Journal of Ethnopharmacology 144 (2012) 328–334

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

# Ethanol extracts from *Hemerocallis citrina* attenuate the decreases of brain-derived neurotrophic factor, TrkB levels in rat induced by corticosterone administration

Li-Tao Yi [a,*], Jing Li [a], Huo-Chen Li [b], Ying Zhou [b], Bi-Fang Su [a], Ke-Feng Yang [b], Meng Jiang [b], Yan-Ting Zhang [a]

[a] Department of Chemical and Pharmaceutical Engineering, College of Chemical Engineering, Huaqiao University, Xiamen 361021, Fujian province, PR China
[b] Department of Biotechnology and Bioengineering, College of Chemical Engineering, Huaqiao University, Xiamen 361021, Fujian province, PR China

ARTICLE INFO

*Article history:*
Received 5 April 2012
Received in revised form
27 August 2012
Accepted 6 September 2012
Available online 18 September 2012

*Keywords:*
*Hemerocallis citrina*
Antidepressant-like effect
Corticosterone
Brain-derived neurotrophic factor
TrkB

ABSTRACT

*Ethnopharmacological relevance:* *Hemerocallis citrina*, a traditional herbal medicine, has been used for the improvement of behavioral and emotional status in Eastern-Asia countries.
*Aim of the study:* Our previous studies have demonstrated that the ethanol extracts of *H. citrina* flowers (HCE) reversed the behavioral alterations and monoamine neurotransmitter dysfunctions in stressed mice. However, the relation of its antidepressant-like action with neurotrophic molecular expressions remains unknown.
*Materials and methods:* To clarify this, we explored the effect of HCE (32.5, 65, 130 mg/kg, p.o.) on the behavior, brain-derived neurotrophic factor (BDNF) and its receptor (TrkB) in depression-like rats induced by exogenous administration of the stress hormone corticosterone (40 mg/kg, s.c.).
*Results:* It was observed that repeated administration of corticosterone induced an elevation on the serum corticosterone levels, which caused the abnormalities observed in the sucrose preference test and forced swimming test (FST). Administration of HCE (65 and 130 mg/kg) reversed the changes above and up-regulated the BDNF and TrkB receptor protein expressions in the brain region of frontal cortex and hippocampus.
*Conclusion:* These findings confirm that HCE produce an antidepressant-like effect in corticosterone-induced depression-like model of rats and this effect is at least partly mediated by BDNF-TrkB signaling in the frontal cortex and hippocampus.



## 1. Introduction

*Hemerocallis citrina* Baroni (Liliaceae) which is widely grown in China, Japan and Korea has been used for food and medicinal purposes for thousands of years. According to the phytochemical and pharmacological studies, *H. citrina* mainly contains flavonoids, polyphenols and essential oils that contribute to the herb's biological effects, such as antibacterial (Zhan et al., 2005), antioxidant (Lang and Luo, 2007) and nitrite-eliminating (Fu et al., 2009) activity. In addition, the effects of *H. citrina* such as relieving gloom and improving sleeping were recorded in the famous pharmaceutical text of "Compendium of Materia Medica", the most complete and comprehensive medical book ever written in the history of traditional Chinese medicine. Clinically, *H. citrina* has been widely used for the treatment of depressive disorders in China (Chen et al., 2008).

In previous studies, we found that the ethanol extracts of *H. citrina* (HCE) administration significantly reduced the immobility time in both the forced swimming test (FST) and tail suspension test (TST) without accompanying changes in locomotor activity in the open-field test (OFT) in mice (Gu et al., 2012). Furthermore, we also demonstrated that HCE enhanced serotonin (5-HT) and noradrenaline (NA) levels in the frontal cortex and hippocampus as well as elevated dopamine (DA) levels in the frontal cortex (Gu et al., 2012). However, until now, the neurotrophic molecular expression underlying the antidepressant-like effect of HCE remains unclear.

Neurotrophic factors are critical regulators of the formation and plasticity of neuronal networks (Lee and Kim, 2010). Brain-derived neurotrophic factor (BDNF), one of important neurotrophic factors, has also been implicated in the etiology of major

*Abbreviations:* ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; DA, dopamine; FST, forced swimming test; GR, glucocorticoid receptor; HCE, ethanol extracts of *Hemerocallis citrina*; NA, noradrenaline; OFT, open-field test; 5-HT, serotonin; TST, tail suspension test

* Corresponding author. Tel./fax: +86 592 6162302.
*E-mail addresses:* litao.yi@yahoo.com, litaoyi@hqu.edu.cn (L.-T. Yi).

0378-8741/$ - see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2012.09.016

depression and the mechanism of antidepressant treatment (Numakawa et al., 2010). Clinical study demonstrates that depression is associated with reduced brain BDNF and its receptor (TrkB) levels and that the reductions can be restored up to the normal value by antidepressant treatment (Thompson et al., 2011). Animal studies have also demonstrated clear evidence that BDNF signaling through TrkB is involved in the mechanisms of action of antidepressant agents (Wang et al., 2010; Kutiyanawalla et al., 2011). BDNF and TrkB levels can therefore be useful markers for antidepressant-like response.

In the present study, we used a rat depression model by repeated corticosterone injections to investigate the antidepressant-like effect of HCE. Furthermore, the study explored whether the HCE could reverse the loss of BDNF and its receptor TrkB mRNA and protein levels in the rat brain regions of frontal cortex and hippocampus.

