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Research article

Root bark of *Morus alba* ameliorates the depressive-like behaviors in diabetic rats

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HIGHLIGHTS

- Root bark of *Morus alba* alleviated obesity, hyperglycemia and hyperlipidaemia in diabetic rats.
- Root bark of *Morus alba* reversed depressant behaviors in diabetic rats.
- The supplement restored BDNF, phosphorylation of ERK and Akt in PFC in diabetic rats.

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ABSTRACT

Diabetes-induced depression is one of the severe chronic complications of diabetes mellitus. Up to now, there are only a few effective medicines to prevent or manage the co-morbidity of diabetes and depression. The present study was to investigate the effect of root bark of *Morus alba* (RBM) on depressive-like behaviors in the diabetic rats established by a high fat diet and a low dose of streptozotocin. Depressive-like behaviors were measured by the open field test, locomotor activity test and forced swimming test. Plasma glucose and lipid parameters were also measured. Expression of Brain-derived neurotrophic factor (BDNF) and phosphorylation of extracellular signal-regulated kinase (ERK) and Akt in the prefrontal cortex (PFC) were assessed. The results showed that a 4-week administration of RBM (10 g/kg, ig) significantly reversed the depressive-like behaviors. BDNF expression and phosphorylation of ERK and Akt were increased in the PFC following RBM treatment in the diabetic rats. The data demonstrated that RBM could improve the depressive-like behaviors induced by diabetes, suggesting a therapeutic potential of RBM for the diabetes-associated depression.

1. Introduction

Diabetes mellitus (DM) has been a major global public health problem, which is becoming increasingly serious. According to the International Diabetes Federation, the number of diabetic people was near 382 million in 2013 [1]. It is predicted to be 592 million in 2035 [2]. Long-term disorders of carbohydrate and lipid metabolism can lead to multiple system injuries, such as diabetic nephropathy, diabetic foot, and diabetic neuropathy. Moreover, in diabetic patients, anxiety, depression and age-related cognitive dysfunction are observed [3]. Depression is thought to be the result of the chronic burden of diabetes [4]. The evidences showed that the prevalence of depression in diabetic patients doubles that in the general population [5]. While in clinic, diabetes and depression are treated separately [6,7], and there are few effective medicines for the co-morbidity of DM and depression.

Root bark of *Morus alba* (RBM) has been used to treat diabetes in traditional Chinese medicine, and also has other biological activities including anti-inflammatory, antimicrobial, and anti-hyperlipidemic properties [8,9]. The preclinical study suggests that *Morus alba* leaves have anxiolytic effects [10], and ethyl acetate soluble fraction of RBM has antidepressant effects on rats in the forced swim test [11]. Given that RBM has the anti-diabetic and antide-

Abbreviations: BDNF, brain-derived neurotrophic factor; ERK, extracellular signal-regulated kinase; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; AUC, area under the curve.

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pressant effects separately, it is interesting to explore whether RBM has therapeutic effects against depression induced by diabetes.

BDNF has both neurotrophic and neuroprotective effects [12,13]. Studies have demonstrated that BDNF expression is significantly lower in patients with depression than healthy people [14,15]. The hippocampus, amygdala and prefrontal cortex (PFC) are crucial regions related to the pathological process of depression [16]. BDNF binds to its receptor tyosine kinase B (TrkB), and then activates the phospholipase Cγ (PLCγ), the phosphatidylinositol 3-kinase (PI3K) and the mitogen-activated protein kinase (MAPK) or ERK pathways [17]. Thus, the present study was not only to investigate whether RBM is effective in treating depression in diabetic rats, but also to observe the expression of BDNF, Akt or ERK in the prefrontal cortex.

2. Materials and methods

2.1. Materials

The high fat diet (HFD) was purchased from Shanghai Laboratory animal Co.Ltd, Shanghai, China. Each 100 g of HFD consists of 54.6 g rat breeding chow, 16.9 g lard, 14 g saccharose, 10.2 g casein, 2.1 g gunk and 2.2 g maltodextrin. RBM was provided by the Zhejiang Chinese Medical University. Streptozotocin (STZ) was purchased from Sigma.

