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Anxiolytic effects of standardized extract of *Centella asiatica* (ECa 233) after chronic immobilization stress in mice

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ABSTRACT

Ethnopharmacological relevance: *Centella asiatica* has long been used for various neurological disturbances in Southeast Asian countries. The present study aims to demonstrate the anxiolytic effect of ECa 233, a standardized extract of *C. asiatica* containing triterpenoids not less than 80%, in comparison to diazepam.

Materials and methods: The test compound was given orally to non-stressed mice and mice subjected to chronic immobilization stress. Anxiolytic effect was assessed by an elevated plus maze (EPM), a dark-light box and an open-field tests.

Results: Anxiolytic effect of ECa 233 was clearly demonstrated in non-stressed mice subjected to acute stress in all behavioral tests employed. In the EPM test, chronically stressed mice showed significant decrease in the number of open arm entries, shortening the time spent in open arms and an increase of the latency to leave the central area, suggesting their release from the stress. In addition, ameliorating effect of ECa 233 was observed on the body weight and serum corticosterone which were adversely affected by immobilization stress. Madecassoside and asiaticoside, equal to their respective contents of the effective doses of ECa 233, exclusively presented anxiolytic effects in EPM, while no distinct effect was observed on the body weight and serum corticosterone.

Conclusions: The present study demonstrated anxiolytic effect of ECa 233 in both acutely and chronically stressed animals. These effects could be mainly accounted by madecassoside and asiaticoside, suggesting a possible use of ECa 233 for the treatment of both acute and chronic anxiety in the pathological state.

1. Introduction

Mental disorders are a global problem and represent one of the biggest challenges for health care systems. Despite the availability of current drugs such as benzodiazepine receptor agonists, newer, better-tolerated and more efficacious treatments including those of alternative/complementary medicines are still needed.

Centella asiatica is a psychoactive medicinal plant that has been used to treat anxiety for centuries in Ayurvedic medicine (Zhang, 2004). The most prominent group of active compounds isolated from *Centella asiatica* is triterpenoid glycosides including asiaticoside, madecassoside, asiatic acid and madecassic acid. As a dietary supplement, *Centella asiatica* has been used to treat sleep disorders in patients with mental health problems. Furthermore, 70% hydroethanolic extract of *Centella asiatica* was found to attenuate anxiety-related disorders and reduce stress phenomenon in clinical study (Jana et al., 2010). Pharmacological evaluation of *Centella asiatica* in different experimental models have demonstrated anti-oxidant effects (Veerendra Kumar and Gupta, 2003) as well as ameliorating effects on learning and memory deficit (Rao et al., 2005). Though methanolic extract of *Centella asiatica* and asiaticoside have demonstrated an anxiolytic-like effect in acutely stressed animals (Chen et al., 2006; Wijeweera et al., 2006), none of them has been tested in the chronically immobilized stress-induced anxiety model.

To avoid a large variation in composition of herbal extracts and to ensure consistency in physical and chemical constituents of *Centella asiatica* end product, a standardized extract of *Centella asiatica* or ECa 233 was prepared by a well-defined and strictly controlled procedures to contain triterpenoid glycosides not less than 80% and the ratio between madecassoside and asiaticoside should be 1.5 ± 0.5.

Previous studies demonstrated neuroprotective effects of orally given ECa 233 on learning and memory deficit induced by transient occlusion of both common carotid arteries (T2VO) in

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mice (Tantisira et al., 2010). Oral administration of ECa 233 in the dose up to 10 g/kg did not cause any lethality in mice and neither significant alteration of blood nor clinical chemistry was noted in subchronic toxicity testing in rats receiving ECa 233 at the doses of 10, 100 and 1000 mg/kg, p. o. (Chivapat et al., 2011).

The present study evaluated the anxiolytic effects of ECa 233 in acutely stressed as well as chronically stressed mice with reference to diazepam, an established anxiolytic drug. In acutely stressed mice, the anxiolytic effect of ECa 233 was assessed by the elevated plus-maze, the dark–light box and the open-field tests. In chronically immobilized mice, the physiological markers of the stress were examined by the serum corticosterone and the body weight measurements in addition to behavioral evaluation with the elevated plus-maze test. In addition, effect of madecassoside and asiaticoside in the amounts equal to those contained in the effective dose of ECa 233 were also investigated in acutely and chronically stressed animals.

