Antidepressant-like effects of albiflorin involved the NO signaling pathway in rats model of chronic restraint stress

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[ABSTRACT] The antidepressant-like effects of albiflorin (AF) were studied on stressed chronic restraint stress (CRS) rats. Experimental rats were subjected to immobilization stress for a daily 6 h-restraining in a plastic restrainer for continuous 21 d and were treated with 30 or 15 mg·kg⁻¹ of AF for 21 d. Control rats were maintained in completely non stressed conditions. Behavioral tests and biochemical analysis were applied to investigating a regulatory mechanism of anti-stress of AF. Treatment with AF significantly restored the depressant-like behaviors. Besides, AF increased the levels of 5-hydroxytryptophan (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline (NE) and dopamine (DA) in the hippocampus and increased the level of brain-derived neurotrophic factor (BDNF) in serum and protein expression in hippocampus. In addition, AF decreased the levels of hypothalamo-pituitary-adrenal (HPA) cascade, reduced the level of NO and cGMP in serum and inhibited the overexpression of 5-HT₁A mRNA and protein expression. Taken together, AF can modulate the NO-mediated network pathway in the hippocampus against stress-induced depressive-like behaviors. These physiological and behavioral changes allow rats to avoid potential deleterious effects of stress that may result from chronically elevated levels of glucocorticosteroids over days.

[KEY WORDS] Paeonia lactiflora; Albiflorin; Chronic restraint stress; Anti-stress; NO-mediated pathway

[CLC Number] R965

Introduction

Depression is one of the most common psychiatric disorders [¹]. Previous studies have demonstrated that stress and monoamine deficiency were two major causes of depression [²]. Stress causes pathological alterations in the HPA axis [³], as a neuroendocrine-immune network hub, mainly effects on the stability in a human body by physiological and psychological reacting to the environmental changes and regulating emotions [⁴]. Study showed that depression patients with overactive HPA axis mainly present elevated levels of corticotropin releasing hormone (CRH) in the central, peripheral nervous system and metabolites, adrenocorticotropic hormone (ACTH) secretion and cortisol level [⁵]. Then, serotonin regulates mood, reactivity to psychological stress, self-control, motivation, and cognitive performance. Meanwhile, 5-HT and 5-HIAA also deeply influence emotions [⁶]. On the other hand, hippocampus plays an important role in memory, emotional expression, and navigation [⁷]. It is also a key structure for studying the neurobiological substrates of depression [⁸] due to its involvement in the regulation of the hypothalamo–pituitary–adrenal (HPA) axis and the high number of 5-HT receptors in this region [⁹]. Previous studies have demonstrated that stress and depression lead to reductions in the total volume of the hippocampus, as well as atrophy and loss of neurons in this structure [⁷-⁹]. Studies have even reported that serum BDNF levels are decreased in depressed patients and they can be normalized by antidepressant treatment [¹⁰]. The involvement of brain-derived neurotrophic factor (BDNF), in the regulation of mood disorders and antidepressant effects also has been under intense investigation [¹¹-¹²].

Moreover, basic researches have reported that acute and chronic unpredictable stresses increase plasma glucocorticoid, and downgrade 5-HT₁A receptor binding and mRNA level [¹³-¹⁴]. The clinical study found in severe depression patients level of 5-HT₁A receptor binding in the frontal cortex, temporal lobe cortex, perirhinal cortex reduced widely by determining the 5-HT₁A receptor in the brain [¹⁵]. Furthermore,
the literature supports an important role for 5-HT and the 5-HT$_{2A}$ receptor system in modulating impulsivity. Nitric oxide (NO) pathway has been suggested as a strong influence on depression and other psychological disorders via interactions with other signaling pathways such as serotoninergic, dopaminergic and cholinergic signaling. Clinical studies showed elevated plasma nitrate levels and increased nitric oxide synthase (NOS) expression in the hippocampus of depressed patients. Nitric oxide production in mammalian cells is a result of the enzymatic oxidation of L-arginine by NO synthases (NOS). Both the inhibition of soluble guanylate cyclase and weakening NOS activity may decrease the level of cGMP and may produce antidepressant-like behaviors. Nitric oxide has been proposed to modulate synaptic transmission in several ways; the commonest one is through activation of guanylate cyclase which is responsible for an increase in cGMP. More importantly, nitric oxide has been involved in the release of several neurotransmitters. In the hippocampus and dorsal raphe nucleus (DRN), NO provides a tonic stimulus to maintain elevated levels of 5-HT release in regulating anxiety-related behaviors.

