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A medicinal herb, *Melissa officinalis* L. ameliorates depressive-like behavior of rats in the forced swimming test via regulating the serotonergic neurotransmitter

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ABSTRACT

Ethnopharmacological relevance: Depression is a serious psychological disorder that causes extreme economic loss and social problems. However, the conventional medications typically cause side effects that result in patients opting to out of therapy. Lemon balm (*Melissa officinalis* L., MO) is an old and particularly reliable medicinal herb for relieving feelings of melancholy, depression and anxiety. The present study aims to investigate the antidepressant-like activity of water extract of MO (WMO) by evaluating its influence on the behaviors and the relevant neurotransmitters of rats performed to forced swimming test.

Materials and methods: Two phases of the experiment were conducted. In the acute model, rats were administered ultrapure water (control), fluoxetine, WMO, or the indicated active compound (rosmarinic acid, RA) three times in one day. In the sub-acute model, rats were respectively administered ultrapure water (control), fluoxetine, or three dosages of WMO once a day for 10 days. Locomotor activity and depression-like behavior were examined using the open field test and the forced swimming test, respectively. The levels of relevant neurotransmitters and their metabolites in the frontal cortex, amygdala, hippocampus, and striatum were analyzed by high performance liquid chromatography.

Results: In the acute model, WMO and RA significantly reduced depressive-like behavior but the type of related neurotransmitter could not be determined. The results indicated that the effect of WMO administration on the reduction of immobility time was associated with an increase in swimming time of the rats, indicative of serotonergic neurotransmission modulation. Chromatography data validated that the activity of WMO was associated with a reduction in the serotonin turnover rate.

Conclusion: The present study shows the serotonergic antidepressant-like activity of WMO. Hence, WMO may offer a serotonergic antidepressant activity to prevent depression and to assist in conventional therapies.

1. Introduction

The World Health Organization (WHO) reported that major depressive disorder (MDD) will be the leading cause of disability and have the highest burden of disease by the year 2030 (Mathers et al., 2008). One of the major pathological hypotheses of depression is neurotransmitter dysregulation (Castren, 2005). Clinical research has indicated that patients with depression have low concentration of monoamines like serotonin (5-HT) in the central nervous system (Stockmeier, 1997; Fava, 2003). Hence, several antidepressants including selective serotonin reuptake inhibitors (SSRIs), nonselective monoamine reuptake inhibitors (tricyclic antidepressants, TCAs), and monoamine oxidase inhibitors (MAOIs) were developed to improve the retention of monoamines in the brain. Though episodes of depression are ameliorated by these medications, undesirable side effects frequently occur and cause patients to opt out of medication. Low acceptability of antidepressants by patients with MDD has been reported (Lin et al., 1995; Gonzalez et al., 2005). In contrast, research has indicated

Abbreviations: WMO, water extract of *Melissa officinalis* L.; RA, rosmarinic acid; FLX, fluoxetine; FST, forced swimming test; 5-HT, serotonin; MDD, major depressive disorder; MAO, monoamine oxidase; HPLC, high performance liquid chromatography

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that patients with MDD show interest in complementary and alternative medicines (CAMs), including herbal remedies (Alderman and Kiepfer, 2003; Hsu et al., 2010). Moreover, people in the pre-disease state of depression cannot be prescribed antidepressants until they are properly diagnosed by a medical doctor. Thus, CAMs offer a feasible way of preventing or complementing conventional MDD therapies.

Lemon balm (*Melissa officinalis* L., MO), an herbal material with a "generally recognized as safe" (GRAS) status, is traditionally used for improving sleep disorders and for de-stressing. Chillemi and Chillemi indicated that MO is a traditional and particularly reliable treatment for relieving feelings of melancholy and depression (Chillemi and Chillemi, 2011). Previous research has identified that MO is composed of phenolic and flavonoid compounds, specifically rosmarinic acid (RA) (Fecka and Turek, 2007). Numerous bioactivities of MO, such as antioxidant (Mimica-Dukic et al., 2004), anti-cancer cell proliferation (Encalada et al., 2011), anti-obesity (Lee et al., 2008) and anti-anxiety (Kennedy et al., 2006) effects, have been reported. For antidepressant-like activity, Taiwo et al. demonstrated that a 10-day administration of MO ethanol extract decreased the immobile time of rats during the FST (Taiwo et al., 2012). Similarly, acute administration of the essential oil or aqueous extract of MO by the intraperitoneal route also decrease the immobility time of rats in the FST (Emamghoreishi and Talebianpour, 2009). Lopez et al. showed that both the ethanol and water extracts of MO (WMO) could inhibit the activity of monoamine oxidase A (MAO_A), the major metabolic enzyme of monoaminergic neurotransmitters (Lopez et al., 2009). In fact, a pharmacokinetic study showed that RA could be detected in the plasma and brains of rats after gavage and intraperitoneal injection (Fale et al., 2011). Previous research indicated that the immobile behavior of mice was reduced by the administration of RA and its metabolite, caffeic acid (Takeda et al., 2002). Moreover, depressive-like behavior was also improved by the administration of RA, which was shown in a chronic animal study for depression (Jin et al., 2013). These results suggest that RA can directly influence the central nervous system and is implicated in the bioactivity of MO in the brain. In this study, we investigated the antidepressant-like effect of WMO in the forced swimming test (FST) and the metabolism of related neurotransmitters.

