Original Article

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Involvement of the central monoaminergic system in the antidepressant-like effect of catalpol in mice

Junming Wang[1,2,*], Ying Cui[1], Weisheng Feng[1,*], Yueyue Zhang[1], Guifang Wang[1], Xingxing Wang[1], Gai Zhou[1]

[1] *Collaborative Innovation Center for Respiratory Disease Diagnosis and Treatment & Chinese Medicine Development of Henan Province, Henan University of Traditional Chinese Medicine, Zhengzhou, Henan, China;*
[2] *College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou, Henan, China.*

Summary **Catalpol is a natural iridoid glycoside with diverse bioactivities that is found in abundance in *Rehmannia glutinosa* Libosch. (Scrophulariaceae). The present study assessed whether catalpol treatment (5, 10, or 20 mg/kg for 14 days by intragastric administration (*i.g.*)) has an antidepressant-like effect on mice performing the forced swim test (FST), tail suspension test (TST), open field test (OFT), and tests for reversal of reserpine-induced ptosis, akinesia, and hypothermia. This study also examined the potential role that catalpol plays in the cerebral monoaminergic system. Results indicated that catalpol administration produced an antidepressant-like effect in mice, as indicated by the reduced duration of immobility in the FST and TST, but it had no effect on locomotor activity in the OFT. Catalpol treatment significantly counteracted the decrease in rectal temperature, akinesia, and eyelid ptosis induced by reserpine. Moreover, catalpol increased levels of serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the brains of mice, but it did not affect levels of norepinephrine (NE) or dopamine (DA). These antidepressant-like effects of catalpol are essentially similar to the effects of the clinical antidepressant fluoxetine hydrochloride (FH). This is the first study to indicate that catalpol has an antidepressant-like effect and that its action may be mediated by the central serotonergic system, and not by noradrenergic or dopaminergic systems.**

Keywords: Catalpol, antidepressant-like effect, monoaminergic system, serotonergic system, reserpine

1. Introduction

Depression is a common but serious illness that can impact people's lives. Various antidepressants, including tricyclic antidepressants, monoamine oxidase inhibitors, and norepinephrine (NE) reuptake inhibitors, are widely available on the pharmaceutical market. Although these drugs have excellent efficacy, most frequently produce undesirable adverse reactions (*1-3*). Thus, an urgent task is to search for more promising antidepressants from natural medicinal plants in order to meet the needs of clinically depressed patients.

The dried root of *Rehmannia glutinosa* Libosch. (Scrophulariaceae) has been widely used as a traditional Chinese medicine to treat behavioral diseases, including depression. Catalpol (Figure 1A) is a major bioactive compound that is found in abundance in the dried root of *R. glutinosa*. This compound has been found to have neuroprotective action, ameliorate cognition deficits, and have potential as a treatment for inflammation-related neurodegenerative diseases (*4-6*). A previous study reported that the powdered dried root of *R. glutinosa* had an antidepressant-like effect in mice (*7*). However, no studies have specifically examined the antidepressant-like effect of catalpol from the dried root of *R. glutinosa*. Moreover, whether the mechanism of catalpol's action is related to the central monoaminergic system has not been known.

**Address correspondence to:*
Dr. Junming Wang and Dr. Weisheng Feng, College of Pharmacy, Henan University of Traditional Chinese Medicine, East Jinshui Road & Boxue Road, Zhengzhou 450046, China. E-mail: mjw98_2010@163.com (Wang JM); fwsh@hactcm.edu.cn (Feng WS)

The present study sought to assess the antidepressant-like effect of catalpol and to behaviorally and pharmacologically determine the role that it plays in the central monoaminergic system.

2. Materials and Methods

2.1. *Experimental animals*

Male Kunming (KM) male mice (18-22 g) were purchased from the Experimental Animal Center of Henan Province (Zhengzhou, Henan Province, China). Animals were given rodent laboratory chow and water *ad libitum* and maintained under controlled conditions with a temperature of 22 ± 1°C, relative humidity of 60 ± 10%, and a 12/12 h light/dark cycle (lights on at 7:00 a.m.). All procedures were in strict accordance with Chinese legislation on the use and care of laboratory animals and with guidelines established by the Institute for Experimental Animals of Henan University of Traditional Chinese Medicine. This study was approved by the committee for animal experiments of Henan University of Traditional Chinese Medicine.

