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Effects of Black Rice Anthocyanin Extract on Depression-Like Behavior in Chronic Unpredictable Mild Stress Model Mice

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Abstract

Objective: To investigate the effects of black rice anthocyanins extract (BSE) on depression-like behavior in mice with chronic unpredictable mild stress (CUMS). Methods: A total of 30 male Kunming mice were randomly divided into Control group, model group (CUMS) and black rice anthocyanin extract group (300 mg/kg). Except the blank group, all mice were treated with CUMS to induce depression. After 21 days of intragastric administration, the behavioral changes of the mice were analyzed by body weight, sucrose preference test, open field test, and water maze test. Enzyme-linked immunosorbent assay (ELISA) was used to detect the levels of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and corticosterone (CORT). Hematoxylin-eosin staining (HE) was used to observe the pathological changes of neurons in hippocampal CA1 and CA3 regions. Cell apoptosis in hippocampus was detected by terminal deoxynucleotidyl transferase-mediated dutP Nick end labeling (TUNEL) staining. Results: Compared with the model group, the brown-rice anthocyanin treatment group showed significantly increased sucrose preference, increased horizontal movement distance and vertical standing times in the open field test, decreased escape latency in the water maze test, increased residence time in the target quadrant and the number of crossing the platform, and alleviated the damage of hippocampal neurons. Plasma ACTH, CORT, and CRH levels were decreased, and apoptosis rates in the hippocampus were decreased. Conclusion: Black rice anthocyanins have a significant antidepressant effect possibly by regulating the function of HPA axis.

Keywords

Black Rice Anthocyanins; Chronic Unpredictable Mild Stimulation; Hypothalamic-Pituitary-Adrenal Axis; Depression.

1. Introduction

Depression is a mental disorder of the central system, which is characterized by low mood, slow thinking, decreased language and action, and despair. It is difficult to cure and prone to recurrent episodes [1]. The pathogenesis is related to the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is an important neuroendocrine feedback regulation axis in the brain. In the face of stress, due to the large release of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and corticosterone (CORT), the HPA axis is over-activated, leading to hyperfunction, and the negative feedback regulation mechanism is impaired. It is accompanied by hippocampal neuron damage [2-3]. At present, the clinical treatment of depression is mainly based on neurotransmitter reuptake inhibitors and psychotherapy, but the efficacy is poor and accompanied by side effects. In the treatment of depression, traditional Chinese medicine can not only relieve symptoms, but also be taken for

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a long time. With the characteristics of multi-components and multi-targets, it can become an important source of antidepressant drugs [4].

Studies have confirmed that anthocyanin monomer components have antidepressant activity, which can improve the regulation disorder of HPA axis and increase the levels of brain-derived neurotrophic factor and monoamine neurotransmitter [6–8]. In this study, a mouse model of depression induced by chronic unpredictable mild stress (CUMS) was used to observe the effects of black rice anthocyanin extract (BRAE) on the behavior of CUMS mice, and to explore the feedback regulation effect of BRAE on HPA axis, so as to explore the possible mechanism of UPGE in the treatment of depression. This study provides new ideas for the development of BRAE antidepressant health care drugs.

2. Materials and Methods

2.1. Materials and Instruments

Thirty healthy male Kunming mice, SPF grade, weighing about 18-22 g, were fed within the standard (0.5d light /0.5d dark, temperature 21±3℃, relative humidity 50%±10%), with diet and water AD libitum. ACTH kit, CORT kit and CRH kit were purchased from Huangshi Yanke Biotechnology Co., LTD.

TM-Vision open field behavioral experiment system, Morris water maze video analysis system (WMT-100S, Taimeng Company); microplate reader (Infinite N200pro, TECAN, USA); high speed refrigerated centrifuge (ST8R, Thermo, USA); fluorescence inverted microscope (CKX53, OLYMPUS); embedding machine (JB-P5, Wuhan Junjie Electronics Co., LTD.); pathological microtome (RM2016, Shanghai Leica Instrument Co., LTD.); tissue spreading Machine (KD-P, Jinhua Kedi Instrument Equipment Co., LTD.); decolorization shaker (WD-9405A, Beijing Liuyi Instrument Factory).

