

Original Research Article

Anti-depressant effect of *Paeonia lactiflora* Pall extract in rats

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Abstract

Purpose: To explore the effect of *Paeonia lactiflora* Pall. extract on depression in rats.

Methods: Various doses (150, 300 and 600 mg/kg) of *Paeonia lactiflora* Pall. extract (PLPE) were orally administered to three groups of rats of 10 each, respectively, suffering from depression for 14 days. Fluoxetine was used as positive control. Tail suspension, forced swimming and monoamine oxidase (MAO) tests were carried out on the rats.

Results: A dose-dependent reduction in rat immobility was observed. The effect of PLPE at the highest dose (600 mg/kg) was more potent than that of the reference antidepressant, fluoxetine. PLPE, at a dose \geq 150 mg/kg significantly inhibited MAO A activity in rat whole brain in a dose-dependent manner ($p < 0.01$); however, only oral administration of PLPE at a dose of 600 mg/kg produced observable MAO B inhibitory activity in rat brain ($p < 0.01$). Only fluoxetine showed a tendency to inhibit both MAO A and B activities in animal brain. Neither PLPE nor fluoxetine, at the doses tested, produced significant effects on locomotor activity.

Conclusion: The results suggest that *Paeonia lactiflora* Pall is a potential agent for the treatment of depression in humans.

Keywords: *Paeonia lactiflora*, Depression, Tail suspension test, Forced swimming test

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INTRODUCTION

Depression is a major disease affecting nearly 13 - 20 % of the population [1]. In spite of the introduction of the tricyclic antidepressants (TCAs), selective reversible inhibitors of monoamine oxidase A (RIMAs), selective serotonin reuptake inhibitors (SSRIs) and specific serotonin-noradrenaline reuptake inhibitors (SNRIs), depression continues to be a major medical problem. However, search for new antidepressant drug continues. According to the theory of the traditional Chinese medicine (TCM), the clinical condition of depression could be mainly classified into liver qi stagnation, the

symptom of which can be described as mental stress, hypochondriac distensive pain, or lumps in the breasts, hernial pain and irregular menstruation. Based on this, many Chinese medicinal plants were successfully used to manage the disorder of depression by dispersing stagnant liver qi and the active principles from some of them were isolated [2,3].

Paeonia lactiflora Pall. is a well-known indigenous herbal medicine for treating depression in China. In the present study, we examined the in vivo antidepressant activities of *Paeonia lactiflora* Pall. extract in rat models of immobility tests as well as MAO activity in rat

whole brain in comparison with the effects of a reference antidepressant: fluoxetine (an SSRI).

EXPERIMENTAL

Material

The herbal samples of *Paeonia lactiflora* Pall. were collected from Shiyan City, Hubei Province in China in October 2015. Taxonomic identification of the plant was performed by Professor Li Hu of Dalian University in China. A voucher specimen of herbarium (no. PLPE 20151008) was deposited in the herbarium of College of Pharmacy, Dalian University, China for future reference. The PLPE was obtained by steeping the dried *Paeonia lactiflora* Pall. in water at 60 °C three times, each for one hour before first drying in an oven and then freeze-drying the last extract thus obtained. One gram powder was equivalent to about 1.5 g crude samples. The yield was 66.67 %.

Animals

Sprague Dawley rats, weighing 180 - 220 g, were provided by the Experimental Animal Center of Liaoning Province (Certificate no. SYXK 2001-0005). The animals had free access to food and water, and were allowed to acclimatize for at least one week before use. The rat experiment was approved by the Animal Care and Use Committee of Dalian University (approval ref no. 20130602) and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [4].

Animal group

The female rats were randomly divided into 5 groups of ten rats: control group [normal saline (0.9 % NaCl)], reference group (fluoxetine 30 mg/kg) as well as the PLPE groups, namely, 150, 300 and 600 mg/kg doses. All drugs were orally administered at 14: 00-15: 00 h every day for 14 days, respectively. The behavioral tests were conducted 1 h after the last treatment. MAO assay was started in rats 1 h was administered after 14-day administration.

Tail suspension test

The tail suspension test was based on the method of Steru [5]. Rats were individually suspended by the tail with a clamp (1 cm distant from the end) for 6 min in a box with the head 5 cm to the bottom. Testing was carried out in a darkened room with minimal background noise. The duration of immobility was observed during the final 4 min interval of the test.