The root of *Paeonia lactiflora* Pall. (family Ranunculaceae, Baishao in Chinese) is often used in Chinese herbal formulas, including Chaihu-Shugan-San (including Baishao), for the treatment of depression-like disorders. In this component, paeoniflorin and albiflorin were deduced as the active constituents of Chaihu-Shugan-San from Baishao, with metabonomics plus chemical fingerprinting.

Albiflorin (AF) is recognized as an isomer different from paeoniflorin (PF). Their chemical structure and HPLC chromatograms were shown in our previous papers and researches showed that PF and AF possessed remarkable effects. PF exert an antidepressant effect in the menopause depression rats via regulating the expressions of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor. Comparatively, AF-mediated antidepressant effect in chronic unpredictable mild stress (CUMS)-induced rat model of depression, it not only regulates the serotonergic system, but also the dopaminergic system. AF also produced significant antidepressant-like effects closely related to the hippocampal 5-HT/NE increase and BDNF expression. However, anti-depressant effect of AF in rat models of chronic restraint stress (CRS) via the NO possible mechanisms is still not included.

In this paper, we hypothesized that anti-stress effect of AF via NO-mediated pathway in the rat model of CRS. The dosages of AF used were 30 and 15 mg·kg$^{-1}$ based on our previous studies. We aim to investigate the antidepressant-like effect of AF in chronic restraint stress (CRS) induced rat model of depression and examine the anti-stress effects of AF. Firstly, changes of the depressant-like behaviors were tested, including the sucrose solution consumption, open field test, and elevated plus maze. Secondly, the serum levels of CRH, ACTH, and corticosterone (CORT), the levels of 5-HT, 5-HIAA, NE, DA, NA, and Homovanillic acid (HVA) in the hippocampus were explored. Furthermore, the levels of NO and cGMP in the hippocampus and the nNOS mRNA expression and protein expression were detected. Moreover, the levels of BDNF and protein expression in the hippocampus were included. In addition, to further analyze the mechanism, the 5-HT$_{1A}$R and 5-HT$_{2A}$R mRNA expression and protein expression were analyzed.

**Materials and Methods**

**Drugs**

Albiflorin (purity = 96.7%, HPLC) was prepared in our library (Patent No. ZL 201110184287, China). AF was separately dissolved in 0.9% normal saline and diluted to the desired concentration on the day of testing.

**Animals**

Sprague Dawley rats (male, 9 weeks, 180–220 g) were obtained from the Vital River Co., Ltd. (Beijing, China) and maintained at a controlled temperature (22 ± 2°C) and humidity (50% ± 10%) with a 12 h light/ dark cycle. All efforts were made to minimize the pain of animals. The experimental protocol was approved by the Ethics Committee of Beijing University of Chinese Medicine (No. BUCM-4-2016012002-1002).

**Chronic restraint stress (CRS)**

After 7 d of acclimatization, rats were divided into five groups ($n = 12/group$): (1) Normal control group (Control), (2) Chronic restraint stress group (Model), (3) Fluoxetine treatment group (a positive control, 2.0 mg·kg$^{-1}$), (4) High-dose AF treatment group (30 mg·kg$^{-1}$) and (5) Low-dose AF treatment group (15 mg·kg$^{-1}$).

