Wild Ginseng Attenuates Anxiety- and Depression-Like Behaviors During Morphine Withdrawal

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The purpose of this study was to evaluate whether wild ginseng (WG) administration could attenuate anxiety- and depression-like behaviors and expression of corticotrophin-releasing factor (CRF) and neuropeptide Y (NPY) following withdrawal from repeated morphine administration in rats. Male rats were administered daily doses of WG (50, 100, or 200 mg/kg, i.p.) for 5 days, 30 min before morphine injection (40 mg/kg, s.c.). The anxiety- and depression-like behavioral responses were measured 72 h after the last morphine injection using an elevated plus maze (EPM) and forced swimming test (FST), respectively. Changes in hypothalamic CRF and NPY expressions were also examined by analyzing their immunoreactivities in the hypothalamus. Daily administration of WG significantly reduced anxiety- and depression-like behavior, and elicited the suppression of CRF expression and the stimulation of NPY expression in the hypothalamus. Our results demonstrated that WG extract might be effective at inhibiting the anxiety and depression responses due to morphine withdrawal by possibly modulating the hypothalamus CRF and NPY systems. Furthermore, these findings imply that WG extract can be used for developing new medication to cure or alleviate morphine withdrawal symptoms and to prevent relapses of morphine use.

Keywords: Morphine, wild ginseng, anxiety, depression, neuropeptide Y, corticotrophin-releasing factor

Morphine, a strong pain reliever, is widely used to treat moderate to severe pain and a number of other pathological indications. This said, the abuse of morphine and its resultant withdrawal cause psychiatric side-effects, including anxiety and depression [20]. Many studies have demonstrated that morphine withdrawal causes anxiety- and depression-related disorders in humans and corresponding behavioral responses in animals [1, 21]. Anxiety and depression associated with morphine withdrawal can be alleviated by the administration of antidepressant or anxiolytic drugs, such as fluoxetine or aminergic [28]. However, some antidepressants exert undesirable side-effects, such as drowsiness, dryness of the mouth, headache, nausea, and sexual dysfunction [8].

Recent studies suggested that Panax ginseng (PG) was found to reduce depression symptoms and anxiety disorders in humans [2]. Some studies have reported that PG showed antidepressant-like activity in the forced swimming test (FST) and also reduced anxiety-like behavior in the elevated plus maze (EPM) test in an animal model [4, 27].

Wild ginseng (WG) is the ginseng (the root of Panax ginseng C.A. Mayer) that naturally grows in the mountains and is distinguished from field-cultivated ginseng. It is known to have more pharmacological efficacy and is thus more expensive than cultivated ginseng. In this study, WG indicates the ginseng that has grown undisturbed in the Korean forest for many years from the seeds initially scattered by humans [9]. In terms of seeding methods, it is also differentiated from truly wild ginseng of which the seeds have been distributed through natural vectors such as birds.

Until now, there are still unresolved questions about the mechanisms underlying WG’s effect as a therapeutic intervention for treating psychiatric side-effects, including the withdrawal symptoms associated with morphine use. The effects of WG on morphine withdrawal-induced anxiety- and depression-like behavioral alterations have not been examined in animal models. In the present study, the pharmacological effects of WG extract on anxiety- and depression-related behaviors following repeated morphine administration and withdrawal were investigated. Morphine withdrawal-induced behaviors were examined using the EPM and the FST. We also tried to elucidate the underlying mechanism of the effect of WG administration on morphine withdrawal.
dependency regarding the alterations of CRF and NPY expressions in the hypothalamus of the rat brain.

**Materials and Methods**

**Animals**

Adult male Sprague–Dawley (SD) rats weighing 260–280 g were obtained from Samtaco Animal Co. (Seoul, Korea). The rats were housed in a limited-access rodent facility with up to five rats per polycarbonate cage. The room controls were set to maintain the temperature at 22°C ± 2°C and the relative humidity at 55% ± 15%. Cages were lit by artificial light for 12 h each day. Sterilized drinking water and standard chow diet were supplied *ad libitum* to each cage during the experiments. The animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised in 1996, and were approved by the Kyung Hee University Institutional Animal Care and Use Committee. All animal experiments began at least 7 days after the animals arrived.

