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Apocynum venetum leaf extract reverses depressive-like behaviors in chronically stressed rats by inhibiting oxidative stress and apoptosis

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Quercetin (CID: 5280343)
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Malondialdehyde (CID: 10964)
Superoxide dismutase (CID: 72941490)
Superoxide anion radical (CID: 5359597)
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ABSTRACT

Background: Major depressive disorder (MDD) is a common but serious psychiatric disorder, but current treatments are inadequate for approximately half of the patients with MDD. Thus, better methods of treatment are urgently needed. This study aimed to investigate the antidepressant-like effects and potential mechanism of *Apocynum venetum* leaf extract (AVLE) in chronic unpredictable mild stress (CUMS) rat model of depression.

Materials and methods: The CUMS rat model of depression was used to investigate the antidepressant-like activity and relevant mechanism of AVLE (30, 60, and 125 mg/kg, i.g.). Behavioral tests, including sucrose preference test (SPT), open field test (OFT), and forced swimming test (FST) were conducted to assess anhedonic, despairing, and spontaneous behaviors, respectively. The activity of the hypothalamic-pituitary-adrenal (HPA) axis was evaluated by measuring the serum adrenocorticotrophic hormone (ACTH) and corticosterone (CORT) concentrations. The underlying mechanism was further explored by assessing oxidative stress parameters, cell apoptosis, and brain-derived neurotrophic factor (BDNF) expression in the rat hippocampus exposed to CUMS.

Results: The AVLE (36, 60, 125 mg/kg) treatment exerted antidepressant-like effects in CUMS-exposed rats similar to fluoxetine (10 mg/kg). The AVLE treatment reduced the serum CORT and ACTH levels in CUMS rats. It also increased the activities and gene expression of antioxidant enzymes (SOD, CAT, and GPx) and decreased the ROS generation levels and the lipid peroxidation marker MDA in the rat hippocampus subjected to CUMS. Additionally, it suppressed the apoptosis of hippocampus cells by modulating Bcl-2/Bax pathways and improved the hippocampal BDNF expressions of CUMS rats.

Conclusion: Our findings suggested that AVLE exerted antidepressant-like effects in CUMS rats, which was possibly mediated by the prevention of oxidative stress, the inhibition of hippocampal neuronal apoptosis, and the upregulation of the hippocampal BDNF level.

1. Introduction

Major depressive disorder (MDD) is a common but serious psychiatric disorder, characterized by continuous sadness, lack of interest, loss of pleasure, low self-worth, impaired concentration, feelings of guilt or fatigue, disturbed appetite or sleep, and even physical pain and suicidal thoughts [1,2]. The global-point prevalence of major depressive disorder was 4.7% (4.4–5.0%), and the pooled annual incidence was 3.0% (2.4–3.8%) [3]. The World Health Organization (WHO) predicted that MDD is projected to become one of the three leading causes of the global burden of disease by 2030 [4]. Nevertheless, the mechanisms underlying the pathogenesis of MDD still remain unknown, and current treatments are inadequate for approximately half of the patients with MDD [5,6]. Furthermore, the limited efficacy and significant side effects of conventional antidepressants lead to poor compliance or premature discontinuation, which ultimately limits the remission in patients to only 60%–70% [7,8]. Thus, better methods of treatment for MDD are urgently needed. Herbal therapy has a long use of history. Medicinal plants, with lower economic costs and fewer side effects, have proven their curative effect in treating many clinical

Abbreviations: AV, *Apocynum venetum* L; AVLE, *Apocynum venetum* leaf extract; CUMS, chronic unpredicted mild stress; SPT, sucrose preference test; OFT, open-field test; FST, forced swimming test; HPA, hypothalamic-pituitary-adrenal; ACTH, adrenocorticotrophic hormone; CORT, corticosterone; BDNF, brain-derived neurotrophic factor; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; MDA, malondialdehyde; TUNEL, TdT-mediated dUTP nick end labeling

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symptoms, including mental illness [9,10]. For example, St John's wort (*Hypericum perforatum*) is one of the most widely used and studied herbal medicine in patients with mild or moderate depression symptoms [11,12].

Apocynum venetum L. ("Luobuma" in Chinese, Apocynaceae) is a wild plant distributed primarily in central and northwestern China [13]. The first record of *Apocynum venetum* can date back to the early 15th century, when it was documented in the ancient Chinese herbal book Jiu-Huang-Ben-Cao (Ming Dynasty, by Zhu Xiao/Su), which recorded the medicinal use of the leaves and tender stems of *Apocynum venetum* served as food and tea. In 1977, Apocynum ventum L. was published in Pharmacopeia of the People's Republic of China, and it has since become a medicine in China. *Apocynum venetum* leaf extract (AVLE) is reported to possess anti-hypertensive, anti-low density lipoprotein oxidation, anti-lipid peroxidation effects, caspase-inhibitory, hepatoprotective, cardiotonic and anxiolytic-like activities [14–18].