Except for the control group, rats were restrained in wire mesh restrainers, secured at the head and tail ends with large binder clips. CRS rats were administered for 6 h daily for 21 d from 10:00 to 16:00. CRS rats were returned to their cages immediately after termination. All animals were sacrificed by decapitation roughly 24 h after the last stress (i.e. between 1300 and 1700 h). Brains were removed and flash frozen on liquid nitrogen and then stored at −80°C until processing.

**Drugs and administration**

All rats except the control and model were intragastrically (i.g.) pretreated with Fluoxetine (2.0 mg·kg$^{-1}$) and AF (30 and 15 mg·kg$^{-1}$) for 21 d. The control and model groups administered an equivalent volume of saline water, once a day for 21 d.

**Behavioral tests**

**Open-field test**

After treatment, in a quiet room, rats were individually placed in the central wood open-field apparatus (80 cm × 80 cm × 40 cm) with a black surface covering the inside walls and the floor divided into 25 squares (16 × 16 cm). When the hind legs of rat crossed the line of the squares, it was considered to have crossed from one square to another.
Table 1  Primers used for quantitative RT-qPCR

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward (5′–3′)</th>
<th>Reverse (5′–3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>TGCTATGTGGCCCTAGACTTCCG</td>
<td>GTGGCATAAGGGTCTTTAGGG</td>
</tr>
<tr>
<td>5-HT1A R</td>
<td>GCGTTGTGGGGTGTCCTAAT</td>
<td>GCGAGAAGTCAGGCACATTG</td>
</tr>
<tr>
<td>5-HT2A R</td>
<td>ATAGCCTGATATGCTGGTGGGTT</td>
<td>AAAGAGCACATCCAGGTAAATCC</td>
</tr>
<tr>
<td>nNOS</td>
<td>TCGAGGGAGGGGAGGTGTTGA</td>
<td>AAGGCGGTTTGTCACCTCATA</td>
</tr>
</tbody>
</table>

(crossing); while the forelegs lift from the floor, the rat was considered to have gotten one point (rearing). The scores of rats during 5 min were recorded. The wood-open-field apparatus was cleaned with an ethanol solution (70%) and dried after occupancy by each rat.

Elevated plus-maze

Rats were placed in the center of the elevated plus-maze facing one of the enclosed arms and allowed to explore for 5 min. The number of entries and the time spent on open or enclosed arms were further analyzed, which were recorded during all experimental session. The maze was cleaned with an ethanol solution (70%) after each rat.

An arm entry was defined as all four paws entering the arm. Ethological measures included the frequency of stretched attend postures, head dipping (exploratory movement in which the animal’s head is protruding over the side of the open arm and down toward the floor), and rearing. The percent of open arm entries (%OAE) and time spent in the open arms (%TOA) were calculated for each rat, considering the following formula: 100% × [open/(open + enclosed)].

Sucrose preference test

Two 200 mL bottles of 1% sucrose water were located in every cage at the first 2 days. After 24 h food and water deprivation, one bottle of 1% sucrose water and one bottle of tap water were given to the rats in the cage. Finally, the consumption amount of tap water and 1% sucrose water were determined in the next 1 h. The sucrose preference index was calculated as follows: sucrose consumption = sucrose consumption/total fluid intake × 100%.

Biochemical analysis

Determination of serum CRH, ACTH, CORT

On the last day of the study, blood samples were collected and centrifuged at 3000 r·min⁻¹ for 15 min to obtain serum. CRH, ACTH, CORT was measured by rat enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer’s instructions. Absorbance was measured at 450 nm.

