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Original Research Article

Effect of polyphenol extract from *Zanthoxylum bungeanum* Maxim. on endocrine hormones and monoamine oxidase activity in a mouse model of climacteric depression

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Abstract

Purpose: *To investigate the effects of polyphenol extract from Zanthoxylum bungeanum Maxim. (ZPPC) on endocrine hormones, monoamine oxidase activity and behavior in a mouse model of climacteric depression.*
Methods: *Institute of Cancer Research (ICR) female albino mice (n = 50) weighing 24 – 26 g (mean wt = 25.0 ± 1.0 g) were randomly assigned to five groups of ten rats each: normal control group, negative control, and ZPPC (50 mg/kg), ZPPC (100 mg/kg) and ZPPC (200 mg/kg) groups. Depression was induced in the mice via oral administration of moclobemide at a dose of 20 mg/kg, and intraperitoneal injection of imipramine (20 mg/kg) 1 h and 30 min, before treatment. Tail suspension, forced swimming and voluntary activity tests were performed on the mice. The activity of monoamine oxidase (MAO) in mouse brain and the levels of endocrine hormones were also determined.*
Results: *Treatment of depressed mice with ZPPC significantly and dose-dependently increased their tail suspension and immobility time ($p < 0.05$). The activity of monoamine oxidase in the brains of mice in the negative control group was significantly higher than that of normal control mice, but was significantly and dose-dependently reduced by ZPPC treatment ($p < 0.05$). Similarly, treatment of depressed mice with ZPPC significantly and dose-dependently reduced their serum adrenocorticotropin and corticosterone levels ($p < 0.05$).*
Conclusion: *The results of this study indicate that ZPPC exerts antidepressant effect via suppression of brain MAO activity.*

Keywords: *Climacteric depression, Endocrine hormones, Menopause, Monoamine oxidase, Polyphenols*

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INTRODUCTION

According to World Health Organization (WHO), depression, which has a lifetime prevalence of more than 15 %, may become the second leading cause of death worldwide after cancer [1]. Climacteric depression is a common mental disorder in women. In addition to emotional

disorders, patients suffering from depression present obvious physical symptoms such as loss of appetite, upper abdominal discomfort, dry mouth, constipation, diarrhea, and in severe cases, suicidal thoughts, and impaired social function [2]. The physiological changes which precede mental symptoms aggravate depression, especially in women [3]. The results of clinical studies show that the incidence of climacteric depression accounts for about 6 % of climacteric population and 78 % of climacteric syndrome, and about 80 % of menopausal women usually experience mood disorders [4]. The incidence of suicide is higher in menopausal women than in men of same age [4].

The involvement of monoamine neurotransmitters in the pathogenesis of depression has been reported [5]. Antidepressants currently used for the management of depression are ineffective and produce adverse reactions, and this has necessitated the search for novel compounds that are safe and effective against depression.

Studies have shown that some preparations used in Traditional Chinese Medicine (TCM) have shown potential as effective antidepressants [6]. They have been reported to protect neurons via regulation of the function of HPA axis and neurotransmitters in the brain [6]. *Zanthoxylum bungeanum,* the mature dry pericarp of *Rutaceae*, is used to relieve pain, repel insects, create conditions of *dryness and dampness*, kill insects and relieve itching. It has been reported that acute administration of ZPPC produces significant antidepressant effect in mouse model of behavioral despair [7].

The present study investigated the effects of ZPPC on endocrine hormones, monoamine oxidase activity and behavior in mouse model of climacteric depression.

EXPERIMENTAL

Drugs and reagents

Moclobemide and imipramine were obtained from Shanghai Jinma Experimental Equipment Co. Ltd. Cloclobin, dihydrobromocanine ureamine and 4-hydroquinoline were products of Sigma-Aldrich (USA). DigBehv Animal Behavior Analysis System was purchased from Jiangsu Cyon Biotechnology Co. Ltd. Fluorescence spectrophotometer (R-5000) was purchased from Shimadu Company (Japan). Refrigerated centrifuge (TGL-16GA) was a product of Hunan Xiangxin Instrument Co. Ltd, while FSH-II high-speed electric homogenizer was obtained from Jintan Jiamei Instrument Co. Ltd. Automatic enzyme marker 550 was purchased from Bio-Rad (USA), while BS110S analytical balance was a product of Shanghai Aozhi Electronic Technology Co. Ltd.