## 2. Materials and methods

### 2.1. Animals

Male Sprague-Dawley rats (220–250 g) were purchased from Laboratory Animal Center, Fujian Medical University, Fujian Province, PR China. Animals were singly housed under a normal 12-h/12-h light/dark schedule with the lights on at 07:00 a.m. and had free access to tap water and food pellets. Ambient temperature and relative humidity were maintained at $22 \pm 2$ °C and at $55 \pm 5$%, and given a standard chow and water ad libitum for the duration of the study. The animals were allowed 1 week to acclimatize themselves to the housing conditions before the beginning of the experiments. All procedures were performed in accordance with the published guidelines of the China Council on Animal Care (Regulations for the Administration of Affairs Concerning Experimental Animals, approved by the State Council on October 31, 1988 and promulgated by Decree No. 2 of the State Science and Technology Commission on November 14, 1988).

### 2.2. Chemicals and reagents

Corticosterone was purchased from TCI Development Co., Ltd. (Tokyo, Japan). Fluoxetine hydrochloride was purchased from Changzhou Siyao Pharmaceuticals Co., Ltd. (Changzhou, PR China). All primers used in this study were designed and synthesized by Sangon Biotech Co. Ltd. (Shanghai, PR China). The anti-BDNF and anti-TrkB antibody and the respective secondary antibody were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, USA). Trizol reagent was purchased from Invitrogen (Carlsbad, USA). Reverse transcriptase Moloney Murine Leukemia Virus (M-MLV) used for cDNA synthesis was from Promega Corporation (Madison, USA). All other reagents used in RT-PCR and western bolt were purchased from Sangon Biotech Co. Ltd. (Shanghai, PR China).

### 2.3. Plant material and HPLC analysis

Flowers of *H. citrina* Baroni (Liliaceae) was purchased from Xiamen Wal-Mart and authenticated by Cheng-Fu Li, Department of Pharmacy, Xiamen Hospital of Traditional Chinese medicine (Voucher specimen number HU/CE-04281).

Since rutin is the main component of *H. citrina* (Yang et al., 2006), a fingerprint was analyzed in HPLC using rutin as an index. A Shimadzu Prominence LC-20A HPLC system equipped with an UV detector was used. Qualitative and quantitative analyses of rutin were performed using an Agilent $C_{18}$ column (4.6 mM × 250 mM, 5 μm), and the column temperatures were kept at 25 °C. A linear gradient elution profile was used in our study [0–20 min: methanol to 0.5% acetic acid water solution (v/v) ratio of 20/80 to 30/70; 20–30 min: 30/70; 30–70 min: 30/70 to 80/20]. Flow rates of elution in both cases were 1 ml/min. The injection volume for the analyte was 10 μl. The detection wavelength was set at 254 nm for rutin. The raw material *H. citrina* was extracted with methanol at 25 °C for 30 min. The content of the marker substance rutin in *H. citrina* was calculated as 0.39% (Fig. 1A and B).

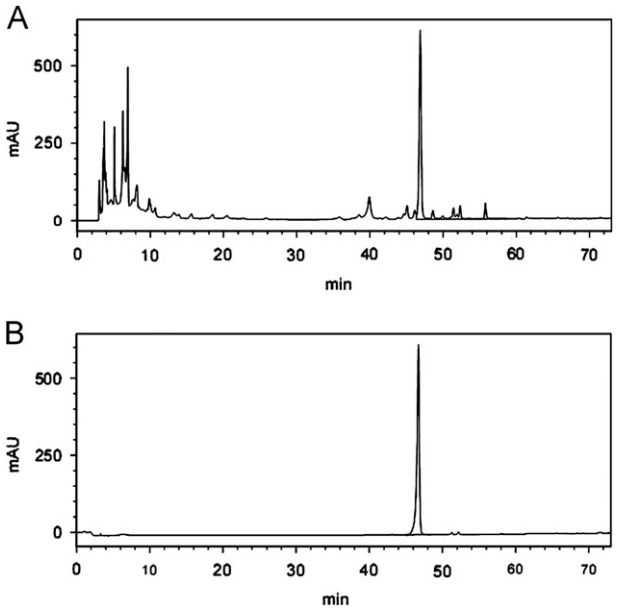


**Fig. 1.** HPLC chromatograms of *Hemerocallis citrina* (A) and the standard compound of rutin (B).

### 2.4. Preparation of the ethanol extracts

Dried sample (500 g) was cut into small pieces and extracted three times in a reflux condenser for 4 h with 3 L of 75% ethanol by sonication at room temperature ($25 \pm 2$ °C). The solutions were combined, filtered, concentrated under reduced pressure and lyophilized into powders. The final yield was 7.65% (w/w).

### 2.5. Drug treatments

Different groups of rats, 7 animals per group, were used for drug treatment and tests. Doses were calculated as mg/kg body weight. All the experimental animals including control and drug-treatment groups were simultaneously deprived food but not water 1 h prior to drug administration. The aim of food withdrawn prior to drug administration was just to ensure the bioavailability of drug. In other time periods, all animals had free access to food.

The standard dose of HCE in rat (130 mg/kg) was based on our previous mouse study (Gu et al., 2012) and calculated on the basis of body surface area ratio between rat and mouse. In addition, considering the long-term treatment used in the present study, the doses of 32.5, 65, 130 mg/kg every 24 h were selected.

The procedure and dose of corticosterone administration was performed as described in Mao et al. (2012). Corticosterone (40 mg/kg), which was dissolved in a saline solution containing 0.1% dimethyl sulfoxide and 0.1% Tween-80, was administrated by subcutaneously (s.c.) in a volume of 5 ml/kg once daily for 21 days. HCE (32.5, 65, 130 mg/kg) and the positive drug fluoxetine (15 mg/kg) were administered by oral (p.o.) gavage in a volume of 10 ml/kg 30 min prior to the corticosterone injection for 21 days.