Determination of monoamine neurotransmitter levels

Levels of hippocampal monoamine neurotransmitters and their metabolites were detected by HPLC-ECD. The tissues of rats were homogenized in an ice-cold tissue lysis buffer including 0.4 mol·L⁻¹ HClO₄ (6.6 μL·mg⁻¹), 0.5 mmol·L⁻¹ Na₂EDTA and 0.1 g·L⁻¹ of L-cysteine, then samples were centrifuged at 15 000 r·min⁻¹ for 15 min, and filtered through a 0.45 μm pore membrane. Firstly, the standard solution or sample was injected into the reversed-phase Sun Fire™ C₁₈ column (5 μm, 250 mm × 4.6 mm). Moreover, a separation was performed in isocratic elution mode at a column temperature of 20 °C using a mobile phase containing 85 mmol·L⁻¹ citric acid and 0.1 mol·L⁻¹ sodium acetate buffer (pH 3.7) with 15% methanol, 0.9 mmol·L⁻¹ sodium octanesulfonate, and 0.2 mmol·L⁻¹ Na₂EDTA at a flow rate of 1.0 mL·min⁻¹. Finally, the contents of the monoamine neurotransmitters and their metabolites were expressed as ng/g wet weight of tissue.

Assessment of hippocampal nitrite level

The hippocampal homogenates were centrifuged and the Griess reagent [1% sulfanilamide, 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride, 2.5% H₃PO₄] was added to the supernatants. To determine the nitrite concentration, a major stable product of nitric oxide metabolism, the absorbance at 540 nm was measured using a standard plate reader, followed by incubation at 37 °C for 30 min.

Assessment of hippocampal levels of BDNF and cGMP

Hippocampal samples were prepared, and the hippocampal levels of BDNF and cGMP were measured using ELISA kits.

Measurement of hippocampal 5-HT₁₄R, 5-HT₂₃R, nNOS mRNA expression

The total RNA was isolated using Trizol (Invitrogen, Carlsbad, CA, USA), according to the manufacturer’s instructions, and the concentration was determined using a BioPhotometer (Eppendorf, Hamburg, Germany). Then total RNA from each sample was reverse-transcribed into cDNA using a Super RT cDNA kit, and the synthesized cDNA was used for RT-qPCR amplification using SYBR Green Real-time PCR Master Mix. Furthermore, the nucleotide sequences of forward and reverse primers used for PCR are shown in Table 1. The cycling conditions were 95 °C for 10 min, followed by 40 cycles of 60 °C for 15 s, 75 °C for the 60 s, and 95 °C, temperature rise once 1 °C per 20 s. The RT-qPCR analysis was performed with the Light Cycler 480 RT-qPCR System (Roche, Basel, Switzerland).The results of relative mRNA expression in each group were semi quantitated using the comparative Ct method.

Measurement of hippocampal 5-HT₁₄R, 5-HT₂₃R, nNOS and BDNF protein expression

The total protein in hippocampus was extracted with lys−is buffer (50 mmol·L⁻¹ Tris, pH 8.0, 150 mmol·L⁻¹ NaCl, 0.1% sodium dodecyl sulfate, 0.5% sodium deoxycholate, 100 mg·mL⁻¹ phenylsulfonyl fluoride, 2 mg·mL⁻¹ aprotonin, 1 mg·mL⁻¹ pepstatin, and 10 mg·mL⁻¹ leupeptin), protein was resolved on a 10% sodium dodecyl sulfate polyacrylamide
gel. The fractionated 50 mg proteins were electrophoretically transferred to an immobilon polyvinylidene difuride membrane and probed with antibody of 5-HT\(_{1A}\)R, 5-HT\(_{2A}\)R, nNOS, BDNF (Biosis, Inc., Beijing, China). Then, the targets were detected and quantified by using the Alpha. The data were normalized using ACTIN to ensure equal loading and were expressed as a ratio of the experimental group to the control group.

**Statistical analyses**

Results are expressed as mean ± SD. Statistical significant differences were determined by one-way analyses of variance and Student’s t-tests. A P value < 0.05 indicates a statistically significant difference.

