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Antidepressant-like effect of active fraction of *Polyrhachis vicina* Roger in a rat depression model

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ABSTRACT

Objective: To investigate the antidepressant-like effect of active fraction of *Polyrhachis vicina* Roger (AFPR) in a rat depression model, and to elucidate the underlying mechanism.
Methods: AFPR was extracted with ethanol followed by petroleum ether. Its antidepressant-like effect was investigated in mice by tail suspension test (TST), forced swimming test (FST) and open field test (OPT). A repeated dose of reserpine (0.5 mg/kg, daily for 14 days) was used to establish a rat depression model. Fluoxetine was used as positive control agent. The effect of AFPR on reserpine-induced ptosis, hypothermia and akinesia, the levels of monoamines and their metabolites, and the activity of monoamine oxidase (MAO) in hippocampus and prefrontal cortex were determined.
Results: Administration of AFPR by gavage at 160 and 320 mg/kg significantly reduced the duration of immobility in the FST and TST, and did not affect locomotor activity in the OPT. In the reserpine-induced depression model, AFPR attenuated anhedonia, demonstrated by reversing hypothermia, akinesia and sucrose consumption. AFPR significantly increased the concentration of monoamines, including dopamine, serotonin, noradrenaline and acetylcholine.
Conclusion: AFPR normalized the metabolism rates of noradrenaline, serotonin and dopamine, and the activity of MAO, which were altered by chronic reserpine exposure. The findings suggest that modulation of the monoaminergic neurotransmitter system likely underlies the antidepressant-like effect of AFPR.

1. Introduction

Depression is a mental condition characterized by depressed mood, changes in behavior, and increased rates of suicide [1]. Major depressive disorder affects nearly 16% of the world population [2]. It is presumed by the World Health Organization that depression will be the second leading public health problem following cardiovascular disease in 2020 [3]. Monoamine neurotransmitter depletion is considered the main pathological mechanism in depression [4,5]. Decreased concentrations of serotonin (5-HT) and noradrenaline (NA) are associated with depression, suggesting that increasing the concentrations of monoamines could alleviate depression [6–8]. Based on this hypothesis, a series of antidepressants were developed, including a 5-HT reuptake inhibitor (fluoxetine), dual reuptake inhibitor (dutoxetine), and MAO inhibitors [9]. However, current antidepressants have failed to achieve optimal therapeutic effectiveness because of major drawbacks in clinical use, such as a delayed onset of action, a high percentage of non-responders and undesired side effects [10,11]. Therefore, there is an urgent need for the development of safe and effective fast-onset antidepressants.

Accumulating evidence has indicated that oxidative stress is involved in the pathology of major depression disorder. Depressed patients have elevated reactive oxygen species [12] and a lower concentration of key antioxidants in plasma, such as superoxide dismutase and coenzyme Q10 [13,14]. Overproduction of reactive

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oxygen species impairs neuronal cell membranes, and the highly unsaturated fatty acids of neuronal cell membranes play an important role in brain cell signaling, including monoamine regulation [15,16]. *Polyrhachis vicina* Roger, also known as black winged ants, has been shown to possess properties benefitting humans, including detumescence, detoxification and immunoregulation, and has been widely used in folk medicine and as a dietary supplement. *Polyrhachis vicina* Roger contains a variety of amino acids, vitamins, trace elements and other nutrients, and has been referred to as the "treasure house of animal nutrition" [17,18]. Furthermore, its antidepressant effect has been demonstrated in chronic depression [19]. Our previous studies have revealed that an active fraction extracted from *Polyrhachis vicina* Roger has anti-inflammatory and antioxidant properties [20]. Gas chromatography–mass spectrometry analysis has shown that 71.14% of AFPR is composed of unsaturated fatty acids, including octadecenoic acid (60.77%), heptadecenoic acid (9.31%) and leinoleic acid (1.06%) [21]. These previous findings suggest that AFPR may be a promising candidate for depression treatment [22].

In the present study, a battery of behavioral tests was employed to investigate the antidepressant-like effect of AFPR. The results showed that AFPR produces an antidepressant-like effect and suggest that modulation of monoaminergic systems may underlie the effect.

2. Methods and materials

2.1. Animals

Adult male Kunming mice and male Sprague-Dawley rats (Vital River Laboratories, Beijing, China) were housed (5 per cage) under standard conditions (12 h light/dark cycle; (22 ± 1) °C ambient temperature; 55% ± 10% relative humidity) with free access to food and water for 7 days of acclimation. After that, they were divided into specific groups according to their body weight. Animal care and experimental procedures complied with the National Institutes of Health for the care and use of laboratory animals and were approved by the Beijing Union Medical College and Chinese Academy of Medical Science.