This research was approved by the Animal Ethical Committee of The First Clinical Medical College of Heilongjiang University of Chinese Medicine, Haerbin, China (the approval no. 201921363), carried out according to Principles of Laboratory Animal Care [8].

Mice

Fifty Institute of Cancer Research (ICR) female albino mice (n = 50) weighing 24 – 26 g (mean weight = 25.0 ± 1.0 g) were purchased from Guangdong Medical Laboratory Animal Center (No. 2018C028). They were housed in metal cages in a greenhouse under standard conditions and had free access to standard feed and water in an environment with equal light/dark periods at 25 ˚C and 60 % humidity. They were acclimatized to the laboratory conditions for three days prior to commencement of the study. The study protocol received approval from the Institutional Animal Ethics Committee of First Clinical Medical College of Heilongjiang University of Chinese Medicine, Haerbin, China and the study procedures were carried out according to the guidelines of the National Institute of Health (NIH) for the use and care of experimental animals. The mice were first subjected to double-strategy ovariectomy before the commencement of study proper.

Animal grouping and treatment

The mice were randomly assigned to five groups of ten rats each: normal control group, negative control, ZPPC (50 mg/kg), ZPPC (100 mg/kg) and ZPPC (200 mg/kg bwt) groups. Depression was induced in the mice via oral administration of moclobemide at a dose of 20 mg/kg and intraperitoneal injection of imipramine (20 mg/kg bwt) 1 h and 30 min, respectively, before treatment. Mice in control group were given an equivalent volume of double-distilled water in place of drug.

Tail suspension test

Exactly 1 h after commencement of treatment, the mice were fixed on an iron shelf about 2 cm away from the tail tip with adhesive tape. The head of each mouse was positioned downward from the box (15 cm from the bottom of the box). At the start, the mouse head was moved from side to side until the number of movements

decreased, and this was taken as intermittent immobility. The behavior of each mouse was noted and recorded within 6 min.

Forced swimming test

Usually, when a mouse is placed in water, it gasps for air, and after some time, it stops struggling, floats in the water and stays still, or does the necessary little movement to keep its head on the surface of the water. Exactly 24 h before commencement of the test, the mice were placed in 1500 mL glass beaker maintained at 25 ^{o}C. Then, the mice were trained to swim for 15 min. One hour after treatment, the mice were again placed in the beaker and forced to swim for 6 min. The time it took each mouse to rest was observed and recorded within 4 min.

Voluntary activity test

The mice were placed in a voluntary activity tester 1 h after drug administration, and the activity of each mouse was recorded using an in-built camera. After the mice acclimatized to the environment for 10 min, their activity time were observed, recorded and taken as index of independent activity.

Determination of monoamine oxidase activity

One hour after drug administration, the mice were decapitated and their brains were quickly isolated on ice, and weighed. Then, 4 mL of cold phosphate buffer (0.05 mol/L) was used to homogenize the brain tissues. Subsequently, 0.4 mL of 20 % Triton, 0.2 mL tissue homogenate and 2.5 mL phosphate buffer were thoroughly mixed. The solution was pre-incubated for 10 min at 38 ^{o}C, and 30 mL of l2.19 mmol/L substrate was thereafter added and pre-incubated for another 30 min at 37 ^{o}C.

About 0.2 mL of 5 mol/L perchloric acid was added, followed by cooling and centrifugation for 10 min at 1500 rpm to obtain supernatant, 0.5 mL aliquot of which was mixed with 2.5 mL of 1.0 mol/L sodium hydroxide (NaOH) solution, and read using fluorescence spectrophotometer. The protein content was measured using Bradford method.