**Results**

**Effects of AF on behavior in rats with chronic restraint stress model**

The effects of AF on bodyweight in CRS rats

On the 21\(^{st}\) day, significant reduction of bodyweight was noted in CRS induced depression-like rats (P < 0.001); meanwhile, on the 21\(^{st}\) day Flu (2.0 mg kg\(^{-1}\)) (P < 0.05), AF 30 and 15 mg kg\(^{-1}\) (P < 0.05) treatment restored the bodyweight to model level (Fig. 1).

The effects of AF on sucrose preference in CRS rats

Sucrose preference is frequently used as a measure of anhedonia in rodents. On the 21\(^{st}\) day, significant reduction of sucrose was noted in CRS induced depression-like rats (P < 0.001); Flu (2.0 mg kg\(^{-1}\)) (P < 0.01), AF 30 mg kg\(^{-1}\) (P < 0.01) treatment increased the sucrose preference to model level (Fig. 2).

The effect of AF on the open-field behaviors in CRS rats

Compared to normal rats, model rats decreased the numbers of crossing (P < 0.01); compared to model rats of depression, Flu and AF 30 mg kg\(^{-1}\) administration increased the numbers of crossing (P < 0.05) respectively. While compared to normal rats, model rats increased the time of rearing (P < 0.01); compared to model groups, Fluoxetine and AF 30 mg kg\(^{-1}\) reduced the time of rearing separately (P < 0.05) (Fig. 3).

The effect of AF on the elevated plus maze behaviors in CRS rats

Exploratory behavior was measured in the elevated plus maze to assess anxiety-like behavior. AF administration at dose of 30 mg kg\(^{-1}\) increased the %OAΕ up to 36.21% (P < 0.05), 2.0 mg kg\(^{-1}\) Flu treatment resulted in 34.28% on the %OAΕ (P < 0.05). AF 30 mg kg\(^{-1}\) increased the %TOA up to 15.12% (P < 0.05), 2.0 mg kg\(^{-1}\) Flu treatment also increased the %TOA to 16.77% (P < 0.01) in the elevated plus maze behaviors test (Fig. 4).

**Effects of AF on levels of CRH, ACTH and CORT in CRS rats**

The serum reproductive hormone results revealed that CORT, ACTH and CRH levels in rats with CRS increased compared with control group (P < 0.001, P < 0.001, P < 0.01). However, when compared with the model group, levels of CORT in both Flu and AF 30 mg kg\(^{-1}\) were significantly decreased (P < 0.01). Compared with the model group, levels of ACTH in Flu and AF 15 mg kg\(^{-1}\) groups were all significantly decreased (P < 0.001). Compared with the model group, levels of CRH in both Flu and AF 30 mg kg\(^{-1}\) were all significantly decreased (P < 0.05) (Fig. 5).

**Effects of AF on the hippocampal monoamine neurotransmitter levels in CRS rats**

The levels of monoamine neurotransmitters and their metabolites detected in the hippocampus are summarized in Table 2. Compared with the control group, the levels of 5-HT (P < 0.01), 5-HIAA (P < 0.01), HVA (P < 0.01), DA (P < 0.001) and NE (P < 0.01) were decreased significantly. The levels of 5-HT (P < 0.01), HVA (P < 0.01), and DA (P < 0.05) were increased after AF (30 mg kg\(^{-1}\)) treatment. Flu (2.0 mg kg\(^{-1}\)) also induced elevations in 5-HIAA (P < 0.01), DA (P < 0.05) and NE (P < 0.05).

**Effect of AF on hippocampus nitrite (NO) level**

Hippocampal NO level in model groups was significantly increased compared with control group (P < 0.001). Administration of AF (30 and 15 mg kg\(^{-1}\)) caused a significant decrease in nitrite levels compared with model group (P < 0.01, P < 0.05). Flu also caused a significant decrease in nitrite levels compared with model group (P < 0.01) (Fig. 6).

