in honokiol group were significantly lower than those in CORT group ($P < 0.05$) (Fig. 4).

3.4 Effects of honokiol on IL-1β and 5-HT levels

The serum concentrations of IL-1β and 5-HT were determined by the ELISA kit to measure the anti-inflammatory effects of honokiol. Compared with the control group, the levels of IL-1β were significantly increased in the serum of the CORT group rats ($P < 0.01$). Treatment with honokiol (10 mg/kg) significantly reduced the levels of IL-1β ($P < 0.01$) (Fig. 5).

The content of 5-HT in the brain tissue of the CORT group was significantly lower in comparison with the control group ($P < 0.001$); however, after treatment with honokiol (10 mg/kg) and fluoxetine (10 mg/kg), the levels of 5-HT were significantly greater ($P < 0.01$) (Fig. 5).

3.5 Effects of honokiol on mRNA expression of hippocampal GR and MR

Real-time quantitative PCR was used to detect the expression of GR and MR mRNA in the hippocampus to assess whether corticosterone-induced depression involved the involvement of GR and MR.

In the CORT group, the expression of GR mRNA in the hippocampus was significantly lower than in the control group ($P < 0.05$) (Fig. 6). After treatment with honokiol, the mRNA expression of GR was significantly greater ($P < 0.05$) (Fig. 6).

The mRNA expression of MR in the hippocampus was significantly higher in the CORT group compared with the control group ($P < 0.05$) (Fig. 6). Compared with the CORT group, there was no difference in mRNA expression of the MR gene after treatment with honokiol and fluoxetine (Fig. 6).

The GR/MR ratio in the CORT group rats was significantly lower than the control group ($P < 0.01$) (Fig. 6). Compared with the CORT group, the GR/MR ratio was significantly greater following honokiol treatment ($P < 0.05$) (Fig. 6).

The above results indicate that honokiol normalizes the HPA axis function in CORT treated rats by mediating the expression of MR and GR in the hippocampus.

3.6 Effects of honokiol on positive expression of hippocampal GR and IL-1βR

Immunohistochemistry was used to assess the expression of GR and IL-1βR in the hippocampus. The expression of GR was significantly lower in the CORT group compared with the control group ($P < 0.05$) (Fig. 7). Compared with the CORT group, the honokiol group and the fluoxetine group had significantly greater hippocampal GR ($P < 0.01$, $P < 0.05$) (Fig. 7).

The expression of IL-1βR was significantly greater in the CORT group compared with that of the control group, suggesting CORT induced inflammation in the CA1 region of the hippocampus ($P < 0.01$) (Fig. 7). Compared with the CORT group, the honokiol group had significantly lower hippocampal CA1 region IL-1βR expression ($P < 0.01$) (Fig. 7).

These results indicate that honokiol can effectively inhibit the GR reduction caused by corticosterone administration. Also, honokiol has a significant anti-inflammatory effect.