Determination of levels of adrenocortico-tropin and corticosterone in the mice

The levels of adrenocorticotropin and corticosterone in mice were determined using radioimmunoassay kit (BoYu biotech, Shanghai, China).

Statistical analysis

Data are expressed as mean ± SD. Statistical analysis was performed using SPSS (15.0). Groups were compared using Rank sum test. Values of $p < 0.05$ were taken as indicative of significance.

RESULTS

Effect of ZPPC treatment on tail suspension time

Treatment of depressed mice with ZPPC significantly and dose-dependently increased their tail suspension time ($p < 0.05$; Figure 1).

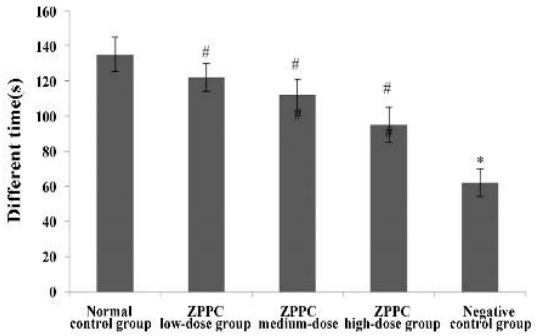

Figure 1: Effect of ZPPC on tail suspension time in depressed mice. *$p < 0.05$, vs normal control; #$p < 0.05$, vs negative control

Effect of ZPPC on immobility time in depressed mice

The immobility time of mice in negative control group was significantly reduced relative to that of normal control group, but was significantly and dose-dependently increased after treatment with ZPPC ($p < 0.05$). These results are shown in Figure 2.

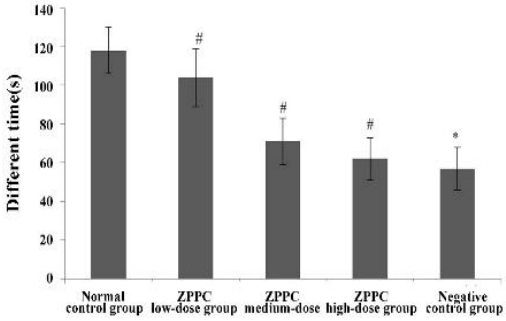

Figure 2: Effect of ZPPC on immobility time of depressed mice. *$P < 0.05$, vs normal control; #$p < 0.05$, vs negative control

Influence of ZPPC on voluntary activities in depressed mice

As shown in Figure 3, there were no significant differences in voluntary activities among the mouse groups ($p > 0.05$).

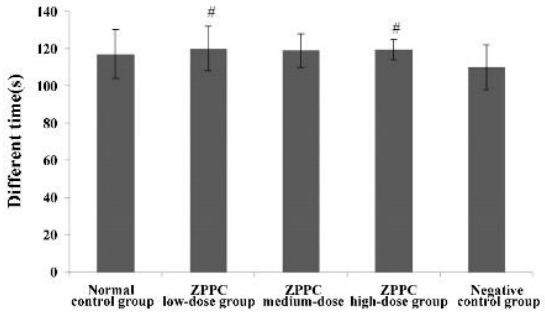

Figure 3: Voluntary activity in depressed mice after treatment with ZPPC. *$P < 0.05$, vs normal control; #$p < 0.05$, vs negative control

Monoamine oxidase activities in brains of depressed mice

The activities of monoamine oxidase in the brains of mice in negative control group were significantly higher than those of normal control group mice, but were significantly and dose-dependently reduced by ZPPC treatment ($p < 0.05$; Figure 4).