**Preparation of the Drugs and the Methanol Extracts of WG and PG**

Wild ginseng roots (adventitious root culture of *Panax ginseng*, WG) were collected in Chonbuk Province in Korea and purchased from Baekjeansam Co. (Mr. Jong-Gu Lee, Jinan-kun, Jinan-up, Yeonjiang-Ri #45-1, Chonbuk, 567-807, Korea). PG (*Panax ginseng*) was purchased from Dongwoodang Pharmacy Co., Ltd (Yeongheon, Korea).

Voucher specimens of WG and PG have been deposited at the herbarium located at the College of Oriental Medicine, Kyung Hee University (No. KH-WG01 for WG and No. KH-PG01 for PG). WG and PG (100 g each) were cut into small pieces and extracted three times with 21 of 80% methanol by sonication in a reflux condenser for 24 h at room temperature (25 ± 2°C), respectively. The solutions were combined, filtered through Whatman No. 1 filter paper, concentrated using a rotary vacuum evaporator (Rotavapor R-124; BUCHI Labortechnik AG, Flawil, Switzerland) under reduced pressure, refrigerated in a recirculating chiller (EYELA CCA-1110; Tokyo Rikakikai Co., Tokyo, Japan) to obtain concentrated extracts, and then lyophilized (EYELA FD-800; Tokyo Rikakikai Co., Tokyo, Japan). The yields of aqueous phases of WG and PG were 11.6% and 20.6% (w/w), respectively. Morphin hydrochloride (Sigma–Aldrich Chemical Co., St. Louis, MO, USA) was obtained from the standard commercial suppliers. Morphin hydrochloride was dissolved in 0.9% saline solution.

**Morphine Treatment and Experimental Groups**

The withdrawal group following repeated morphine administration was given morphine (40 mg/kg-body weight, s.c., MOR group, n=6) twice a day for 5 consecutive days. No drugs were injected within 72 h after the last morphine injection, and behavioral responses were tested during this period. The vehicle-treated rats (as a negative control of the addiction withdrawal model development) were administered with saline (0.9% NaCl, s.c.) instead of morphine in the same way (SAL group, n=6). The WG- or PG-treated groups were divided as follows: 50 mg/kg WG plus morphine-treated group (WG50+MOR, n=6), 100 mg/kg WG plus morphine-treated group (WG100+MOR, n=6), 200 mg/kg WG plus morphine-treated group (WG200+MOR, n=6), and 500 mg/kg PG plus morphine-treated group (PG500+MOR, n=6). The WG or PG treatments were given intraperitoneally 30 min prior to the injection of morphine for 5 consecutive days, as the development phase. The anxiety- and depression-like behavioral responses were measured 72 h after the last morphine injection in all groups using the EPM and FST tests. The experimental schedule of all drug administration and behavioral tests are shown in Fig. 1.

**Measurement of Elevated Plus Maze (EPM)**

The EPM test has been used to assess internal conflict between voluntary approaches and withdrawal tendencies as a rodent model for human anxiety [18]. Because this test is based on a natural fear of open and elevated spaces, the number of entries into the open arms and the time spent in open arms are negatively correlated with the anxiety level of the subject. The EPM consists of a plus-shaped plastic apparatus with two open and two enclosed arms, each with an open roof. The apparatus was painted with black enamel and was elevated 50 cm from the floor. All arms were 10 cm in width and 50 cm in length and joined at the center to form a 10 cm² central platform. Two closed arms opposite each other were surrounded by 40 cm high plastic walls with an open roof, and the other two open arms were without any walls. Exploration of the open arms was encouraged by testing under indirect dim light (2×60 W).

At the start of testing, the rat was placed on the central platform facing the given open arm, and was allowed to move freely for 5 min. Each rat was placed in the center of the maze, after which the cumulative time spent on each arm and the numbers of entries into the open or closed arms were recorded during a 5 min test session 72 h after the last injection of morphine or saline. Entry into an arm was defined as beginning when the animal placed all four paws in that arm. The maze was cleaned with alcohol after each rat was tested. The behavior in the maze was recorded using a video camera mounted on the ceiling above the center of the maze and was relayed to the S-MART program (PanLab, Barcelona, Spain), which included a standard measure of anxiety-like behaviors, and several additional assessments of open-arm exploration. The standard measure of anxiety was indicated by the time ratio, which is the time spent in the open arms of the maze divided by the total time spent in any arm of the maze. A smaller ratio indicates less open-arm exploration or more “anxiety.” Additional measures of activity and exploration included head dips and rearing, which were independently scored in the closed arms, open arms, and central

![Fig. 1. Experimental schedule of morphine withdrawal-induced anxiety- and depression-like behaviors in rats.](image-url)