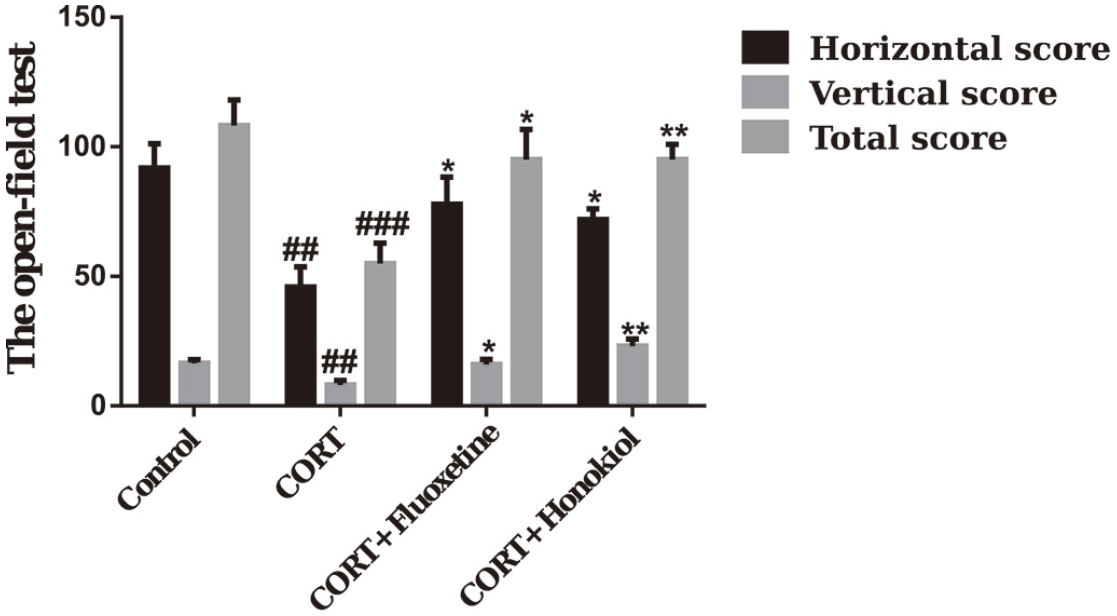

Fig. 3. The effects of honokiol on the open field test. Honokiol can increase the horizontal movement score, vertical movement score, and total movement score of corticosterone-induced depressive rats. Data are expressed as mean ± SEM (n = 10/group).$^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$ vs. the control group; $^{**}P < 0.01$, $^{*}P < 0.05$ vs. the CORT group.

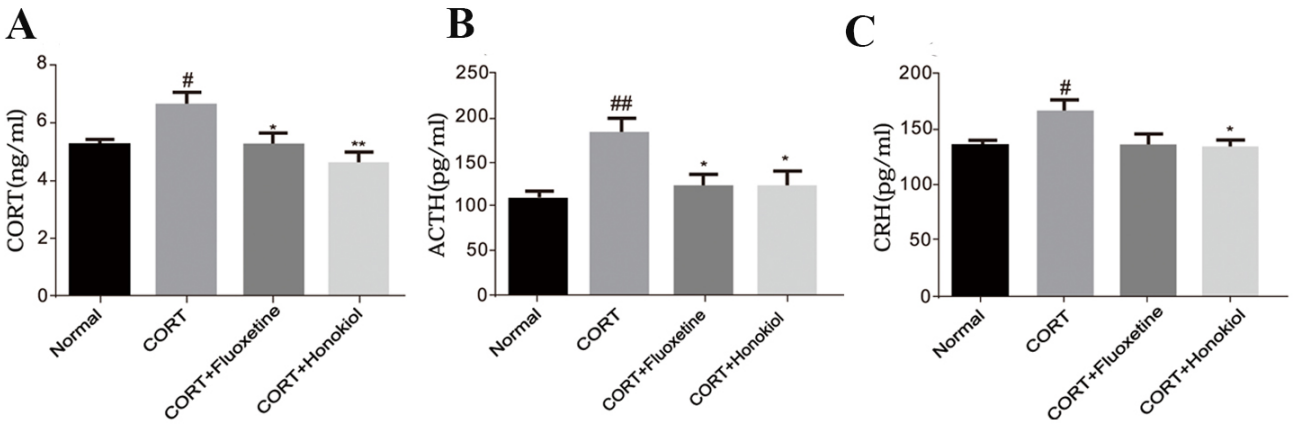

Fig. 4. The effects of honokiol on adrenocorticotropic hormone (ACTH), corticosterone (CORT), corticotrophin-releasing hormone (CRH) levels in serum. Honokiol can significantly reduce the levels of CORT, ACTH, and CRH in the serum. (A) levels of CORT in serum; (B) levels of ACTH in serum; (C) levels of CRH in serum. Data are expressed as mean ± SEM (n = 10). $^{**}P < 0.01$, $^{*}P < 0.05$ vs. the control group; $^{**}P < 0.01$, $^{*}P < 0.05$ vs. the CORT group.

4. Discussion

The rodent CORT induced model of depression is suitable for exploring the molecular mechanisms of depression related to HPA axis dysfunction and can simulate depression caused by exposure to stress in humans (Gourley et al., 2013; Thibaut, 2019). The sucrose preference and exploratory activity significantly decreased after the rats received repeated corticosterone injections. These results indicate the successful application of the CORT induced depression model. Additionally, sucrose preference and exploratory activity increased after treatment with honokiol, demonstrating honokiol has antidepressant-like effects on depressed rats. Honokiol can ameliorate depression-like effects of chronic CORT, similar to the widely used antidepressant, fluoxetine.