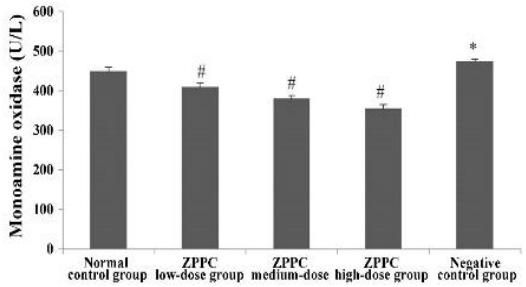

Figure 4: Effect of ZPPC on monoamine oxidase activities in brain tissues of depressed mice. *$P < 0.05$, vs normal control; #$p < 0.05$, vs negative control

Levels of endocrine hormones in depressed mice

Treatment of depressed mice with ZPPC significantly and dose-dependently reduced their adrenocorticotropin and corticosterone levels ($p < 0.05$; Table 1).

DISCUSSION

Depression, a mental health disorder characterized by persistently depressed mood or loss of interest in activities, causes significant impairment to daily life. It is common in perimenopausal women. Studies have shown that impaired ovarian function after menopause contributes significantly to the onset of climacteric depression [9].

Table 1: Effect of ZPPC on levels of endocrine hormones in depressed mice

Group	Adrenocorticotropin (mg/kg tissue)	Corticosterone (mg/kg tissue)
Normal control	18.61 ± 1.51	10.21 ± 0.71
Negative control	98.41 ± 6.41*	47.21 ± 2.31*
ZPPC (50 mg/kg)	85.91 ± 4.01#	41.41 ± 0.81#
ZPPC (100 mg/kg)	74.61 ± 3.81#	34.41 ± 1.81#
ZPPC (200 mg/kg)	46.01 ± 4.21#	17.81 ± 1.11#

*$p < 0.05$, vs normal control; #$p < 0.05$, vs negative control

Forced swimming test (FST), a simple, easy, reliable and effective animal model of depression, is used to screen drugs with potential antidepressant effects [10]. Other behavioral despair models have been established, which take clues from FST, the most prominent of which is the mouse tail suspension test (MTST) [11]. In this test, there is usually significant reduction in the number of survival activities due to disappointment and the mice appear to be in an intermittent static state, manifested as desperate state. Immobility is a kind of adaptive behavior which occurs after depressed animals try to escape, but fail, and it is considered to be an indicator of depression [12]. The results of this study showed that the immobility time of mice in negative control group was significantly longer than that of normal control group, but was significantly and dose-dependently shortened by ZPPC treatment. These results suggest that pretreatment of mice with ZPPC may confer some antidepressant effect on them.

In order to distinguish antidepressants from non-antidepressants using a mouse model, it is important to observe spontaneous activity of the mice. In this study, there were no significant differences in total exercise distance among the treatment groups, an indication that ZPPC may have no effect on spontaneous behavior of the mice. In an earlier study, it was hypothesized that estrogen withdrawal for the first time could contribute to the onset of climacteric depression [13]. The gradual decline in ovarian function in climacteric women leads to significant reduction

in estrogen secretion, and the resultant low level of estrogen contributes significantly to the onset of depression or aggravates the condition.

Clinical studies have shown that estrogen and progesterone affect monoamine oxidase denaturation and serotonin metabolism [14]. Both hormones regulate the expression of serotonin receptor. The pathogenesis of climacteric depression may be related to the activity of postsynaptic serotonin receptor [15]. Estrogen promotes the growth and development of neurons and synapses at the genetic level [16]. In this study, treatment of depressed mice with ZPPC significantly and dose-dependently reduced their adrenocorticotropin and corticosterone levels. It is suggested that the antidepressant effect of ZPPC pretreatment may be related to suppression of brain MAO activity.

CONCLUSION

The results of this study indicate that ZPPC exerts antidepressant effect via suppression of brain MAO activity, and therefore could find clinical application in the treatment of depression.

DECLARATIONS

Acknowledgement

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors, all authors read and approved the manuscript for publication. Yu Li conceived and designed the study, Aiying Song, Qiang Zhang, Xiaoqing You, Xiangni Zou, Xiao Han, Yu Li, Yin Tang collected and analysed the data, Aiying Song wrote the manuscript.

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