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Short communication

Antidepressant activity of aqueous extracts of *Curcuma longa* in mice

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Abstract

Curcuma longa (turmeric) is a well-known indigenous herbal medicine. The aqueous extracts, when administered orally to the mice from 140 to 560 mg/kg for 14 days, were able to elicit dose-dependent relation of immobility reduction in the tail suspension test and the forced swimming test in mice. The effects of the extracts at the dose of 560 mg/kg were more potent than that of reference antidepressant fluoxetine. The extracts, at the dose of 140 mg/kg or above for 14 days, significantly inhibited the monoamine oxidize A (MAO) activity in mouse whole brain at a dose-dependent manner, however, oral administration of the extract only at a dose of 560 mg/kg produced observable MAO B inhibitory activity in animal brain. Fluoxetine showed only a tendency to inhibit MAO A and B activity in animal brain in the study. Neither the extracts of *C. longa* nor fluoxetine, at the doses tested, produced significant effects on locomotor activity. These results demonstrated that *C. longa* had specifically antidepressant effects in vivo. The activity of *C. longa* in antidepression may mediated in part through MAO A inhibition in mouse brain.

Keywords: *Curcuma longa*; Antidepressant activity; Immobility; MAO A; MAO B

1. Introduction

Depression is a major disease affecting nearly 13–20% of the population (Licinio and Wong, 1999). 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, 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 (Kong et al., 2001a,b; Luo et al., 2000). As a follow-up to our previous research, the rhizomes of *Curcuma longa* L. (Zingiberaceae), which was indicated for liver *qi* stagnation in Chinese medicine, was selected. The extract made from the rhizomes of this species has been shown to have a powerful antioxidant, anti-inflammatory, lipid reducing, immunodulatory and sedative actions (Antony et al., 1999; Stano et al., 2000; Kamal-Eldin et al., 2000). Previous report suggested that curcumin isolated from *C. longa* as food constituent attenuates the activity of C_6 glial cells monoamine oxidize (MAO), which plays a central role in several psychiatric and age-related neurological disorders, including clinical depression and Parkinson's disease (Mazzio et al., 1998). In the present study, we examined the in vivo antidepressant activities by the aqueous extracts of *C. longa* in mouse models of immobility tests as well as MAO activity in mouse whole brain in comparison with the effects of reference antidepressant fluoxetine (SSRI).

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2. Materials and methods

2.1. Preparation of extracts

The rhizomes of *C. longa* L. (Zingiberaceae) were purchased from Medicinal Materials Co. of Jiangsu Province and authenticated by Professor L.X. Zhang, School of Life Sciences, Nanjing University. A voucher specimen (NU-78001) has been deposited in the Herbarium of Nanjing University, Nanjing University, Nanjing 210093, People's Republic of China.

The air-dried rhizomes of *C. longa* (1000 g) were extracted with 8 l of hot water for 2 h. The procedure was repeated twice. The extracts were filtered and then concentrated *in vacuum* into residues and lyophilized into powder. We obtained the water extract of *C. longa*. The yield of the extract was 5.4% (w/w).

Fluoxetine (Sigma) were suspended in normal saline (0.9% NaCl).

2.2. Chemicals

5-Hydroxytrylamine and β-phenylethylamine were purchased from Sigma (USA). All other reagents used in the study were of analytical grade.

2.3. Animals

Male ICR mice weighing 22–26 g, were purchased from the Laboratory Animal Center (Nanjing, Jiangsu Province, China) and were housed in plastic cages. They were housed in a quiet room under a 12-h light:12-h dark cycle at 25±2 °C for 5 days before experimentations. All the animals were given standard chow and water *ad libitum*, except during observation periods.

2.4. Drug administration

The animals were randomized into control and experimental groups and divided into five groups of 12 animals each. Animals in group 1 were administered with normal saline (0.9% NaCl). Animals in groups 2, 3 and 4 were administered with the extracts of *C. longa* at the doses of 140, 280 and 560 mg/kg. Animals in group 5 were administered with fluoxetine at the dose of 20 mg/kg. 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 was started in mice 1 h was administered after 14-day administration.

2.5. Tail suspension test

The tail suspension test was based on the method of Steru (Steru et al., 1985). Mouse was individually suspended by the tail with clamp (1 cm distant from the end) for 6 min in a box (25 × 25 × 30 cm) 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.

2.6. Forced swimming test

The studies were carried out on mice according to the method of Porsolt (Porsolt et al., 1977a). Briefly, mouse was individually 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±1 °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.