2.2. Experimental Methods

2.2.1. CUMS Mouse Model

The male Kunming mice were adaptively fed for one week, and their body weight was recorded. Before grouping, the mice were trained to adapt to sugar water, and the sugar water preference test was performed after 24 hours of water deprivation. The remaining mice were randomly divided into three groups: blank group (Control), model group (CUMS) and black rice anthocyanin extract group (300mg/kg). The mice in the normal group were fed normally without any stimulation, and the other groups were given CUMS mice model. The specific procedures were as follows: water deprivation for 24h, swimming in hot water at 45℃ for 5min, wetting bedding for 24h, shaking the cage for 15min, clamping the tail for 5min, electric shock to the foot once (3mA, 5s), swimming in cold water at 8℃ for 5min, restraint for 3h, tilting the cage at 45° for 24h, fasting for 24h, and reversal of day and night for 24h. One type of stimulation was given randomly and noncontinuously at 9 a.m. each day, and the mice were not aware of it. The body weight, sucrose preference and open field test were recorded before the model establishment. After modeling, the mice showed decreased body weight, sucrose preference, and decreased spontaneous activity and exploratory behavior, indicating that the model was successfully established. Three weeks after modeling, CUMS was continued simultaneously with intragastric administration. The normal group and the model group were given the same dose of normal saline by gavage for 3 weeks.

2.2.2. General Condition and Body Weight

Food intake, water intake, hair color smoothness and mental state of mice were observed, and the body weight of mice was recorded every 7 days.

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2.2.3. Sugar Preference Test

Anhedonia, a core symptom of depression, was reflected by changes in sugar intake. All mice underwent sucrose preference test before and after modeling. After the first day, mice were given 2 bottles of 1% sucrose solution (200 ml). On the 2nd day, one bottle of 1% sucrose water (200mL) and one bottle of ordinary drinking water (200mL) were given; On day 3, after 23h of fasting and water, 1 bottle of 1% sucrose water (200mL) and 1 bottle of ordinary drinking water (200mL) were exchanged after 0.5h, removed and weighed again.

Sucrose preference ratio (%) = sucrose consumption/total fluid consumption ×100. (1)

2.2.4. Open Field Experiment

The status of the mice was evaluated by the spontaneous activity and exploratory activity in the unfamiliar environment, reflecting the loss of interest. Each mouse was placed gently in the middle of the open field experiment box, and the movement trajectory of the mouse in 5 minutes was recorded by the camera above. The horizontal movement score (total distance of horizontal movement) and vertical score (number of standing) were analyzed. Before each mouse was tested, the bottom of the box was thoroughly cleaned with 75% ethanol to avoid odor interference with the experimental results of the next mouse.

2.2.5. Morris Water Maze Test

Morris water maze (MWM) test, which can reflect the hippocampal dependent spatial learning and memory ability of mice, placed mice in a circular pool with a height of 50cm and a diameter of 160cm (temperature 24-26 ℃). The pool was divided into quadrants A, B, C and D4. In quadrant A, the platform was placed at a depth of 2cm, and the mice were gently placed in the water facing the wall of the pool. The escape latency (time of climbing on the platform) was recorded. Two trials per day were conducted to ensure the possibility of observing short-term memory generated on the day, with continuous measurements of 3d. After the completion of the Morris water maze test, the underwater platform was transferred, and the mice were placed into the water from the back to the platform. The swimming movement of the mice within 300s was recorded, and the residence time in the A quadrant, that is, the residence time in the target quadrant, was recorded.

3. Results

3.1. Effect of BRAE on General State and Body Weight of CUMS Mice

Observation of the general state of mice: the mice in the blank group were in good mental state, with shiny hair, normal feces, and more exploration behaviors. The mice in the model group had dim and messy hair color, decreased appetite, poor mental state, few exploratory behavior, and often in a state of immobility and curled up. Compared with the model group, the mice in the BRAE treatment group were more active, with bright eyes and shiny hair. Changes in body weight of mice: the body weight of mice in each group was basically the same before modeling. Compared with the blank group, the body weight of the model group grew slowly and the change range was significantly reduced. Compared with the model group, the body weight and growth rate of the BRAE group were significantly increased after gavage on day 21. At the end of the administration, compared with the model group, the body weight of the mice in the administration group increased significantly.