HPA axis dysfunction is a critical etiological factor in depression. The current data support these findings, as the corticosterone group exhibits HPA axis hyperactivity and depressive-like behavior compared with the control group. The levels of CORT, ACTH, and CRH in the serum of depressed rats caused by repeated corticosterone injections were higher than those of the control group but were lower after treatment with honokiol. CORT, ACTH, and CRH are all hormones essential to the regulation of the HPA axis. The HPA axis can maintain a steady-state of the internal environment through the negative feedback mechanism (van Bodegom et al., 2017). When the body is under intense stress, the HPA axis is stimulated, and neurons in the paraventricular nucleus of the hypothalamus synthesize and secrete CRH. CRH is delivered to the

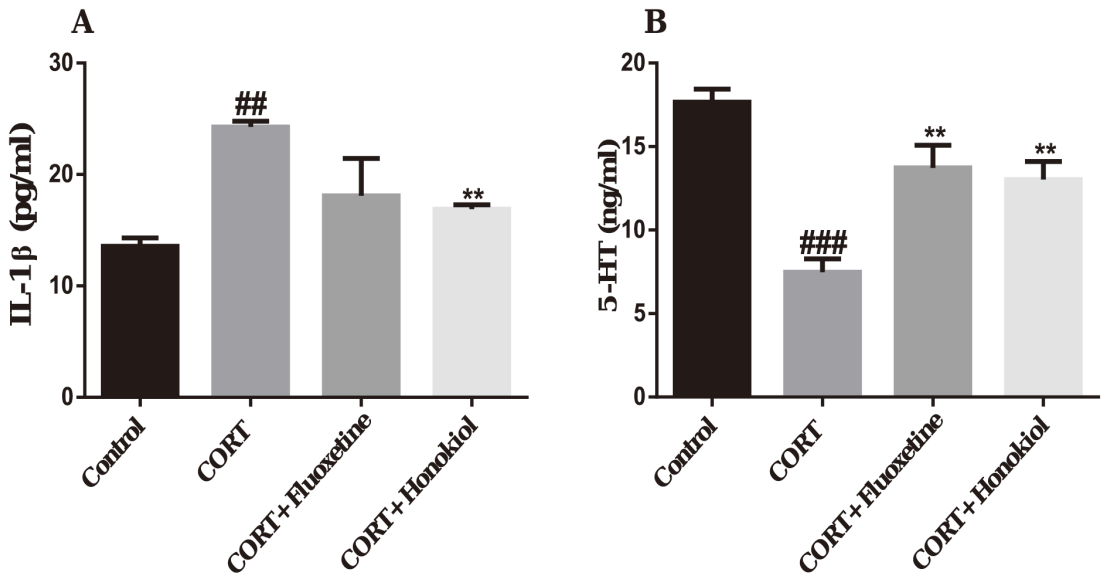

Fig. 5. The effects of honokiol on interleukin-1β (IL-1β) and 5-hydroxytryptamine (5-HT) levels. Honokiol can significantly reduce the levels of IL-1β in serum and increase the levels of 5-HT in brain tissue. (A) The levels of IL-1β in serum; (B) The levels of 5-HT in brain tissue. Data are expressed as mean ± SEM (n = 10). $^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$ vs. the control group; $^{**}P < 0.01$ vs. the CORT group.

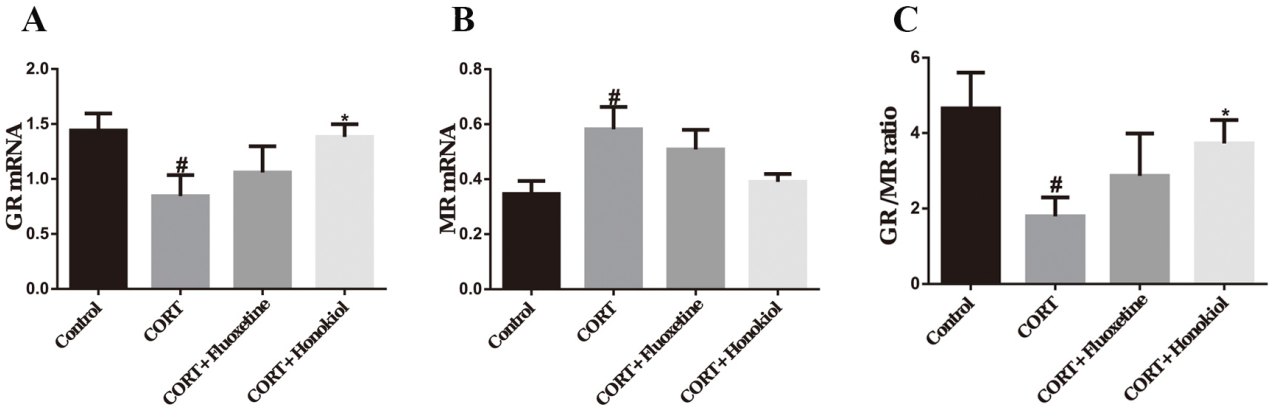

Fig. 6. The effects of honokiol on mRNA expression of hippocampal GR and MR. (A) mRNA expression of GR in the hippocampus; (B) mRNA expression of MR in the hippocampus; (C) The GR/MR ratio in the hippocampus. Data are expressed as mean ± SEM (n = 5). $^{\#}P < 0.05$ vs. the control group; $^{*}P < 0.05$ vs. the CORT group.

