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Original research article

Behavioral and biochemical evidences for antidepressant-like activity of palmatine in mice subjected to chronic unpredictable mild stress

Dinesh Dhingra *, Arun Bhankher

Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India

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ABSTRACT

Background: In the present study, antidepressant-like activity of palmatine was evaluated in unstressed and stressed young male Swiss albino mice.
Methods: The animals were subjected to unpredictable mild stress daily for 21 successive days to induce depression-like behavior. Palmatine (0.25, 0.5, 1 mg/kg, *ip*) was administered for 21 successive days to unstressed and stressed mice. The antidepressant-like activity was evaluated using the tail suspension test, forced swim test and sucrose preference test.
Results: Palmatine (0.5 and 1 mg/kg, *ip*) significantly decreased immobility periods of unstressed and stressed mice in the forced swim test and tail suspension test, thus indicating its significant antidepressant-like activity. Only the highest dose (1 mg/kg) of palmatine significantly reversed the stress-induced decrease in sucrose preference. There was no significant effect on locomotor activity of the mice by palmatine and fluoxetine. The antidepressant-like activity of palmatine was found to be comparable to fluoxetine (10 mg/kg) administered for successive 21 days. Palmatine (0.5 and 1 mg/kg, *ip*) significantly reversed the stress-induced increase in brain catalase levels, MAO-A activity, lipid peroxidation, plasma nitrite and corticosterone levels.
Conclusions: Palmatine showed significant antidepressant-like activity in unstressed and stressed mice probably through inhibition of MAO-A activity, decrease in plasma nitrite levels and due to its antioxidant activity. In addition, palmatine also showed antidepressant-like activity in stressed mice probably through decrease in plasma corticosterone levels.

Introduction

Depression is one of the major mental disorders and involves a triad of symptoms with low or depressed mood, anhedonia and low energy or fatigue [5]. The primary causes of depression include depletion of monoamines, like serotonin, noradrenaline and dopamine by monoamine oxidase overactivation, oxidative stress and hyperactivity of HPA-axis. There was a strong relationship between MAO activity and the HPA axis function in depressed patients [28]. Stress has been observed to play an important role in the etiology of psychiatric disorders [9]. Stressful experiences have been reported to favor the development of depression in humans [18]. Animal stress models are widely used in pre-clinical evaluation of antidepressants [10]. In rats and mice, application of chronic unpredictable mild stress procedures resulted in a variety of behavioral, neurochemical, neuroendocrine and neuroimmune alterations, resembling some of the dysfunctions observed in human depression [35]. In rodents, CUMS elicits depression-like symptoms such as a lack of sucrose preference interpreted as anhedonia, a core symptom of major depression [37]. Animals exposed to CUMS show signs of increased activity of the HPA axis leading to hypersecretion of corticosterone [1]. St. John's wort, a clinically employed herbal antidepressant; and fluoxetine (a selective serotonin reuptake inhibitor) attenuated stress-induced increase in corticosterone [12]. Nitric oxide, an important neurotransmitter in the nervous system, regulates many behavioral, cognitive, and emotional processes, including depression [13]. Nitric oxide production is increased in depression [34]. Oxidative damage due to CUMS has been reported in rats [3]. Fluoxetine attenuated stress-induced increase in oxidative parameters [39]. There is a correlation of depressive disorders in humans with oxidative stress either in the brain or blood [4].

Palmatine is a quaternary protoberberine alkaloid. It is typically yellow in color and is an active constituent of a number of plants, such as *Coptidis rhizoma* [17]. Palmatine has been reported to possess sedative [15] and antioxidant [17] activities. It is also

Abbreviations: CUMS, chronic unpredictable mild stress; FST, forced swim test; HPA, hypothalamic–pituitary–adrenal axis; MAO, monoamine oxidase; TST, tail suspension test.

* Corresponding author.
E-mail addresses: din_dhingra@rediffmail.com, din_dhingra@yahoo.com (D. Dhingra).

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reported to be an inhibitor of acetylcholinesterase and butyrylcholinesterases; beta site APP cleaving enzyme 1 [17] and monoamine oxidase [21]. Since MAO inhibitors are classical antidepressant drugs, so palmatine has potential in the management of depression. Antidepressant-like activity of palmatine has not been reported in the literature. Therefore, the present study was designed to explore the antidepressant-like effect of palmatine in mice subjected to chronic unpredictable mild stress.

Materials and methods

Experimental animals

Male Swiss albino mice (3 months old, weighing around 20–25 g) were purchased from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana, India). Since estrogens (female sex hormones) have been found to have antidepressant effect, so we excluded female mice and used only male mice for the study [23]. Animals were housed separately in groups of 10 per cage (polycarbonate cage size: 29 cm × 22 cm × 14 cm) under laboratory conditions with alternating light and dark cycle of 12 h each. The animals had free access to food and water. The animals were kept fasted 2 h before and 2 h after drug administration. The animals were acclimatized for at least five days before behavioral experiments which were carried out between 09:00 and 17:00 h. The experimental protocol was approved by Institutional Animal Ethics Committee and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forests, Government of India (Registration no. 0436).

Drugs and chemicals

Palmatine, fluoxetine hydrochloride (Sigma Aldrich, USA), sulfanilamide, N-(1-naphthyl)ethylenediamine dihydrochloride, and meta-phosphoric acid (Hi-Media Laboratories Pvt., Ltd., Mumbai, India) were used in the present study. Fluoxetine hydrochloride was dissolved in normal saline (0.9% (w/v) sodium chloride).

Selection of doses

Doses of palmatine and fluoxetine hydrochloride (10 mg/kg) were selected on the basis of literature [15,20]. The volume of vehicle or drug solution administration was 10 ml/kg.

Chronic unpredictable mild stress procedure

The mice were subjected to chronic stress as described by Mao et al. [30] and Kumar et al. [20]. Animals were subjected to stress paradigm once a day over a period of 3 weeks between 09:00 and 14:00 h. The order of stressors used was as follows:

Weeks	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 1	I	E	F	O	T2	X	T1
Week 2	I	O	X	E	T2	C	F
Week 3	O	F	T1	S	C	I	F

I—Immobilization for 2 hours; E—Exposure to empty water bottles for 1 hour; F—Exposure to foreign object for 24 hours (e.g. piece of plastic); O—overnight illumination; T2—tail pinch (60 s); X—Tilted cage at 45 degree for 7 hours; T1—tail pinch (30 s).

Mice subjected to CUMS procedure were called as stressed mice. Unstressed mice were exposed to behavioral tests, and not subjected to CUMS procedure. Drugs were administered 30 min before CUMS procedure in case of stressed group. