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LC–MS/MS based studies on the anti-depressant effect of hypericin in the chronic unpredictable mild stress rat model

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ABSTRACT

Ethnopharmacological relevance: St John's Wort (*Hypericum perforatum*, SJW) is a widely used herbal medicine in western countries but also an important Uygur drug in China. Hypericin (HY) is the main components in SJW extracts, which is used to treat fatigue, weakness, and mild depression. The aim of this study was to investigate the anti-depression effects of HY on chronic unpredictable mild stress (CUMS) model rats and identify the possible mechanisms.

Materials and methods: In this study, the protective effects of HY on CUMS-induced depression in rats were investigated by using a combination of behavioral assessments and urinary metabolites analysis. Urinary metabolites analyses were performed using LC–MS/MS in conjunction with principal components analysis (PCA) after oral administration of either HY or Venlafaxine (VF) for 27 days. During the procedure of experiment, food consumption, body weight, adrenal gland, thymus and spleen indices, behavior scores, sucrose consumption, and stress hormone levels were measured.

Results: Changes in the classic behavioral tests and pharmacological biochemical indices reflected that HY alleviated the symptoms of depression in a shorter period than VF, which was used as positive control for antidepression. Metabolites analysis of urine revealed that HY affected excitatory amino acids and monoamine neurotransmitter metabolites. Remarkably, urinary valine was increased remarkably by HY, even much higher than CUMS group. These results provide important mechanistic insights into the protective effects of HY against CUMS-induced depression and metabolic dysfunction.

Conclusion: As the most important active ingredient in SJW extracts, HY possesses the better protective effect against CUMS-induced depression symptoms and metabolic disturbances.

1. Introduction

Depression, a psychiatric disorder characterized by emotional suppression, pessimism, and frustration, exists in modern society. The number of patients with depression has increased notably recently and most of the patients manage this mental disorder by anti-depressant drugs (Kemp, 2014; Yin et al., 2015). Many synthetic antidepressants are used in the clinic which carries a high risk of relapse and side effects (Assadi et al., 2011; Klingenstein et al., 2006). Natural and herbal sources are thought to have antidepressant qualities with fewer side effects (Schulz, 2006).

St John's Wort (*Hypericum perforatum*, SJW), which is a flowering plant, is a widely used herb for the treatment of mild-to-moderate depression for centuries in Europe and the USA (Barnes et al., 2001; Pirotta et al., 2014). In Germany, SJW has been approved by the Commission E since 1984 for the treatment of anxiety, depression, and insomnia (Russo et al., 2014). SJW is also an important traditional medicinal herb in China which was recorded as Uygur drug. Based on Uygur tradition, SJW was also used to treat wounds, ulcers, varicose, hemorrhoids, myalgia, sciatica, rheumatism, lumbago and cramps (Chinese Pharmacopoeia Commission, 1999).

SJW is characterized by the low side-effect incidence in comparison with other commonly used antidepressants. However, SJW has been proven to be a potent inducer of hepatal and intestinal cytochrome P450 monooxygenases (CYP450), particularly CYP3A4 and CYP2C9, and/or intestinal ATP-binding cassette (ABC) transporter P-glycoprotein (Markert et al., 2015; Weber et al., 2004). Therefore, serious clinically relevant interactions of SJW extracts with co-administered drugs (Davis et al., 2014; Zhou et al., 2004) have been reported. Such as cancer patients who often suffer from depressive disorders, co-administration with SJW may be hazardous (Goey et al., 2014; Haefeli and Carls, 2014).

Although >150 discrete ingredients that exhibit many additive, synergistic, and partly antagonistic effects have thus far been identified in this plant (Butterweck and Schmidt, 2007), SJW's most characteristic constituents are flavonoids (rutin, quercitrin, hyperoside); phloroglucinols (hyperforin, adhyperforin); and naphtodianthrones (hypericin, pseudohypericin) (Butterweck and Schmidt,

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Table 1
CUMS schedule.

Stressors	Days of CUMS experiments		
Forced swimming at 40 °C for 5 min	1	16	25
Water deprivation for 24 h	2	15	21
Food deprivation for 24 h	3	12	24
Tail squeezing for 1 min	4	10	23
Electric shock for 10 s	5	11	22
Reversal of day and night	6	14	20
exposure to 40 °C for 5 min	7	13	19
Exposure to 0 °C for 10 min	8	17	26
2 h behavior restriction	9	18	27

2007; Grundmann et al., 2006). Hypericin (HY), the most important active ingredient in SJW extracts, is used to treat fatigue, weakness, and mild depression (Caccia, 2005; Chang and Wang, 2010). The concentration of HY varies among different parts of the plant (0.02–2.5%) (Russo et al., 2014; Nahrstedt and Butterweck, 1997). However, there are insufficient number of studies addressing the efficacy of HY as well as the differences between synthetic antidepressants and HY in treating depression.