2.2. *Reagents*

Catalpol with a purity of 99.5% according to high-performance liquid chromatography (HPLC) was provided by Shanghai Jinsui Biotechnology Co., Ltd. (Shanghai, China). Reserpine with a purity of more than 98% according to HPLC was purchased from Xi'an Senzhuo Biotech Co., Ltd. (Xi'an, Shanxi Province, China). Fluoxetine hydrochloride (FH) was purchased from Changzhou Siyao Pharmaceuticals Co., Ltd. (Changzhou, Jiangsu Province, China).

Mouse norepinephrine (NE), dopamine (DA), serotonin (5-HT), and 5-hydroxy-indoleacetic acid (5-HIAA) enzyme-linked immunoassay (ELISA) kits were purchased from R&D Systems China Co., Ltd. (Shanghai, China).

2.3. *Animal treatment protocol*

Male mice were randomly divided into several groups of 10 mice each. Mice in the treated groups were orally administered catalpol (5, 10, or 20 mg/kg) or the positive drug FH (10 mg/kg) daily for 14 consecutive days by intragastric administration (*i.g.*). Mice in the vehicle group (*i.e.* not treated with catalpol) were orally administered (*p.o.*) 0.5% CMC-Na daily. A tail suspension test (TST), forced swim test (FST), open field test (OFT), and tests for reversal of reserpine-induced ptosis, akinesia and hypothermia were used to observe the antidepressant-like effects of catalpol in mice 1 h after final administration. After the OFT, TST, and FST, animals were immediately sacrificed by cervical dislocation. Brain tissue was quickly removed and kept on ice to analyze levels of the central monoamine neurotransmitters NE, DA, and 5-HT and the 5-HT metabolite 5-HIAA.

2.4. *Tail suspension test (TST)*

The total duration of immobility induced by tail suspension was measured in accordance with the methods of Steru *et al*. (*8*). Acoustically and visually isolated mice were suspended 50 cm above the floor with adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded during the last 4 min of the 6 min testing period. Mice were considered immobile only when they hung passively and they were completely motionless.

2.5. *Forced swim test (FST)*

The forced swim test was similar to tests described previously (*9,10*). The mice were indi-vidually placed in glass cylinders (height, 25 cm; diameter, 10 cm) containing 10 cm of water (22 ± 1°C). The duration of immobility was defined as the time the mouse spent without struggling, floating motionless or making only small movements necessary to keep its head above water during the last 4 min of the 6 min testing period. The water was replaced following each trial.

2.6. *Open field test (OFT)*

Locomotor activity was observed *via* a slightly modified form of the open field test (OFT) (*11,12*). Briefly, the locomotor activity of the mice was measured using a box (40 × 60 × 50 cm) with the floor divided into 12 identical squares illuminated with light from the ceiling. Mice were placed in the central square and the total number of squares entered was recorded for 3 min. The open field arena was cleaned following each trial.

2.7. *Tests for reversal of reserpine-induced ptosis, akinesia, and hypothermia*

Tests for reversal of reserpine-induced ptosis, akinesia, and hypothermia were in accordance with the methods of Qiu *et al*. (*13*). Mice were treated with reserpine (2.5 mg/kg, *i.p.*) 1 h after the administration of catalpol. Three parameters of akinesia, the degree of palpebral ptosis, and the rectal temperature were recorded 4 h after the administration of reserpine. The degree of palpebral ptosis was evaluated according to the following rating scale: 0, eyes open; 1, eyes one-quarter closed; 2, eyes half-closed; 3, eyes three-quarters closed; and 4, eyes completely closed. To measure akinesia, mice were placed in the center of a circle (diameter, 7.5 cm). The total time the mice remained within the circle during a 1 min period was measured.

2.8. *Measurement of monoamine neurotransmitter levels*

Brain levels of NE, DA, 5-HT, and the 5-HT metabolite 5-HIAA were measured with ELISA kits according to the manufacturer's protocols (*14-16*). The levels of NE, DA, 5-HT, and 5-HIAA were expressed as ng/g in wet weight tissue. Sample preparation was as follows: 100 mg of tissue was rinsed with 1× phosphate buffered solution (PBS), homogenized in 1 mL of 1× PBS, and stored overnight at -20°C. After two freeze-thaw cycles were used to rupture the cell membranes, the homogenates were centrifuged for 5 min at 5000× g, 4°C. The supernate was assayed and immediately removed. The sample was thawed and centrifuged again before measurement. Repeated freeze-thaw cycles were avoided.

2.9. *Statistical analysis*

All experimental data are expressed as the mean ± standard error of mean (SEM). Significant differences among experimental groups were compared using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test ($p < 0.05$) using the Statistics Package for Social Science (SPSS) program Version 13.0.