2. Materials and methods

2.1. Animals

The protocol of animal housing and treatment used in this study was approved by the Ethic Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University (Approval no. 10-33-005). Adult male ICR mice (20–25 g) were purchased from the National Laboratory Animal Center of Mahidol University, Nakornpathorm, Thailand. They were housed in groups ($n=6$) in polypropylene cages (24 cm × 34 cm × 16 cm) with wood shavings. Standard laboratory diet (C.P. mice food, Thailand) and tap water were provided *ad libitum*. They were acclimatized in the ventilated room of laboratory at the ambient temperature of 25 ± 2 °C on a natural light/dark cycle for at least 1 week prior to the experiment.

2.2. Chemicals and test compounds

ECa 233 was prepared by Assoc. Prof. Ekarin Saifah and collaborates at the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand using a well-defined procedure (patent pending). The total triterpenoids of the extract in the present study were 85% of which 53% and 32% were madecassoside and asiaticoside, respectively. ECa 233 (10, 30, 100 and 300 mg/kg), madecassoside (16 mg/kg), asiaticoside (10 mg/kg) and diazepam (Atlanlic Labs, Thailand) (1, 2, and 10 mg/kg), were suspended in distilled water with 0.5% carboxymethyl cellulose (CMC). The test compound was orally given to the animals.

2.3. Analysis of triterpenoids in ECa 233

Madecassoside and asiaticoside were analyzed by HPLC. The standards, madecassoside and asiaticoside (Extrasynthese, France) and ECa 233 were dissolved in 50% HPLC-grade methanol. The HPLC system was a Shimadzu, equipped with a UV detector at 210 nm. The reverse phase was a Luna® C_{18} column (5 μm, 4.6 mm ID × 250 mm), thermostatic at 25 °C. The mobile phases were (A) methanol and (B) water. 70% A and 30% B were eluted under isocratic conditions. Fig. 1 shows the chromatogram of triterpenoids in ECa 233, which contained 53% madeccasoside and 32% asiaticoside.

2.4. Drug administration

The anxiolytic effect of the test compounds was initially examined in non-stressed mice using three tests including elevated plus maze, dark–light box and open-field tests. ECa 233 (30, 100 or 300 mg/kg), diazepam (1 or 2 mg/kg) or 0.5% CMC (10 ml/kg) was orally administered 1 h prior to the behavioral testing. The anxiolytic effect of the test compound was further investigated in stressed mice using elevated plus maze. The test compound including ECa 233 (10, 30 or 100 mg/kg), diazepam (2 or 10 mg/kg), madecassoside (16 mg/kg), asiaticoside (10 mg/kg) or 0.5% CMC (10 ml/kg) was orally given to the animals twice a day (8:00 am and 5:00 pm) for 10 consecutive days during stress-exposure period. In addition, 0.5% CMC was given to non-stressed mice in the same manner. No drug treatment was made on day 11, all animals were subjected to the EPM test during 10:00 am–12:00 am and then left undisturbed in their home cage until the day of their sacrifice (Belzung and Griebel, 2001).

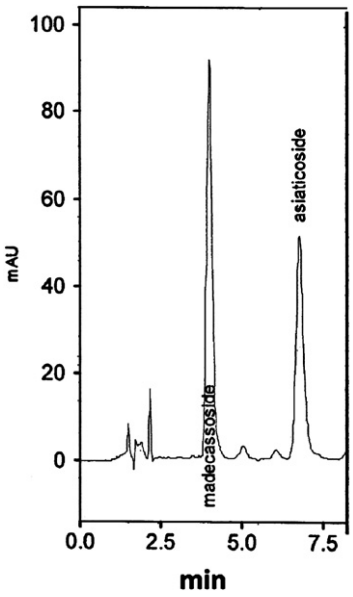

Fig. 1. Chromatogram of triterpenoids in ECa 233.

2.5. Exposure of animals to stress

The stress-exposure was conducted according to the method previously described (Vyas et al., 2006; Roozendaal et al., 2009). Briefly, the mice were subjected to chronic immobilization stress that consisted of complete immobilization (10:00 am–12:00 am/day) in the restraint cage (cylinder diameter 3 cm × height 8 cm, homemade) with no access to either food or water. The stress was given to animals at the same time of the day for ten consecutive days.

2.6. Behavioral studies

All the behaviors were monitored via a closed-circuit video camera mounted on the ceiling of the test apparatus, which was surrounded by black curtains to minimize undue distraction. The apparatus was cleaned with 96% ethanol before each animal was tested.

2.6.1. Elevated plus-maze

The elevated plus-maze apparatus was a four-arm maze made of plastics. It consisted of two open arms with no wall (30 cm length × 5 cm width), two enclosed arms with a black wall (30 cm length × 5 cm width × 16 cm height of the wall) and a central platform (5×5 cm^2), arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated 40 cm above the floor. After the drug administration, each animal