Chronic unpredictable mild stress (CUMS), a well-validated model of depression, has been used widely for studying depression and evaluating anti-depression effects of drugs. In CUMS, animals are exposed to different kinds of mild stressors that mimic the stressors in human life (Tang et al., 2015). Recent studies revealed that the concentrations of amino acids in urine significantly changed when CUMS induced depression rat model (Chen et al., 2014; Liu et al., 2012). On the other side, in clinic, hypercortisolism and impaired HPA axis feedback regulation are a common finding in depressed patients (Dubey et al., 2015; Foland-Ross et al., 2014). Therefore, we hypothesized that HY will attenuate the stress induced variations in classic behavioral tests and the contents of urinary amino acids and neurotransmitters in rat CUMS model, which would be further confirmed by the analysis of corticosterone (CORT) in plasma. To certify the anti-depression effect of HY and to explore the possible pharmacological mechanisms in the rat CUMS model, we evaluated therapeutic effects of HY comprehensively by means of urinary metabolites analyses along with classic physiological and behavioral outcomes.

Venlafaxine (VF) has been used in the clinic for the treatment of depression for nearly half a century which acts primarily as serotonin-norepinephrine reuptake inhibitors (SNRIs) by blocking the serotonin transporter (SERT) and the norepinephrine transporter (NET) (Cravello et al., 2009; Serres et al., 2012). In this study, VF was used as positive control and the anti-depression effects of the two kinds of antidepression agents (HY and VF) were compared in an animal model of depression using LC–MS/MS with the aims to achieve a more in-depth understanding of the antidepressant mechanism of HY.

2. Materials and methods

2.1. Animal handling and sampling

This study was conducted in accordance with the Chinese national legislation and local guidelines, and was performed at the Centre of Laboratory Animals, Huazhong University of Science and Technology, Wuhan, P.R. China. SD rats, male, 180–220 g, were obtained from the Laboratory Animal Center of the Tongji Medicine College of Huazhong University of Science and Technology (Wuhan, Hubei, China). Experimental protocols were approved by the Animal Care Committee of Huazhong University of Science and Technology. The rats were housed individually in stainless steel wire mesh cages and given free access to food and water in a temperature and humidity controlled room (24 ± 1 °C and $45 \pm 15\%$) with a 12 h light/dark cycle. The rats were acclimated to the new environment in metabolic cages for 2 weeks before experiments were performed.

Rats were weighted and randomly divided into four groups ($n=6$): negative control group (control), CUMS model group (model), hypericin group (HY, 1.56 mg/kg) and positive control group (VF, 7.81 mg/kg). A 24 h baseline urine sample was collected from each animal on day 0. Hypericin (> 96%) was purchased from Aladin Ltd. (Shanghai, China). Venlafaxine-hydrochloride tablets were purchased from Beijing Wyeth Pharma (China). HY and VF were dissolved with saline to the required concentration, which was equivalent to medium doses used clinically, and administered to the HY group and VF group, respectively. The model group was given vehicle at the same volume once a day from day 1 to day 27.

Animals in the control group were healthy rats which were not exposed to CUMS, while the rats in model, HY and VF groups were subjected to the following stressors according to the CUMS schedule in Table 1 every day between 9 and 11 a.m.: forced swimming at 40 °C for 5 min, water deprivation for 24 h, food deprivation for 24 h, tail squeezing for 1 min, electric shock for 10 s, reversal of day and night, exposure to 40 °C for 5 min, exposure to 0 °C for 10 min, and 2 h behavior restriction. Body weight, and food consumption were recorded every day, and the results of sucrose intake and an open-field test were recorded for each rat on days 0, 9, 18, and 27.

A 24-h urine sample from each animal was collected on days 0, 9, 18, and 27. The urine samples were centrifuged at 6000*g* for 10 min at 4 °C to remove particle contaminants, and the supernatants were stored at −80 °C until analysis. Plasma of all animals were collected at the end of the experiment on day 27. The weights of the adrenal gland, thymus, and spleen were recorded and their ratios to bodyweight values were calculated as the adrenal, thymus, and spleen indices.

2.2. Behavioral assessment of CUMS group

An open-field test was performed between 1:00 and 3:00 p.m. in a quiet room on days 0, 9, 18 and 27. The apparatus was a four-sided 100 cm × 100 cm × 40 cm rectangular arena whose floor was marked with a grid divided into 25 equal-size squares. Each rat was gently placed in the central square of the apparatus and observed for 5 min, and the following behaviors were recorded: the number of ambulations (grid lines crossed with at least three paws) and the amount of time spent grooming and rearing (defined as standing upright on hind legs). Every incidence of grooming or rearing counted as one point, every grid crossed counted as one point, and the behavioral score was the total number of points. The apparatus was cleaned between each test.

2.3. Sucrose preference test

Before the experiment was carried out, all rats were exposed to a 1% sucrose solution for 24 h to avoid neophobia. Then, two bottles, one containing 1% sucrose solution and the other containing tap water, were weighed and presented to each rat for 1 h. The position of the plain water bottle and sucrose solution bottle were randomly determined. Sucrose and water consumptions (g) were measured, and the sucrose preference was calculated using the following equation:

$$\text{Sucrose preference} = \text{sucrose consumption (g)} / (\text{sucrose consumption(g)} + \text{water consumption(g)}) \times 100\%$$

2.4. Food consumption and body weight

Every day, rat chow was weighted and added to the feed bucket for each rat at 5 p.m. After 24 h, the uneaten food was removed