3.2. Effect of BRAE on Sugar Preference Rate in CUMS Mice

At the end of the modeling experiment, compared with the blank group, the mice in the model group consumed less sucrose water. Compared with the model group, the sucrose preference rate in the BRAE group was significantly increased, had a certain improvement effect on the depression of mice, and the difference was statistically significant, see Figure 1.

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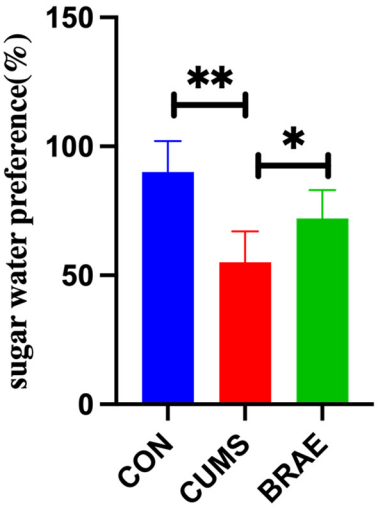

Figure 1. Sugar water preference

3.3. Effects of BRAE on Horizontal and Vertical Movement in CUMS Mice

Before modeling, the horizontal and vertical scores of the experimental groups were basically the same. From the third week after modeling, compared with the normal group, the horizontal movement distance and vertical movement scores of the model group were significantly decreased. Compared with the model group, the horizontal and vertical activity behaviors of the mice in the BRAE group were improved to some extent, as shown in Figure 2.

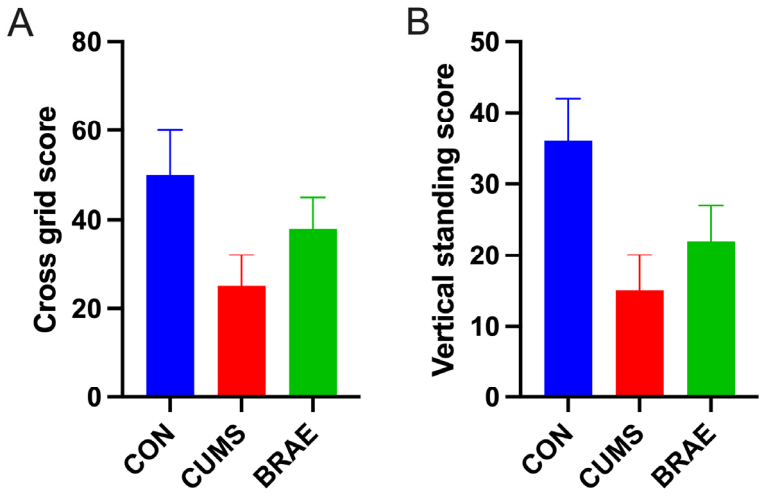

Figure 2. Effect of UPGE on autonomic activity of CUMS mice

3.4. BRAE on Escape Latency and Spatial Exploration in CUMS Mice

Effect In the Morris water maze test, compared with the normal group, the escape latency of the model group was significantly increased; Compared with the model group, the escape latency of the BRAE treatment group was decreased. Compared with the normal group, the number of crossing the platform in the target quadrant and the retention time in the target quadrant in the model group were significantly reduced. Compared with the model group, the number of crossing the platform and the time of staying in the target quadrant of the mice in the BRAE treatment group were significantly increased

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4. Conclusion

The CUMS scheme used in this study has been widely used in the establishment of animal depression models. This method can better simulate the complex and changeable social pressure stimulation and people's stress psychology. The sucrose preference test, open field test and Morris water maze test used in this study can be used to evaluate whether the depression mouse model is successfully established. The results showed that compared with the model group, the mental state of the mice in the BRAE group was improved, the hair was shiny, the body weight growth rate tended to be normal, the clinical manifestations similar to depression were significantly improved, and the behavioral indicators were recovered to varying degrees. In the sugar-water preference test, BRAE treated mice showed increased preference for sugar-water and improved anhedonia. In the open field test, the mice in the BRAE group had increased horizontal movement distance, improved movement ability in the new environment, and increased vertical standing times, which proved that the mice had enhanced independent exploration ability and curiosity in the unfamiliar environment. In the Morris water maze test, the escape latency of mice was shortened, and the number of crossing the target quadrant and the stagnation time were increased when the platform was not present, indicating that the spatial learning and memory ability of mice was improved to a certain extent. BRAE treatment can improve the depressive behavior of CUMS-induced mice and has an antidepressant effect.

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