Forced swimming test

Before drugs were administered, rats were forced to swim individually for 6 min, in glass cylinders (20 cm in height; 14 cm in diameter), containing fresh water up to a height of 10 cm at 25 °C. After 6 min, they were removed and dried with a towel. They were again forced to swim in a similar environment for a period of 6 min 24 h later. The duration of immobility was measured during the final 4 min interval of the test.

MAO assay

The mitochondrial fraction suspended in 10 milliliter of cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose), was mingled at 4 °C for 20 min. The mixture was centrifuged at 15 000 \times /g for 30 min at 0 °C and the pellets were re-suspended in the same buffer. The protein concentration was adjusted to 1 mg/ml. Protein concentration was estimated by the Lowry method [6] using bovine serum albumin as the standard. MAO activity was assessed as described previously [7]. The assay mixtures contained 4 mM 5-HT or 2mMb-PEA as specific substrates for MAO A and B, respectively, 250 ml solution of the mitochondrial fraction and 100 mM sodium phosphate buffer (pH 7.4) upto a final volume of 1 ml. The reaction was allowed to proceed at 37 °C for 20 min, and stopped by adding 1 M HCl (200 ml), the reaction product was extracted with 5 ml of butyl acetate (for MAO A assay) or cyclohexane (for MAO B assay), respectively. The organic phase was measured at wavelength of 280 nm for MAO A assay and 242 nm for MAO B assay with spectrophotometer respectively. Blank samples were prepared by adding 1 M HCl (200 ml) prior to reaction, and worked up subsequently in the same manner.

Statistical analysis

Data are expressed as mean \pm standard deviation (SD). Significant differences between the groups were analyzed using one-way analysis of variance (ANOVA) followed by two-paired Student's t-test. $p < 0.05$ was considered statistically significant.

RESULTS

Effect of PLPE on duration of immobility

As shown in Table 1, PLPE showed no change after 1 day treatment, and had the tendency to reduce the immobility time only after 7-day treatment. After a 14-day treatment, PLPE at the

doses of 150, 300 and 600 mg/kg significantly decreased the duration of immobility in a dose-dependent manner, resulting in 33.4, 41.5 and 56.3 % immobility reduction, respectively ($p < 0.05$). However, the reference antidepressant fluoxetine at the dose of 30 mg/kg resulted in reduction. PLPE at the doses of 300 and 600 mg/kg appeared to be more potent than that of fluoxetine after 14-day treatment in the study.

As shown in Table 2, PLPE showed no change after 1 day treatment. PLPE at the dose of 600 mg/kg exhibited a reduction in immobility after 7-day treatment ($p < 0.05$). The extract at all doses tested decreased the duration of immobility in a dose-dependent manner, resulting in 51.3, 61.4 and 67.8 % immobility reduction for the 150, 300 and 600 mg/kg after 14-day treatment, respectively. Fluoxetine at the dose of 30 mg/kg significantly produced a time-dependent immobility reduction. PLPE at the dose of 600

mg/kg appeared to be more potent than that of fluoxetine after 14-day treatment.

Effect of PLPE on MAO A and B activities in rat whole brain

The effect of PLPE and fluoxetine for 14 days on the MAO A and B activities in rat whole brain is shown in Table 3. The MAO A and B activities in normal group were 24.8 ± 1.6 nmol/mg protein h and 20.4 ± 1.3 nmol/mg protein h, respectively. Oral administration of the extract at the doses of 150, 300 and 600 mg/kg inhibited MAO A activity in a dose-dependent manner, providing 21.6, 34.7 and 44.3 % inhibition ($p < 0.05$). However, only the extract at a dose of 600 mg/kg inhibited MAO B activity, producing 35.4 % inhibition ($p < 0.05$). Fluoxetine at the dose of 30 mg/kg showed a tendency to reduce the MAO A and B activity, but the effects were not significant in the study.