anterior pituitary by the portal vein system of the pituitary tract and stimulates ACTH release by adrenocorticotropic hormone cells. ACTH reaches the adrenal gland through systemic circulation and promotes the release of GC from the adrenal gland (cortisol in primates, corticosterone in rodents) (Ulrich-Lai and Herman, 2009; Watson and Mackin, 2006). Our results indicate that the HPA axis activity of depressed rats is increased, and honokiol is likely to improve depression-like behavior by reducing levels of CORT, ACTH, and CRH. Abnormal elevations of CORT, ACTH, and CRH can induce depressive symptoms and signs (Sun et al., 2020). This may be due to an impairment of learning and memory caused by the increase of CRH and ACTH, as excessive exposure to CORT can cause learning deficits and emotional instability.

The mRNA expression of GR in the hippocampus of depressed rats was lower than controls but returned to control levels after treatment with honokiol. These results indicate that honokiol can reduce HPA axis activity by regulating GC and GR mediated negative feedback to exert antidepressant effects in depressed rats. The hippocampus is a key regulatory center of HPA axis activity and the stress response, which can mediate the recovery from hyperactive HPA axis activity resulting from chronic stress (Belleau et al., 2019). There are two steroid hormone receptors in the hippocampus, GR and MR. MR has a strong affinity for GC, and its expression in the central nervous system is mainly concentrated in the marginal area. It maintains the basal level of cortisol in circulation when the body is in a normal state (Micale and Drago, 2018). GR has a much weaker affinity for GC than MR. GR contributes to the circadian secretion of cortisol and the regulation of stress-related cortisol secretion (Odeon et al., 2017). The hippocampus maintains HPA axis activity stability through the regulation of MR and the negative feedback mechanism mediated by GR and GC. The body exhibits HPA axis hyperactivity and secretes large amounts