3. Results

3.1. *Catalpol shortens the duration of immobility in the TST and FST*

The duration of immobility in the TST and FST was 93.8 ± 4.9 s and 102.4 ± 6.8 s, respectively, for the vehicle group. Compared to the vehicle group, catalpol (5, 10, and 20 mg/kg, *i.g.*) significantly shortened the duration of immobility both in TST (Figure 1B) and in FST (Figure 1C). Fluoxetine hydrochloride (FH, 10 mg/kg, *i.g.*) that was used as a positive control also caused a significant shortening of the duration of immobility in both the TST and FST (Figures 1B and 1C). There was no significant difference in the duration of immobility caused by a similar dose of catalpol or FH. Results indicated that catalpol causes antidepressant-like action and that catalpol is comparable to the positive drug FH.

3.2. *Catalpol has no effect on locomotor activity*

The effect of catalpol on the locomotor activity of mice is shown in Figure 2. The locomotion measured in the OFT was 80.3 ± 6.7 for the vehicle group. Neither catalpol nor FH affected locomotor activity at doses that significantly shortened the duration of immobility in the TST and FST.

3.3. *Catalpol attenuates reserpine-induced ptosis, akinesia, and hypothermia*

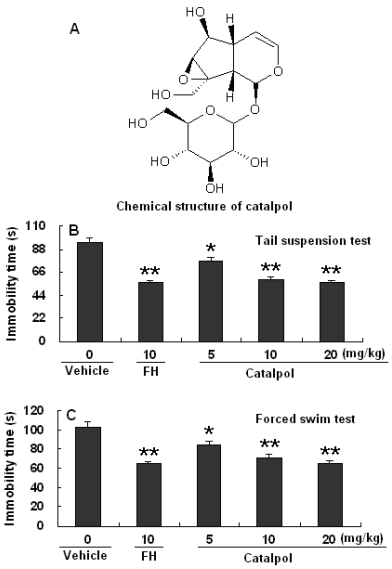

Figure 1. Chemical structure of catalpol (A), and its effect on the duration of immobility of mice in the tail suspension (B) and forced swim (C) tests. Data are expressed as the mean ± SEM ($n = 10$). Significant differences compared to the vehicle group were indicated by * $p < 0.05$ and ** $p < 0.01$.

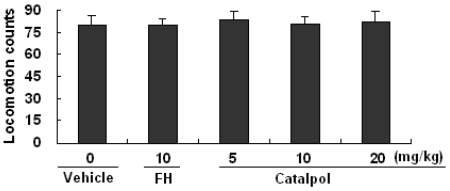

Figure 2. Effect of catalpol on the locomotor activity of mice in the open field test. Data are expressed as the mean ± SEM ($n = 10$).

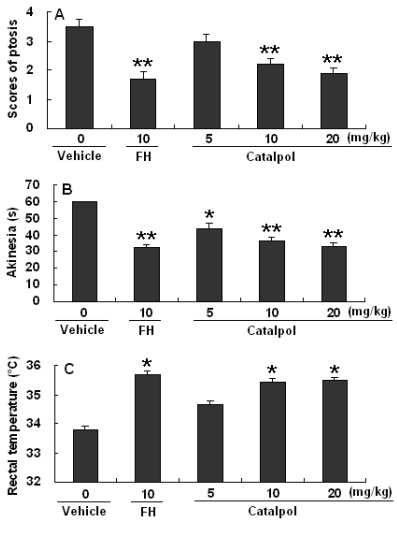

Figure 3. Effects of catalpol on reserpine induced ptosis, akinesia, and hypothermia in mice. Data are expressed as the mean ± SEM ($n = 10$). Significant differences compared to the vehicle group were indicated by * $p < 0.05$ and ** $p < 0.01$.

The effects of catalpol on reserpine induced ptosis, akinesia, and hypothermia are shown in Figure 3. Reserpine induced ptosis was 3.5 ± 0.3, akinesia was