2. Materials and methods

2.1. Plant material

Leaves of *Melissa officinalis* L. were purchased from the Hualien District Agricultural Research and Extension Station in Hualien, Taiwan. The taxonomy was confirmed by Prof. T. L. Chang in the Department of Horticulture and Landscape Architecture at National Taiwan University. The voucher specimens (voucher number: 388449) have been kept at the Herbarium of Taiwan Forestry Research Institute.

2.2. Extraction and composition determination of WMO

To obtain the WMO, the freeze-dried MO leaves were crushed and blended into a powder using a blender. The MO powder (1 kg) was stirred with 2 L of ultrapure water at 80 °C for 2 h. The insoluble materials were subsequently removed through centrifugation at 10,000 g for 30 min. Finally, the resulting supernatant was filtered and freeze-dried. Fluoxetine (FLX) was obtained from Eli Lilly Company (Taiwan). RA (PubChem CID: 5281792) was purchased from Sigma (St. Louis, MO, USA).

LC-NET II/ADC (JASCO, Easton, MD, USA), a high performance liquid chromatography (HPLC) system consisting of a pump (PU-2089-PLUS) and a UV detector (UV-2075-PLUS), with a Phenomenex Luna C18 column (250 × 4.6 mm, 5 μm, Torrance, CA, USA) and the Chrompass™ program was used for analyzing the RA content in WMO. The mobile phase consisted of 2% acetic acid (A) and 7/3 2% acetic acid/acetonitrile (B), and a linear gradient from 90% to 40% A in 40 min was applied for elution. The flow rate was 1 mL/min and the column effluent was monitored by UV detection at 320 nm.

2.3. Animals and treatments

Male Sprague-Dawley rats (6-week-olds) were purchased from BioLasco Taiwan Company (Taipei, Taiwan) and housed individually with controlled day/night cycle (12 h light/dark), temperature (23 ± 2 °C), and humidity (50 ± 10%). Food and water were provided and could be freely accessed. Two experimental protocols were designed in this study: (i) sub-acute administration (0, 19 and 24 prior to the test) and (ii) sub-chronic administration (10 days). The habituation for each experiment was one week. For the acute experiment, the rats were separated into 4 groups: control (CTL, ultrapure water), WMO (300 mg/kg body weight), RA (36 mg/kg body weight), and FLX (18 mg/kg body weight). The dosage of RA was equal to its content in WMO. In the acute experiment, after the first FST for 15 min, oral administration occurred at three time points, 0, 19 and 24 h. For the sub-acute model, the rats were separated into 5 groups: CTL (ultrapure water), low dosage WMO (L-WMO, 30 mg/kg body weight), medium dosage WMO (M-WMO, 100 mg/kg body weight), high dosage WMO (H-WMO, 300 mg/kg body weight), and FLX (18 mg/kg body weight). The dosages of WMO usd in the sub-acute model were based on the results in the acute model and on previous reports by Taiwo et al. (2012) and Emamghoreishi and Talebianpour (2009). Oral administration in the sub-acute model was initiated after habituation and proceeded for 10 days once a day. For each model and treatment, there were six to eight per group. The protocol complied with the guidelines described in the "Animal Protection Law", which was amended on June 29, 2011 to Hua-Zong- (1)-Yi-Tzi-10000136211 by the Council of Agriculture, Executive Yuan, Taiwan.

2.4. Open field test (OFT)

The autonomous behavior of rats was evaluated by the OFT after 10 days of administration. The open field apparatus was divided into central and peripheral areas. The rats were placed individually in the central area of the open field (76 × 57 × 35 cm) for 5 min and videotaped. The bouts (the number of central and peripheral squares entered), duration, distance, and velocity were analyzed using the TOP SCAN v2.0 software (Clever Sys Inc., Reston, VA, USA).

2.5. Forced swimming test (FST)

The FST was developed by Porsolt et al. (1979). This well-established model being used in the present study was described previously (Lin et al., 2014). Following the standard protocol, the rats were forced swim for 15 min on the final day of administration. After 24 h, the rats were forced swim again for 5 min, and they were videotaped for further behavioral analyses such as calculating the duration of immobility, swimming, and struggling by the ForcedSwimScan™ software (CleverSys, Reston, VA, USA). The definition of these different behaviors is based on the activity of the four limbs and the ratio of body area that was under or above the water surface.