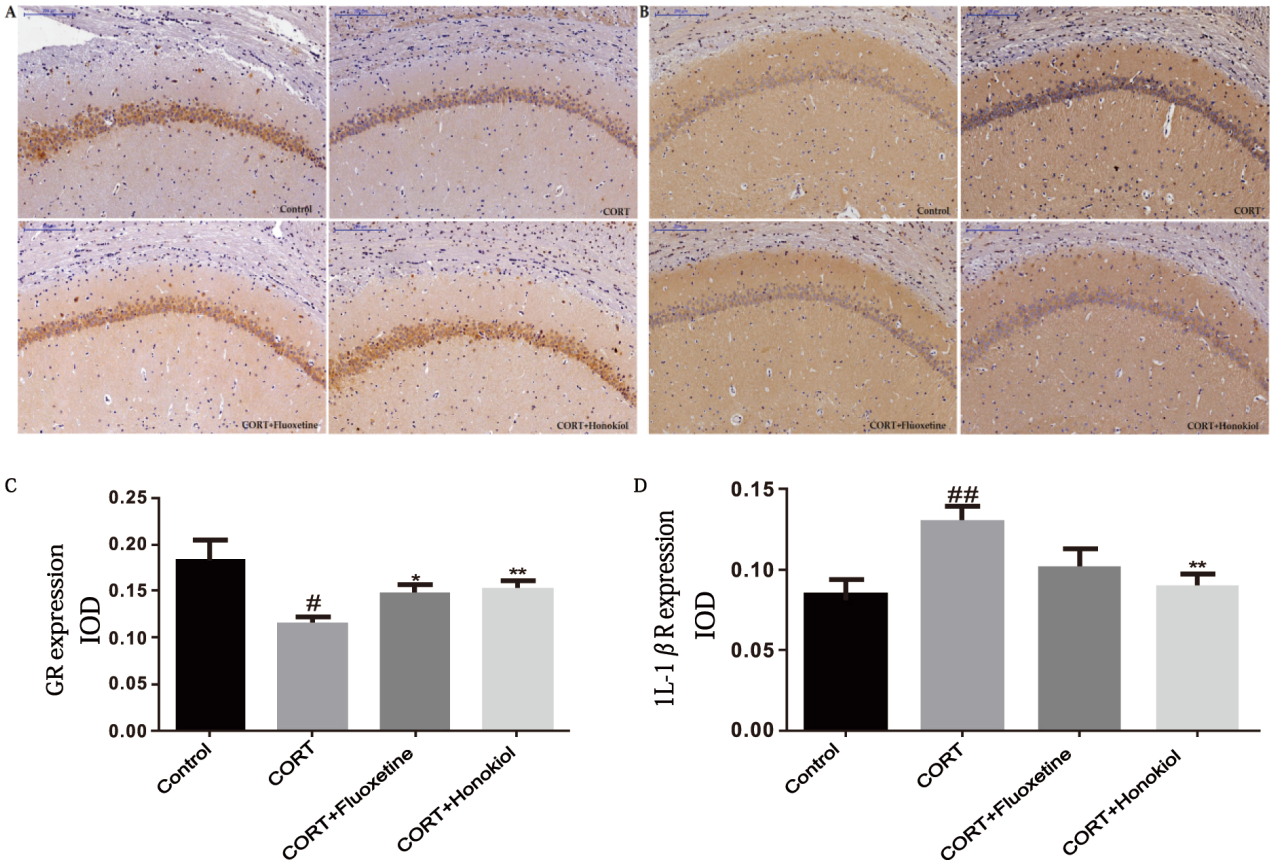

Fig. 7. The effects of honokiol on the expression of GR and IL-1βR in the hippocampal CA1 region of rats. Honokiol significantly increased the positive expression of GR and decreased the expression of IL-1β in the hippocampus. (A) GR expression was assessed by immunohistochemistry (magnification 50×, Scale bar = 200 μm); (B) IL-1βR expression was examined by immunohistochemistry (magnification 50×, Scale bar = 200 μm); (C) Bar graph for semi-quantitative analysis of GR by quantitative integrated optical density (IOD); (D) Bar graph for semi-quantitative analysis of IL-1βR by quantitative integrated optical density (IOD). Data are expressed as mean ± SEM (n = 5). $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ vs. the control group; $^{**}P < 0.01$, $^{*}P < 0.05$ vs. the CORT group.

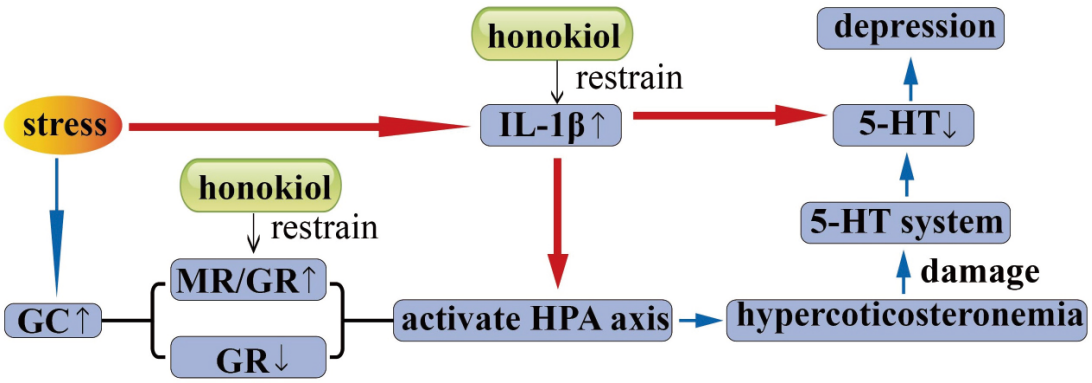

Fig. 8. Graphical abstract. Honokiol regulates HPA axis activity by restoring the GR-mediated negative feedback mechanism and regulating the balance between GR and MR, thereby exerting an antidepressant effect. The antidepressant effect is also related to the suppression of inflammatory responses. CORT, corticosterone; GC, glucocorticoids; MR, mineralocorticoid receptor; GR, glucocorticoid receptor; HPA, hypothalamus-pituitary-adrenal axis; IL-1β, interleukin-1β; 5-HT, 5-hydroxytryptamine.

of glucocorticoids when it is subjected to chronic stress.