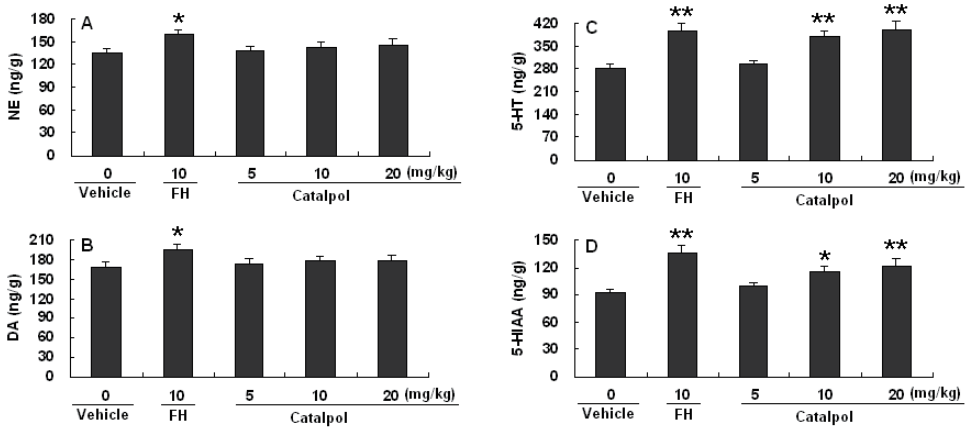

Figure 4. Effect of catalpol on central monoamine neurotransmitters and a major metabolite. Data are expressed as the mean ± SEM (n = 10). Significant differences compared to the vehicle group were indicated by * $p < 0.05$ and ** $p < 0.01$.

60 s, and hypothermia was 33.8 ± 0.1°C in the vehicle group. Compared to the vehicle group, catalpol (10, 20 mg/kg) significantly decreased reserpine-induced ptosis and significantly counteracted reserpine-induced hypothermia. Catalpol (5, 10, and 20 mg/kg) significantly reduced the duration of reserpine-induced akinesia in comparison to that in the vehicle group. FH (10 mg/kg) also significantly counteracted the hypothermia, ptosis, and akinesia induced by reserpine.

3.4. *Catalpol increases levels of monoamine neurotransmitters and a major metabolite in mice*

The effect of catalpol on central monoamine neurotransmitters and a major metabolite of 5-HT in mice is shown in Figure 4. The levels of 5-HT, 5-HIAA, NE, and DA in the brains of mice in the vehicle group were 280.3 ± 11.7, 92.2 ± 3.9, 134.7 ± 5.8, 168.8 ± 7.6 ng/g, respectively. Compared to the vehicle group, catalpol (10, 20 mg/kg) treatment significantly increased the levels of 5-HT and 5-HIAA, but not NE or DA, in the brains of mice, while FH treatment significantly affected levels of NE, DA, 5-HT, and 5-HIAA.

4. Discussion

Both the TST and FST are widely used to screen drugs for antidepressant activity (*8-10*). In animals, immobility as a result of the forced swim and tail suspension is similar to human depression and is amenable to reversal by antidepressant drugs (*8-10*). These models are based on the despair or helpless behavior of animals in an inescapable situation or a confined space. Results from these models revealed that catalpol has significant antidepressant-like effects in a dose-dependent manner.

In these behavioral tests, false-positive results are occasionally obtained with agents that stimulate locomotor activity (*17*). Therefore, the present study determined whether catalpol had excitatory or inhibitory action on the central nervous system. Catalpol had no effect on the spontaneous locomotor activity of mice, indicating that catalpol had no excitatory or inhibitory action on the central nervous system at the doses used. This rules out the possibility of false positive results on the TST and FST. Catalpol shortened the duration of immobility in the TST and FST; this is likely due to antidepressant-like action and not psychomotor-stimulant action.

Reserpine is an antihypertensive drug that depletes neuronal storage granules of biogenic amines in the brains of rodents and that produces a clinically significant depression-like state (*13*). Mice become hypothermic, akinetic, and diarrhetic, with eyelid drooping, in response to reserpine. Reserpine irreversibly inhibits the vesicular uptake of monoamine neurotransmitters such as NA, DA, and 5-HT (*13-17*). The symptoms are reversed by major classes of antidepressant drugs. The present results revealed that catalpol counteracts, in a dose-dependent manner, the ptosis, akinesia, and hypothermia induced by reserpine in mice. This indicates that catalpol has an antidepressant-like effect and may have an effect on monoamine neurotransmitters. Therefore, this study further explored the antidepressant action of catalpol by using ELISA to determine the levels of monamine neurotransmitters in the brains of mice. Results indicated that catalpol increased the levels of central 5-HT and its metabolite 5-HIAA but did not affect levels of NE and DA in mice, suggesting that the antidepressant-like effects of catalpol may be mediated by the central serotonergic system and not by the noradrenergic or dopaminergic systems.

In conclusion, this is the first study to reveal that catalpol has an antidepressant-like effect in mice and that its action may involve upregulation of the central serotonergic system.

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