Table 1: Effect of PLPE on the duration of immobility in rat tail suspension test (mean \pm SD, n = 10)

Group	Dose (mg/kg)	Duration of immobility (s)		
		Day 1	Day 7	Day 14
Control	-	82.3 \pm 3.2	89.5 \pm 3.6	84.1 \pm 2.8
Fluoxetine	30	64.2 \pm 1.9	53.6 \pm 2.5	54.8 \pm 2.7
PLPE-H	600	81.2 \pm 4.2	57.3 \pm 3.1	35.4 \pm 1.8**
PLPE-M	300	79.3 \pm 3.1	69.2 \pm 2.6	47.1 \pm 1.9**
PLPE-L	150	84.2 \pm 2.7	82.8 \pm 2.1	52.3 \pm 3.5*

PLPE-H: High dose of PLPE; PLPE-M: Middle dose of PLPE; PLPE-L: Low dose of PLPE; * $p < 0.05$, ** $p < 0.01$ when compared with control groups

Table 2: Effect of PLPE on duration of immobility in mouse forced-swimming test (mean \pm SD, n = 10)

Group	Dose (mg/kg)	Duration of immobility (s)		
		Day 1	Day 7	Day 14
Control	-	79.6 \pm 2.5	75.3 \pm 2.7	77.0 \pm 2.9
Fluoxetine	30	42.6 \pm 1.8	35.2 \pm 1.4	33.4 \pm 1.6**
PLPE-H	600	73.5 \pm 2.3	52.4 \pm 1.7	26.6 \pm 1.5**
PLPE-M	300	76.4 \pm 1.9	71.3 \pm 2.2	34.3 \pm 1.7**
PLPE-L	150	74.6 \pm 2.6	72.2 \pm 2.0	38.8 \pm 2.4*

PLPE-H: High dose of PLPE; PLPE-M: Middle dose of PLPE; PLPE-L: Low dose of PLPE; * $p < 0.05$, ** $p < 0.01$ when compared with control groups

Table 3: Effect of PLPE on MAO activity in mouse whole brain (mean \pm SD, n = 10)

Group	Dose (mg/kg)	MAO activity (nmol/mg protein h)		MAO inhibition (%)	
		A	B	A	B
		Control	-	24.8 \pm 1.6	20.4 \pm 1.3
Fluoxetine	30	16.5 \pm 1.4	17.3 \pm 0.9	14.3	13.5
PLPE-H	600	15.3 \pm 1.1**	18.3 \pm 1.4**	44.3	35.4
PLPE-M	300	12.6 \pm 1.4**	16.5 \pm 0.8	34.7	14.2
PLPE-L	150	10.7 \pm 1.3*	13.7 \pm 0.6	21.6	7.5

PLPE-H: High dose of PLPE; PLPE-M: Middle dose of PLPE; PLPE-L: Low dose of PLPE; * $p < 0.05$, ** $p < 0.01$ when compared with control groups

DISCUSSION

The tail suspension and forced swimming tests are two behavioral tests in rodents that predict the clinical efficacy of many types of antidepressant treatments [8,9]. We studied PLPE on the immobility behaviors in rats. The extract at oral doses from 150 to 600 mg/kg for 14 days significantly decreased the duration of immobility in the tail suspension test and the forced swimming test in rats. These behavioral effects of PLPE at the dose of 600 mg/kg were more potent than that of fluoxetine after 14-day treatment. Neither PLPE nor fluoxetine, at the doses tested, produced significant effects on locomotor activity. These data in the present study has shown that PLPE has antidepressant effects in rat models of immobility tests.

MAO is an important enzyme in the metabolism of a wide range of monoamine neurotransmitters, including noradrenaline, dopamine, and 5-hydroxytryptamine. MAO exists in two forms, A and B. MAO A is more important than MAO B in the metabolism of the major neurotransmitter monoamines. MAO A inhibitors have been accepted to treat depression [10,11]. In the present investigation, we have demonstrated that the extract of *Paeonia lactiflora* Pall. significantly inhibited *in vivo* MAO A activity in rat whole brain in a dose-dependent manner, however, only the extract at a dose up to 600 mg/kg exhibited MAO B inhibitory activity. These findings suggested that anti-depressant effects of *Paeonia lactiflora* Pall. in animal models of immobility tests may be related to the inhibitory activity of MAO, especially to that of MAO A.

CONCLUSION

This study suggests that *Paeonia lactiflora* Pall. is a potential source of therapeutic substances

for the treatment of depression, which would be used for treatment of depression in clinical.

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