Furthermore, glucocorticoids will be maintained at high levels during chronic stress exposure. The high concentration of glucocorticoids repeatedly stimulates GRs, inducing GR dysfunction, with deficits in the negative feedback mechanism. The result is ongoing HPA axis hyperactivity and damage to the hippocampus, inducing a maladaptive cycle (Bellavance and Rivest, 2014; Gądek-Michalska et al., 2013). This process is considered to be the most

substantial physiological and biochemical basis of stress-induced depression. In general, GR-mediated negative feedback regulation plays a vital role in maintaining the typical activity of the HPA axis. Repeated corticosterone injections can reduce GR mRNA expression in the hippocampus in rats, accompanied by HPA axis hyperactivity and depression-like behavior. However, these adverse effects of CORT are successfully treated with honokiol. This indicates that the antidepressant effects of honokiol are related to the restoration of GR mediated negative feedback.

The results also demonstrated that the ratio of GR/MR in depressed rats was significantly lower, which was ameliorated with honokiol. These results suggest that honokiol can restore HPA axis activity by regulating the balance between GR and MR, and exhibits an antidepressant-like effect in depressed rats. The ratio of GR/MR plays a vital role in neuronal excitement, stress response, and adaptability to stress, and the pathogenesis of depression. The regulatory functions of the two types of receptors are closely interrelated (Pariante and Miller, 2001). Studies have shown that defects in MR can cause a more rapid corticosterone response and produce a more pronounced GR-mediated effect.

Moreover, the imbalance of the GR/MR ratio stimulates hippocampal neurons to transition to a vulnerable state where they are prone to stress-related diseases, including depression (de Kloet et al., 2018; Lucassen et al., 2001). An imbalance in GR and MR may result in damage to the hippocampus. The hippocampus itself has an inhibitory effect on the HPA axis activity, and this may be a factor in increased HPA axis activity. Repeated injections of corticosterone can cause a decrease in the GR/MR ratio in rats, accompanied by depression-like behaviors. After treatment with honokiol treatment prevents the development of this pathological state. Therefore, the mechanism of the antidepressant effect of honokiol may be related to regulating the balance between GR and MR.

Damaged to the GR negative feedback mechanism and GR/MR imbalance will result in excessive activation of the HPA axis, resulting in increased CORT, which is the final metabolite of the HPA axis, leading to hypercortisonemia. There is a key functional interaction between the HPA axis and the 5-HT system. Elevated CORT levels can damage the 5-HT neural pathway of the raphe nucleus-hippocampus system, resulting in an array of depression symptoms such as depression, inattention, memory loss, and insomnia (Pérez-Ortiz et al., 2013; Snyder et al., 2011).

The antidepressant effect of honokiol is likely to be related to its anti-inflammatory effect. Repeated corticosterone injections can increase IL-1β levels in serum and positive expression of IL-1βR in the hippocampus, which is prevented after treatment with honokiol. Proinflammatory cytokines, especially IL-1β, have regulatory actions on the HPA axis. IL-1β is a vital messenger substrate in the HPA axis. It can act on CRH neurons in the hypothalamus and promote CRH release, thereby activating the HPA axis and causing elevated glucocorticoid levels. Long-term accumulation of high levels of glucocorticoids is a direct cause of depression (Zheng et al., 2019). Also, high levels of IL-1β in the brain can directly damage neurons or alter the internal environment in which neurons survive, impairing the ability of neurons to regenerate and repair and causing depression (Kurczewska et al., 2019; Zhang et al., 2019). IL-1β can also affect the 5-HT system, and one possible mechanism for these effects is that IL-1β mediated reduction of 5-HT concentrations in synaptic space through the promotion of 5-HT reuptake in midbrain and striatum (Zhang et al., 2007). 5-HT is a neurotransmitter which is widely studied in the field of depression. It not only regulates emotion, energy levels, and memory but also reduces the damaging effects of excitatory neurotoxins on neurons. Decreased 5-HT concentrations can lead to depression (Jang et al., 2019).

The potential mechanisms by which honokiol exerts antidepressant-like effects in the CORT induced rat model of depression are shown in (Fig. 8).

5. Conclusions

In conclusion, the results reveal that honokiol has significant antidepressant-like effects on corticosterone induced depressive rats. The mechanism of these effects may be that honokiol regulates HPA axis activity by restoring the GR-mediated negative feedback mechanism and regulating the balance between GR and MR. This antidepressant-like effect may also be related to the suppression of inflammatory responses.

Ethics approval and consent to participate

The Experimental Animal Ethics Committee of the Academic Committee of Beijing University of Chinese Medicine approved all the steps involving animals in the experiment (project identification code: BUCM-4-2019021502-1012).

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Conflict of Interest

The authors declare no competing interests.

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