**Effect of AF on hippocampus cGMP level**

Hippocampal cGMP level in model groups was significantly increased compared with control group (P < 0.001). Administration of AF (30 and 15 mg kg\(^{-1}\)) caused a significant decrease in nitrite levels compared with model group (P < 0.001, P < 0.05). Flu also caused a significant decrease in nitrite levels compared with model group (P < 0.05) (Fig. 7).

**Effects of AF on mRNA expression and protein level of nNOS in hippocampus**

As shown in Fig. 8, nNOS mRNA and protein expression in brains of model group increased significantly in comparison with that in control group (P < 0.001, P < 0.05), while nNOS mRNA expression in brains of Flu and AF 30 mg kg\(^{-1}\) group were increased in comparison with those in model group (P < 0.05). However, AF 30 mg kg\(^{-1}\) had no effect on the nNOS protein expression.

**Effects of AF on mRNA expression of 5-HT\(_{1A}\)R, 5-HT\(_{2A}\)R and protein levels in hippocampus**

As shown in Fig. 8, 5-HT\(_{2A}\)R mRNA expression of model group increased significantly compared with control group (P < 0.001), while 5-HT\(_{2A}\)R mRNA expression in brains of Flu, AF 30 and 15 mg kg\(^{-1}\) groups were decreased remarkably in comparison with those in model group (P < 0.01, P < 0.01, P < 0.001). While 5-HT\(_{2A}\)R protein expression was increased in comparison with that in control group (P < 0.05), Flu and AF 30 mg kg\(^{-1}\) were decreased remarkably in comparison with those in model group (P < 0.01, P < 0.05).

**Effect of AF on hippocampus BDNF level and protein level**

As shown in Fig. 8, Hippocampal BDNF level in model groups was significantly declined compared with control group (P < 0.01). Administration of AF (30 mg kg\(^{-1}\)) caused...
a significant decrease in BDNF level compared with model group \( (P < 0.05) \). Fluoxetine also caused a significant decrease compared with model group \( (P < 0.01) \). BDNF protein expression in brains of model group decreased significantly in comparison with that in control group \( (P < 0.01) \), while BDNF protein expression in brains of Flu and AF 30 mg\( \cdot \)kg\(^{-1} \) group were increased in comparison those in model group \( (P < 0.01) \). BDNF protein expression in brains of model group decreased significantly in comparison with that in control group \( (P < 0.01) \), while BDNF protein expression in brains of Flu and AF 30 mg\( \cdot \)kg\(^{-1} \) group were increased in comparison with those in model group \( (P < 0.01) \).

**Discussion**

NO/cGMP signal pathway is known to possess biologically beneficial effects on blood vascular [22]. Although lots of studies have investigated \( P. \) lactiflora could ameliorate depression, no research involved in anti-stress effects of AF via NO-mediated signal pathway. It was obvious from our results that chronic restraint stress-induced depression-like effects, e.g. inhibition in total body weight gain, increased hypothalamo-pituitary-adrenal (HPA) cascade levels, un-normal monoamine neurotransmitter levels and changed receptors expression in hippocampus. Thus, it is fair to postulate from the results obtained that AF can function as a potential agent that suppresses depressive-like behavior via NO-mediated mechanism associated with stress.

The first experimental evidence of AF’s anti-stress effect was provided via behavioral tests. Body weight was also decreased as expected after CRS (Fig. 1). AF increased the body weight of the rats and sucrose solution consumption. Similar to fluoxetine, AF significantly improved behavioral disorder. Furthermore, effect of AF on locomotion was evaluated by the open-field test. In our study, AF increased the numbers of crossing and reduced the time of rearing (Fig. 3). Meanwhile, AF increased the %OAE and increased the %TOA (Fig. 4). Thus, a reliable model of depression was established. More importantly, treatment of AF before stress exposure significantly ameliorated these behavioral changes, as well as dramatically inhibited the hyperfunction of HPA axis induced by CRS. Depression patients with overactive HPA axis mainly present elevated levels of CRH in the central, peripheral nervous system and metabolites, ACTH secre-
tion and cortisol level. In the study, the content of CRH, ACTH, CORT in model rats’ serum were significantly higher than those in control group, consistent with the report on HPA function changes in researches. AF significantly increased the model rats’ weight and sucrose consumption, made the abnormal behavior obviously improved and corrected the HPA axis hyperfunction.