2.7. Open-field test

The studies were carried out on mice according to a slightly modified method (Archer, 1973). The open-field apparatus consisted of a circular base (80 cm in diameter, 20 cm high wall) having three concentric circles of 14, 28, and 42 cm radius, divided into 36 U without walls. The center was illuminated with a 60 W electric bulb, hung directly 40 cm above it. During all the experiments, the laboratory room was dark. Mouse was placed individually into the center of the arena and allowed to explore freely. The ambulations (the number of crossing sector lines with all four paws), rearing (number of times mouse stood on its hind limbs) and numbers of grooming were recorded for 3 min.

2.8. MAO assay

Mouse brain mitochondrial fraction was prepared following the procedure described previously (Schurr and Livne, 1976). Briefly, the mitochondrial fraction suspended in 10 vol. 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 × *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 (Lowry et al., 1951) using bovine serum albumin as the standard. MAO activity was assessed spectrophotometrically as described previously (Charles and McEwen, 1977). The assay mixtures contained 4 mM 5-HT or 2 mM β-PEA as specific substrates for MAO A and B, respectively, 250 μl 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 °C for 20 min, and stopped by adding 1

M HCl (200 μl), 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 μl) prior to reaction, and worked up subsequently in the same manner.

2.9. Statistics

Values are given as mean ± S.E.M and significances calculated using one-way analysis of variance following by Duncan's *t*-test.

3. Results

3.1. Effects of the aqueous extract of C. longa on the duration of immobility time in the mouse tail suspension test

Effects of oral administration of the aqueous extract of *C. longa* and fluoxetine on the duration of immobility in the mouse tail suspension test were shown in Table 1. The extract showed no any change after 1 day treatment, and had the tendency to reduce the immobility time after 7-day treatment. After a 14-day treatment, the extracts at the doses of 140, 280 and 560 mg/kg significantly decreased the duration of immobility in a dose-dependent manner, resulting in 35.6, 40.8 and 58.1% immobility reduction, respectively. However, the reference antidepressant fluoxetine at the dose of 20 mg/kg resulted in significant reduction. The effects of *C. longa* at the doses of 280 and 560 mg/kg appeared to be more potent than that of fluoxetine after 14-day treatment in the study.

3.2. Effects of the aqueous extract of C. longa on the duration of immobility time in the mouse forced swimming test

Effects of oral administration of the aqueous extract of *C. longa* and fluoxetine on the duration of immobility in the mouse forced swimming test were shown in Table 2.

The extract showed no any change after 1 day treatment. The extract at the dose of 560 mg/kg exhibited to show significant immobility reduction after 7-day treatment. The extracts at doses of 140, 280 and 560 mg/kg significantly decreased the duration of immobility in a dose-dependent manner, resulting in 53.2, 63.1 and 69.7% immobility reduction after 14-day treatment, respectively. Fluoxetine at the dose of 20 mg/kg significantly produced a time-dependent immobility reduction. The effect of *C. longa* at the dose of 560 mg/kg appeared to be more potent than that of fluoxetine after 14-day treatment.

3.3. Effects of the aqueous extract of C. longa on mouse open-field behavior test

In this study, the extracts of *C. longa* at the doses of 140, 280 and 560 mg/kg, and fluoxetine at the dose of 20 mg/kg, which significantly reduced immobility time in mouse tail suspension and in mouse forced swimming tests, produced no significant difference in ambulation, rearing and the numbers of grooming in the open-field behavior test, compared with controls (results not shown).

3.4. Effects of the aqueous extract of C. longa on MAO A and B activities in mouse whole brain

The effects of the aqueous extract of *C. longa* and fluoxetine for 14 days on the MAO A and B activities in mouse whole brain were shown in Table 3. The MAO A and B activities in normal group were 21.67 ± 1.8 nmol/mg protein h and 22.29 ± 1.58 nmol/mg protein·h,

Table 1
Effects of the aqueous extract of *C. longa* and fluoxetine on the duration of immobility in the mouse tail suspension test (mean ± S.E.M.)

	Dose (mg/kg)	Number of mice	Duration of immobility (s)		
			Day 1	Day 7	Day 14
Control		12	77.6 ± 3.5	82.3 ± 3.2	77.5 ± 2.0
C. longa					
	140	12	79.3 ± 2.2	78.3 ± 1.8	49.9 ± 1.6**
	280	12	72..2 ± 3.3	67.6 ± 2.4	45.9 ± 1.6**
	560	12	76.7 ± 4.5	59.4 ± 3.6	32.5 ± 1.7***
Fluoxetine					
	20	12	61.7 ± 1.8*	50.7 ± 2.8*	51.2 ± 2.4*

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ when compared with control groups.

