Tropical Journal of Pharmaceutical Research April 2017; 16 (4): 867-871
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v16i4.17

Original Research Article

Determination of antidepressant activity of Cyperus rotundus L extract in rats

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Received: 13 August 2016 *Revised accepted: 4 March 2017*

Abstract

Purpose: *To investigate the antidepressant effect of Cyperus rotundus L. extract (CRLE) in rats.*
Methods: *A rat model of depression was prepared for behavioral tests including tail suspension test or forced swimming test. Wistar rats were randomly divided into six groups (n = 10): normal group (0.9 % NaCl), model group (0.9 % NaCl), positive drug group (fluoxetine 30 mg/kg) as well as CRLE groups, namely, 200, 400 and 800 mg/kg doses. All drugs were orally administered at 14: 00 - 15: 00 h for 1, 7 and 14 days, respectively. Tail suspension and forced swimming tests were conducted 1 h after the last treatment. Monoamine oxidize A (MAO) assay was commenced in rats after 14-day administration. Except for the rats in normal group, other group participated in the behavioral tests.*
Results: *The effect of 800 mg/kg CRLE was more potent than the positive drug fluoxetine. CRLE (≥ 200 mg/kg) treatment for 14 days significantly inhibited brain MAO activity in rats ($p < 0.01$) in a dose-dependent manner. Oral administration of 800 mg/kg CRLE produced MAO B inhibitory activity in rat brain ($p < 0.01$). Fluoxetine inhibited both brain MAO A and B activities in rats ($p < 0.01$).*
Conclusion: *The results suggest that CRLE produces significant antidepressant effect in rats, and therefore, may be useful for the treatment of depressed patients, but further studies are required to ascertain this.*

Keywords: *Cyperus rotundus, Antidepressant, Tail suspension test, Forced swimming test, Monoamine oxidase, Fluoxetine*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Depression is a major disease affecting nearly 13 - 20 % of people in the world. [1]. In spite of the introduction of the tricyclic antidepressants (TCAs), selective reversible inhibitors of monoamine oxidize 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, hernia pain and irregular menstruation. Based on this, many Chinese medicinal plants were successfully used to manage the disorder of depression using the active principles of some plants [2,3].

Cyperus rotundus L. is a well-known indigenous herbal medicine for treating depression in China. In this study, we examined the *in vivo* antidepressant activity of *CRLE* in rat models of immobility tests as well as MAO activity in rat whole brain in comparison with the effects of reference antidepressant fluoxetine (SSRI).

EXPERIMENTAL

Plant material and extraction

All the herbal samples of *Cyperus rotundus L.* were collected from Changsha City, Hunan Province in China in March 2016. Taxonomic identification of all the plants were performed by Professor Shan Li of Shandong University in China. A voucher specimen (no. CRLE 20160306) was deposited in the herbarium of College of Pharmacy, Shandong University, China for future reference.

The whole plant of *Cyperus rotundus L.* was dried in a drying oven at 100 ^{o}C for 12 h. CRLE was obtained by steeping the dried *Cyperus rotundus L.* in water at 60 ^{o}C three times, each for 1 h before first drying in an oven and then freeze-drying the last extract thus obtained. One gram powder was obtained from about 1.6 g dried sample, i.e., a yield of 62.5 %.

Animals

Wistar rats weighing 150 - 180 g were provided by the Experimental Animal Center of Shandong Province (certificate no. SYXK 2003-0007). 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 Shandong University (approval ref no. 20110403) and was carried out in compliance with Directive 2010/63/EU on the Handling of Animals Used for Scientific Purposes [4].

Animal model and groups

The rat model of depression was prepared by the behavioral tests including tail suspension test and forced swimming test. The rats were randomly divided into six groups of ten rats in each: normal group (0.9 % NaCl), model group (0.9 % NaCl), positive drug group (fluoxetine 30 mg/kg) as well as CRLE groups, 200, 400 and 800 mg/kg doses. All drugs were orally administered at 14: 00 - 15: 00 h for 1, 7 or 14 days, respectively. The behavioral tests were conducted 1 h after the last treatment, respectively. MAO assay started in rats after 14-day administration. Except for the rats in normal group, all other rats participated in the behavioral tests.

Tail suspension test

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

Forced swimming test

Rats were individually forced to swim 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 ^{o}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 counted during the final 4 min interval.

MAO assay

The mitochondrial fraction suspended in 10 vol. of cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose), was mixed at 4 ^{o}C for 20 min. The mixture was centrifuged at 15 000×/g for 30 min at 0 ^{o}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 2 mM b-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) up to a final volume of 1 mL. The reaction was allowed to proceed at 37 ^{o}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 were 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

Values are expressed as mean ± SD (n = 10). 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 CRLE on duration of immobility

Rat tail suspension

As shown in Table 1, CRLE reduced the immobility time after 7-day treatment. After a 14-day treatment, CRLE at the doses of 200, 400 and 800 mg/kg significantly decreased the duration of immobility in a dose-dependent manner, resulting in 31.7, 43.6 and 52.7 % immobility reduction, respectively ($p < 0.05$). However, the reference antidepressant fluoxetine at the dose of 30 mg/kg resulted in significant reduction ($p < 0.05$). CRLE at the doses of 400 and 800 mg/kg appeared to be more potent than that of fluoxetine after 14-day treatment.