The hippocampus is closely associated with human emotion, with rich glucocorticoid receptors, an inhibitory effect on the HPA axis. Continuing high CORT level can damage hippocampal neurons, which impaired the inhibitive function of the HPA axis, resulting in the occurrence of affective disorders. The mechanisms of behavioral variation involve the low levels of serotonin and norepinephrine, these substances in the central nervous system will be closely related to depression. Studies have shown that serotonin (5-HT) system plays a very important role in the onset of mental ill-

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Fig. 4 The effects of AF on the elevated plus maze behaviors in rats model of CRS (Mean ± SD, n = 12). *P < 0.05, **P < 0.01, ***P < 0.001 vs the control group; † P < 0.05, ‡ P < 0.01, §§ P < 0.001 vs the model group

Fig. 5 The effects of AF on the serum levels of CRH, ACTH, CORT in rats model of CRS (Mean ± SD, n = 10). *P < 0.05, **P < 0.01, ***P < 0.001 vs the control group; † P < 0.05, ‡ P < 0.01, §§ P < 0.001 vs the model group

Table 2 The effects of AF on the hippocampal monoamine neurotransmitter levels in CUS rats (Mean ± SD, n = 8)

<table>
<thead>
<tr>
<th>Groups</th>
<th>5-HT (ng·g−1)</th>
<th>5-HIAA (ng·g−1)</th>
<th>HVA (ng·g−1)</th>
<th>DA (ng·g−1)</th>
<th>E (ng·g−1)</th>
<th>NE (ng·g−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.75 ± 2.00</td>
<td>2467.15 ± 394.49</td>
<td>25.48 ± 6.89</td>
<td>1672.96 ± 354.20</td>
<td>1588.04 ± 244.08</td>
<td>38.98 ± 9.29</td>
</tr>
<tr>
<td>Model</td>
<td>3.37 ± 0.97**</td>
<td>1611.61 ± 313.61</td>
<td>16.21 ± 6.31*</td>
<td>1009.53 ± 106.41**</td>
<td>1201.57 ± 400.88</td>
<td>26.05 ± 5.11***</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>4.67 ± 2.30</td>
<td>2162.82 ± 425.75**</td>
<td>26.67 ± 6.73</td>
<td>1361.27 ± 435.66**</td>
<td>1525.37 ± 308.32</td>
<td>35.56 ± 10.79**</td>
</tr>
<tr>
<td>AF 30 mg·kg−1</td>
<td>6.38 ± 2.25**</td>
<td>2042.06 ± 390.48</td>
<td>27.38 ± 8.42**</td>
<td>1410.73 ± 397.43**</td>
<td>1528.72 ± 528.52</td>
<td>34.55 ± 6.65</td>
</tr>
<tr>
<td>AF 15 mg·kg−1</td>
<td>6.64 ± 1.55**</td>
<td>2249.91 ± 772.06**</td>
<td>24.42 ± 4.16**</td>
<td>1260.94 ± 309.81</td>
<td>1377.39 ± 396.73</td>
<td>36.66 ± 7.79**</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001 vs the control group; † P < 0.05, ‡ P < 0.01, §§ P < 0.001 vs the control group

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Fig. 6 The effects of AF on the hippocampal levels of NO in rats model of CRS (Mean ± SD, n = 10). *P < 0.05, **P < 0.01, ***P < 0.001 vs the control group; † P < 0.05, ‡ P < 0.01, §§ P < 0.001 vs the model group