2.2. Animal experiment

Male Sprague-Dawley rats at 2 months of age were obtained from the Experimental Animal Center of Zhejiang. After a week adaptation (1 week), a total of 24 rats were randomly divided into four groups (n = 6) as follows: normal fat diet control (NFD control), NFD + RBM (RBM) group, HFD + STZ (DM) group, HFD + STZ + RBM (DM + RBM) group. After 8 weeks of high fat diet feeding (9 week), diabetes was induced by one injection of STZ (25 mg/kg, ip). The rats with non-fasting plasma glucose $\geq$ 16.7 mmol/L were considered diabetic [18]. A week after STZ injection (10 weeks), both DM + RBM and RBM groups received intragavagely with RBM at dose of 10 g/kg twice a day for 4 weeks (14 weeks), while the other groups received the same volume of saline by gavage. The animal experimental protocol was approved by the Institutional Ethics Committee on Animal Care and Experimentation at Ningbo University (Certificate No. 0012371). The rats were kept in a controlled environment with a 12 h:12 h light-dark cycle and a temperature of 22 ± 1 °C, and they had access to water and food ad libitum.

2.3. Measurements of plasma glucose

Non-fasting plasma glucose from the tail vein was measured at a week after STZ injection and 4 weeks after RBM management by One Touch Glucose Monitor (Johnson&Johnson, Shanghai, China). The oral glucose tolerance test (OGTT) was performed once at 10 days after STZ injection, and again at the end of the study. Blood glucose from the tail vein was measured at 0, 0.5, 1 and 2 h after glucose administration, which was given at the dose of 2 g/kg (ig) at 8 a.m. after an overnight fasting.

2.4. Measurements of lipid parameters

After a 12-h fasting, blood was collected the day when rats were sacrificed and plasma was obtained by centrifugation at 3000 rpm for 10 min. Total cholesterol (TC), triglycerides (TG), HDL-C and LDL-C were measured by using commercially available kits immediately.

2.5. Open field test (OFT)

OFT was performed as previously described with modifications [19]. Rats were placed individually in a white Plexiglas box (100 × 100 × 40 cm) with the bottom divided into four identical squares on the arena in a dim room. Rat was placed in the center of the cage. The frequencies of line crossing (four paws placed into a new square) and rearing (with both front paws raised from the floor) were recorded over 5 min. All behaviors were recorded using a video camera located 150 cm above the arena. OFT was conducted after four weeks RBM administration.

2.6. Locomotor activity assessment (LA)

Each rat was placed in a black box (100 × 100 × 40 cm) without cover, which was enclosed in a sound proof box. There are several infrared beams around the wall, and a video camera located 150 cm above the arena. Horizontal distance was measured by the sequential breaking of infrared beams. Movement time was incremented when a rat was active for 1 s. The average speed of movement was obtained by dividing the total horizontal distance traveled by the total time in motion in the 60 min testing period. LA was taken two days after OFT.

2.7. Forced swimming test (FST)

FST was conducted in a sound-attenuated room that was dimly illuminated as described previously [20], rat was allowed to swim in the cylinder for 5 min under the conditions in which escape was not possible. The duration of immobility, which was defined as a lack of motion of the whole body except for small movement necessary to keep the head above water, was recorded. FST was performed two days after LA.

2.8. Western blot analysis

Rats were sacrificed 24 h after the behavioral tests and the PFC regions were carefully removed on ice. The tissue was homogenized, incubated, centrifuged, and the supernatant was collected. Protein concentrations were measured using the Bicinchoninic Acid Kit (Beyotime, Jiangsu, China). Proteins were separated by 12% SDS-PAGE electrophoresis and transferred to PVDF membranes. The membranes were blocked by 5% bovine serum albumin (Cwbiotech, Beijing, China) for 1 h and then incubated with relevant primary antibodies overnight at 4 °C followed by appropriate secondary antibodies for 1 h. The primary antibodies used were as follows: anti-β-actin(1:5000), anti-ERK1/2(1:5000) and anti-pERK1/2(1:500) from Cell Signaling Technology, anti-Akt (1:1000) and anti-pAkt(1:2000) from Signaling way, anti-BDNF(1:200) from Santa Cruz Biotechnology, Densities of the BDNF band were normalized to that of β-actin, while the pERK1/2 and pAkt band densities were normalized to that of ERK and Akt, respectively.

2.9. Statistics

All data were expressed as means ± S.E.M. and analyzed by Analysis of Variance (ANOVA) with SPSS Statistics 17.0. Two-way ANOVA was used to assess the effect of diabetic models, RBM supplement and the interaction of these two variables. Bonferroni test was used to compare the differences between two groups. Significance was set as $p < 0.05$.