AVLE was commercially prepared as a standard water-soluble extract as brown-colored powder form under the trade name VENETRON. It contained more than 15 kinds of phenolic constituents, and two malonated flavonol glycosides (Hyperoside and Isoquercitrin) were identified as active componentsas, which have been proved to be the important actives for the antidepressant effects [19]. A randomized, placebo-controlled, double-blind study of 8-weeks treatment of *Apocynum venetum* leaf extract (VENETRON, 50mg/day) was conducted in the US on 39 patients with mild depression. As a result, 50% of the VENETRON group showed a decrease of 50% or greater on Hamilton Depression Rating Scale (HAM-D), suggesting a significant improvement in the symptoms of depression. Also, serum serotonin concentration increased in 50% of the patients treated with VENETRON (increase of 67%; 10.6 ± 6.3 ng/mL to 17.7 ± 7.2 ng/mL), while plasma 3- methoxy-4-hydroxyphenylglycol (MHPG) concentration decreased in 65% of the subjects in VENETRON group, demonstrating biochemical evidence of improvement. Furthermore, no severe adverse effects were observed. Thus, VENETRON is a standardized herbal extract for use as antidepressant, and has been registered under USA patent No. US 6,737,085 [20,21]. Moreover, recent studies have indicated that hyperoside and isoquercitrin, the predominant active components of AVLE, exhibit potent antioxidant activities [22,23]. Additionally, the antioxidant activities of quercetin have been proven to contribute to its antidepressant-like effects in bulbectomized mice [24].

MDD is thought to originate from the interaction of causative genes with environmental events, in particular stress [25]. Exposure to stress may be associated with increased production of reactive oxygen species (ROS) [26]. Oxidative stress is defined as an imbalance of pro-oxidants and anti-oxidants, leading to an elevated release of ROS and a disruption of redox signaling and control [27,28]. A growing body of evidence from preclinical and human postmortem studies supports the oxidative stress hypothesis of depressive disorder [29–31]. Furthermore, emerging evidence for the influence of antidepressants on oxidative stress suggested an anti-oxidative role of antidepressants [31–34].

The chronic unpredictable mild stress (CUMS) procedure has a unique combination of predictive validity (pathological state), face validity (phenomenological similarity), and construct validity (theoretical rationale) as an animal model of depression [35,36]. In previous studies, the antidepressant effect of AVLE was principally evaluated in the screening models, such as tail suspension test (TST) and FST [37,38].

In view of the abovementioned facts, the present study was designed to investigate the effects of the chronic administration of AVLE on some depressive behavioral changes induced by CUMS in rats: Additionally, we investigated the involvement of oxidative stress, cell apoptosis, and the BDNF expression on the antidepressant effect of AVLE. Here, we demonstrated that behavioral disturbances induced by CUMS were reversed by AVLE, which coincided with reduced oxidative stress damage and inhibition of apoptosis. Therefore, our results may reveal a novel mechanism for AVLE amelioration of depressive-like behavior induced by CUMS, and provide theory gist for AVLE to be an alternative or adjuvant therapeutic approach in the treatment of MDD.

2. Material and methods

2.1. Animals

Adult male Wistar rats (42 days old) weighing 180–220 g were provided by the Experimental Animal Centre of Shanghai University of Traditional Chinese Medicine. The rats were housed singly in cages in a room with a 12-h light/dark cycle (lights on at 7:00 a.m., lights off at 7:00 p.m.), equivalent temperature (22 ± 2 °C), and relative humidity (55 ± 5%). A standard diet and water were available ad libitum except during the periods of food and water deprivation required by the CUMS protocol or before assessment of sucrose preference. All experiments and procedures were approved by the local ethics committee for animal research (Approval number: SZY 201604001) and conducted according to the National Institutes of Health guide for care and use of laboratory animals.

2.2. Sucrose consumption

After an initial habituation period of 7 days, all animals were trained to consume a 1% sucrose solution (*w/v*): two bottles of 1% sucrose solution were placed in each cage for 24 h, and the 1% sucrose in one bottle was replaced with tap water for another 24 h. After the adaptation, the sucrose test was conducted to obtain a baseline sucrose preference (SP). The rats were deprived of water for 18 h prior to the test, starting at 6:00 p.m. Each animal was given free access to two preweighed bottles containing either 1% sucrose solution or tap water for six hours. Six hours later, the bottles were weighed and recorded again [39,40]. SP was calculated by the formula as previously described [41]: sucrose preference = sucrose consumption / (water consumption + sucrose consumption) × 100%. The sucrose preference test (SPT) was run once per week during the CUMS protocol.

2.3. Groups and drug treatment

On the basis of the sucrose preference test, rats were divided into two matched groups: (1) vehicle group (n = 10); (2) CUMS group (n = 60). The CUMS rats were treated simultaneously with CUMS. Four weeks later, the CUMS group was divided into five matched subgroups (n = 10 each) with SP distributed evenly across subgroups, including the CUMS model group (deionized water), the Fluoxetine (10 mg/kg) group, and the AVLE (30, 60, 125 mg/kg) groups. Fluoxetine hydrochloride (FLX) was purchased from Eli Lilly Pharmaceuticals. Powdered AVLE, under the trade name VENETRON (drug/extract ratio: 25/2 prepared by Tokiwa Phytochemical Co., Ltd., Chiba, Japan) was used. All drugs were dissolved in deionized water by sonication and given in a volume of 10 ml/kg by oral gavage once daily between 8:00–10:00 A.M. during the last 4 weeks of the CUMS. Vehicle-treated rats were given a constant volume of deionized water (10 ml/kg).The doses were chosen on the basis of previous studies [38,42].

2.4. CUMS paradigm

The chronic stress regimen was achieved as presented previously and is a variant of the chronic mild stress procedures described by Willner [35]. Briefly, CUMS rats were daily subjected to various mild stressors in accordance with an unpredictable schedule over a total period of 8 weeks. The stressors included: food or water deprivation, 45° tilted cage, forced swimming, physical restraint, soiled caged, pair-housing, reversal of light/dark cycle and stroboscopic illumination in accordance with an unpredictable schedule (Table 1). Stressors were unpredictable in the nature, duration, and frequency. Control rats were housed undisturbed in a separate animal room. Body weight and SP