Fig. 7 The effects of AF on the hippocampal levels of cGMP in rats model of CRS (Mean ± SD, n = 10). *P < 0.05, **P < 0.01, ***P < 0.001 vs the control group; † P < 0.05, ‡ P < 0.01, §§ P < 0.001 vs the model group
The synthesis, release, reuptake and metabolic disorders of 5-HT and their receptors are closely related to social relationship deterioration, affective disorder and lack of pleasure. Results have obtained in the present study demonstrate that AF antagonizes the ptosis, akinesia, and hypothermia induced by reserpine in mice, which indicates that...
AF has an antidepressant-like effect and may have an effect on monoamine neurotransmitters. We then explored the anti-stress mechanism of AF by determining the levels of monoamine neurotransmitters in the hippocampus by HPLC-ECD. This result was in line with the anti-depressant effect observed with AF modulating 5-HT, 5-HIAA and DA levels. However, no similar result was found in the E group after 3 weeks of AF.

Evidence shows that 5-HT1A receptor hyperfunction is particularly close to anxiety and depression. A set of basic researches reported that acute and chronic unpredictable stresses increase plasma glucocorticoid, and down-regulate 5-HT1A receptor binding and mRNA level \(^\text{[6]}\). On the other hand, 5-HT2A receptors are densely found in the hippocampus, amygdala, prefrontal cortex and the performance area of olfactory cortex, which have the close connection with suicide, depression, and schizophrenia \(^\text{[39]}\). Studies have reported that depression is due to pathologically improving 5-HT2A receptor function in brain marginal zone. The long-term use of tricyclic antidepressants (TCA) and SSRIs can reduce the number of 5-HT2A receptor \(^\text{[10, 37]}\). The study also demonstrated that blockade of 5-HT2A receptors with M100907 optimizes response inhibition modulated by both glutamatergic and monoaminergic mechanisms.

The study showed that the function of the 5-HT2A receptor is hyperfunction. After AF treatment, the 5-HT2A receptor is down-regulated, resulting in anxiety and antidepressant. However, no obvious result was found in the 5-HT1A. The mechanisms of AF treats depression are not only the disorder of HPA axis but also adjust the 5-HT receptor subtypes in rat’s hippocampus.

To further study the signaling pathways associated with the anti-depressant effects of AF, we examined the expression of BDNF. Some clinical studies that measured the serum BDNF levels in drug-free depressive patients have shown that serum levels are significantly lower in these than in healthy participants, and chronic antidepressant treatment increased the serum BDNF levels in depressed patients \(^\text{[10, 37]}\). Taking into account its importance in depression, we investigated the effect of chronic treatment with AF in the hippocampal levels of BDNF. AF increased the hippocampal BDNF levels and protein expression. That may be suggested that at least some of the anti-depression effects of AF via enhanced hippocampal neurogenesis.

Animal studies have demonstrated that by regulating the effect of cGMP \(^\text{[38]}\), NO produces depression-like state \(^\text{[19]}\). Due to the fact that a decrease of NO level within the hippocampus and NO-mediated through the reduction of cGMP \(^\text{[45]}\) produces antidepressant-like effects \(^\text{[46]}\). In this study, we showed that AF decreased the NO level and cGMP level in the hippocampus but no effect on the cGMP protein expression. Furthermore, numerous studies indicate that NOS inhibitors exert antidepressant effects in animal studies \(^\text{[42-43]}\). Indeed, nNOS derived NO was demonstrated to exert a negative control on the hippocampal neurogenesis \(^\text{[44]}\). In this study, AF decreased nNOS mRNA expression but no protein expression.

Conclusions

Our findings suggest that AF produced an anti-stress effect in CRS rats by a mechanism that modulated the inhibition of the NO-mediated network pathway against stress-induced depressive-like behaviors. This effect is likely partially mediated through increased hippocampal BDNF levels, suggesting a possible involvement of neurogenesis and it might be correlated with its neuroprotective action related to 5-HT2A receptors.

References

[15] Sargent PA, Kiaer KH, Bench CJ, et al. Brain serotonin 1A receptor binding measured by positron emission tomography with...