Table 2
Effects of the aqueous extract of *C. longa* and fluoxetine on the duration of immobility in the mouse forced swimming test (mean ± S.E.M.)

	Dose (mg/kg)	Number of mice	Duration of immobility (s)		
			Day 1	Day 7	Day 14
Control	12		80.3 ± 2.2	76.4 ± 2.3	78.0 ± 3.1
C. longa					
	140	12	72.5 ± 2.3	79.4 ± 1.8	36.5 ± 2.4*
	280	12	74.3 ± 1.8	70.3 ± 2.1	28.8 ± 1.4***
	560	12	73.2 ± 2.5	43.6 ± 1.4*	23.6 ± 1.4***
Fluoxetine					
	20	12	45.6 ± 1.6*	36.7 ± 1.3*	31.2 ± 1.7**

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ when compared with control groups.

respectively. Oral administration of the extract at the doses of 140, 280 and 560 mg/kg significantly inhibited MAO A activity in a dose-dependent manner, providing 20.6, 36.5 and 46.8% inhibition. However, only the extract at a dose of 560 mg/kg was significantly exhibited to inhibit MAO B activity, producing 37.3% inhibition. Fluoxetine at the dose of 20 mg/kg showed a tendency to reduce the MAO A and B activity, but the effects were not significant in the study.

4. Discussion

The tail suspension and forced swimming tests were two behavioral tests in rodent that predicted the clinical efficacy of many types of antidepressant treatments (Porsolt et al., 1977b, 1978; Butterweck et al., 1998). *C. longa* (Turmeric) was a well-known indigenous herbal medicine and its major constituents curcumin, exhibited a wide range of biologic activities (Khanna, 1999). In contrast, very little information was available about the antidepressant activity of *C. longa*. We studied the aqueous extract of *C. longa* on the immobility behaviors in mice. The extract at oral doses from 140 to 560 mg/kg for 14 days significantly decreased the duration of immobility in the tail suspension test and the forced swimming test in mice. These behavioral effects of *C. longa* at the dose of 560 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, mice were tested in the open filed test. Neither the extracts of *C. longa* nor fluoxetine, at the doses tested, produced significant effects on locomotor activity. These data in the present study has shown that *C. longa* has antidepressant effects in mouse 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 (Wouters, 1998; Knoll, 1997). In the present investigation, we have demonstrated that the aqueous extract of *C. longa* significantly inhibited in vivo MAO A activity in mouse whole brain in a dose-dependent manner, however, only the extract at a dose up to 560 mg/kg exhibited to have the MAO B inhibitory activity. These findings suggested that anti-

Table 3
Effects of the aqueous extract of *C. longa* and fluoxetine on MAO activity in mouse whole brain (mean ± S.E.M.)

Group	Dose (mg/kg)	Number of mice	MAO activity (nmol/mg protein·h)		MAO inhibition (%)	
			A	B	A	B
Control	12	21.67 ± 1.80	22.29 ± 1.58			
C. longa						
	140	12	17.21 ± 0.96*	21.15 ± 1.14	20.6	5.1
	280	12	13.77 ± 1.48**	19.48 ± 0.54	36.5	12.6
	560	12	11.53 ± 1.14***	13.98 ± 0.88***	46.8	37.3
Fluoxetine						
	20	12	18.95 ± 1.29	19.75 ± 0.87	12.5	11.4

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ when compared with control groups.

depressant effects of *C. longa* in animal models of immobility tests may be related to the inhibitory activity of MAO, especially to that of MAO A.

Evidence is accumulating that major depression is associated with dysfunction of immunity (Mendelovic et al., 1999). A variety of immune parameters such as mitogen response, natural killer cell activity and the numbers, T-cell, and T-cell subpopulations were in relation to depression. *C. longa* exhibited to have immunostimulatory activity (Antony et al., 1999), inhibiting TNF-α induced expression of ICAM-1, VCAM-1 and E-selectin on human umbilical vein endothelial cells (Gupta and Ghosh, 1999) and Th1 cytokine profile in CD4±T cells by suppressing interleukin-12 production in macrophages (Kang et al., 1999). Although the relevance of these psychoimmune relationships remains in question, from clinical studies, more evidences showed that patients with depression could exploit their immune system for suicide (Mendlovic et al., 1997). It is, therefore, suggested that *C. longa* may be as a possible therapeutic and protective use in the immune-associated depression. Additional study of the depressant mechanism and related active constituents are in progress.

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