2.2. Preparation of AFPR solution

Dried *Polyrhachis vicina* Rogers were provided by a local vendor of Chinese materia medica in Nanning city (Guangxi, China). The voucher specimen (PR-201505) was identified by Professor Xianbiao Zeng from Guangxi Institute of Chinese Medicine and Pharmaceutical Science and was deposited in the Herbarium of Guangxi Institute of Chinese Medicine and Pharmaceutical Science. Dried *Polyrhachis vicina* Rogers (1000 g) were milled and extracted three times with 10 L of ethanol/water (95:5, v/v) (Sigma-Aldrich, St. Louis, MO, USA) in a reflux system (each extraction for 1 h). The extracts were merged and filtered, followed by a water bath at (75 ± 5) °C to obtain a crude aqueous ethanol extract that was extracted three times by petroleum ether (Sigma-Aldrich). The extracts were again merged and filtered, followed by a water bath at (75 ± 5) °C to obtain the active fraction, with a yielded weight of 3.96% of dried *Polyrhachis vicina* Rogers (w/w). To facilitate the administration by gavage, the petroleum ether extract was emulsified by tween 80 (2.5%) and was administrated at a volume of 10 mL/kg.

2.3. Experiment design and drug treatment

Experiment 1 examined the antidepressant-like activity of AFPR in mice by FST, TST and OPT [23]. Mice were randomly assigned to into five groups ($n = 10$): (a) control (vehicle); (b) fluoxetine (3.6 mg/kg, Lilly, Indianapolis, Indiana, USA); (c) AFPR, 80 mg/kg; (d) AFPR, 160 mg/kg; (e) AFPR, 320 mg/kg. Animals were administered vehicle (water:tween 80 = 97.5%:2.5%) or drugs by gavage for 10 days, and the behavioral tests were carried out within 1 h of dosing on the 11th day.

In experiment 2, according to the method by Antkiewicz-Michaluk et al. [24] adult male rats were subjected to reserpine for 14 days at a dosage of 0.5 mg/kg (ip) to establish the model of depression. Rats were randomly divided into five groups ($n = 10$): (a) control (vehicle); (b) model (reserpine + vehicle); (c) reserpine + fluoxetine (1.8 mg/kg, Lilly); (d) reserpine + AFPR (80 mg/kg); (e) reserpine + AFPR (320 mg/kg). Animals were administered vehicle (water:tween 80 = 97.5%:2.5%) or drugs daily for 14 days. Fluoxetine and AFPR were administered by intragastric injection. Body weight was measured on the first day, seventh day, and 14th day. Behavioral tests were carried out on the 14th day, including the measurement of ptosis, hypothermia, akinesia and a sucrose preference test (SPT). After behavioral testing, rats were placed on a flat board under anesthesia by diethyl ether, and the prefrontal cortex and hippocampus were dissected on ice for later neurochemical assessment.

2.4. Tail suspension test

Mice were tested in the TST as previously described [25]. Briefly, 1 h after drug administration, mice were suspended 50 cm above the floor for 6 min by adhesive tape placed approximately 1.5 cm from the tip of the tail. Immobility time during the final 4-min interval was recorded. Mice were considered immobile only when they hung passively and were completely motionless.

2.5. Forced swimming test

The FST was carried out in mice according to previous reports with a slight modification [26]. Briefly, mice were subjected to a 15-min pre-swim on the day before test day. On the experiment day, 1 h after drug administration, mice were individually placed into a glass cylinder (25 cm in height, 15 cm in diameter) filled with water 10 cm high [(25 ± 1) °C]. The water was changed after each trial. All animals were forced to swim for 6 min, and the immobility time during the final 4-min interval of the test was recorded. A mouse was judged to be immobile whenever it remained floating in the water, in an upright position, making only small movements to keep its head above the water.

2.6. Open-field test

To assess the effect of AFPR on locomotor activity, an OPT was conducted as previously described [27,28]. The mice were placed individually in an open field apparatus (50 cm × 50 cm × 40 cm), with the floor divided into 25 identical squares marked by black lines. Each mouse was placed in the center square and then tested for 5 min; the number of square crossings (with three paws in one square) and the number of rearing events (posture standing on two hind paws) were recorded. The apparatus was cleaned with alcohol (70%) before and after each session.

2.7. Antagonism of reserpine-induced ptosis, hypothermia and akinesia

The procedure was performed according to previous studies [29,30]. On the 14th day, rats were injected intraperitoneally with 0.5 mg/kg reserpine 1 h after drug administration. Ptosis, rectal temperature and akinesia were recorded at 1 h after the administration of reserpine. Rats were placed on a shelf (20 cm above the table top) and the degree of ptosis was recorded according to

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