Rat forced swimming

As shown in Table 2, CRLE showed no change after 1 day treatment. CRLE at the dose of 800 mg/kg exhibited to show significant immobility reduction after 7-day treatment. The extracts at doses of 200, 400 and 800 mg/kg significantly decreased the duration of immobility in a dose-dependent manner, resulting in 49.8, 60.6 and 65.4 % immobility reduction after 14-day treatment, respectively. Fluoxetine at the dose of 30 mg/kg significantly produced a time-dependent immobility reduction. CRLE at the dose of 800 mg/kg appeared to be more potent than that of fluoxetine after 14-day treatment.

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

The effects of CRLE and fluoxetine for 14 days on the MAO A and B activities in rat whole brain was shown in Table 3. The MAO A and B activities in normal group were 31.6 ±/1.9

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

Group	Dose (mg/kg)	Duration of immobility (s)		
		Day 1	***Day 7***	***Day 14***
Normal	-	75.3±2.4	95.2±3.1	84.2±2.4
Model	-	78.6±2.6	65.6±2.4$^{\Delta}$	40.5±3.2$^{\Delta\Delta}$
Fluoxetine	30	80.1±1.8	50.5±2.6**	44.3±2.6**
CRLE-H	800	77.5±3.5	66.4±3.1**	46.8±1.9**
CRLE-M	400	76.3±2.7	68.7±2.2**	44.3±2.4**
CRLE-L	200	77.9±3.1	59.4±2.1	37.2±3.1

CRLE-H: High dose of CRLE; CRLE-M: Middle dose of CRLE; CRLE-L: Low dose of CRLE. $^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$ when compared with normal groups, $^{*}p < 0.05$, $^{**}p < 0.01$ when compared with model groups

Table 2: Effects of CRLE on the duration of immobility in the rat forced swimming test (mean ± SD, n = 10)

Group	Dose (mg/kg)	Duration of immobility (s)		
		Day 1	**Day 7**	**Day 14**
Normal	-	84.2±2.1	56.4±2.7	37.4±3.1
Model	-	83.5±1.7	79.3±2.3$^{\Delta\Delta}$	50.2±2.9$^{\Delta\Delta}$
Fluoxetine	30	86.1±1.8	70.6±2.5*	37.6±1.6**
CRLE-H	800	79.4±2.3	58.6±2.4*	36.8±1.4**
CRLE-M	400	75.3±3.4	69.3±2.3	38.3±1.5**
CRLE-L	200	77.6±2.6	72.2±1.8	46.4±2.4*

CRLE-H: High dose of CRLE; CRLE-M: Middle dose of CRLE; CRLE-L: Low dose of CRLE; $^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$ when compared with normal groups, $^{*}p < 0.05$, $^{**}p < 0.01$ when compared with model groups

Table 3: Effect of CRLE on MAO activity in rat whole brain (mean ± SD, n = 10)

Group	Dose (mg/kg)	MAO activity (nmol/mg protein h)		MAO inhibition (%)	
		A	***B***	***A***	***B***
Normal	-	31.6±1.9	25.8±1.5	-	-
Model	-	8.4±2.3$^{\Delta\Delta}$	14.4±1.6$^{\Delta\Delta}$	-	-
Fluoxetine	30	19.5±1.8**	17.3±0.9**	16.2	13.4
CRLE-H	800	17.2±1.5**	18.6±1.7**	44.7	37.3
CRLE-M	400	13.6±1.7*	15.9±1.7*	33.4	17.6
CRLE-L	200	10.5±1.6	14.2±1.5	24.2	8.5

CRLE-H: high dose of CRLE; CRLE-M: medium dose of CRLE; CRLE-L: low dose of CRLE. $^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$ when compared with normal groups, $^{*}p < 0.05$, $^{**}p < 0.01$ when compared with model groups

nmol/mg protein h and 25.8 ±1.5 nmol/mg protein h, respectively. Oral administration of the extract at doses of 200, 400 and 800 mg/kg significantly inhibited MAO A activity in a dose-dependent manner, providing 23.3, 32.6 and 45.2 % inhibition. However, only CRLE at a dose of 800 mg/kg significantly inhibited MAO B activity, producing 37.3 % inhibition. Fluoxetine at the dose of 30 mg/kg also reduced the MAO A and B activity significantly ($p < 0.01$).

DISCUSSION

The tail suspension and forced swimming tests are two behavioral tests in rodent that predicted the clinical efficacy of many types of antidepressant treatments [8-10]. CRLE at oral doses from 200 to 800 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 CRLE at the dose of 800 mg/kg were more potent than that of fluoxetine after 14-day treatment. As changes in immobility may be due to changes in locomotor activity caused by central nervous system stimulating agents, rats were tested in the open field test. These data in the present study showed that CRLE 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 [11-13]. 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 regarded as drugs for treating depression in clinical [14-16]. In the present investigation, we have demonstrated that the CRLE significantly inhibited *in vivo* MAO A activity in rat whole brain in a dose-dependent manner. However, only CRLE at a dose up to 800 mg/kg exhibited to have the MAO B inhibitory activity. These findings suggest that anti-depressant effects of CRLE in rat models of immobility tests may be related to the inhibitory activity of MAO.

CONCLUSION

The findings of this study suggest that *CRLE* exhibits significant antidepressant effect in rats, and therefore can be potentially developed for clinical application for the treatment of depression of depression.

DECLARATIONS

Acknowledgement

This study was supported by Science and Technology Development Project of Linyi, namely, “Health-related Quality of Life and Its Influence Factors in Patients with First-ever Post-stroke Depression” (no. 201013018).

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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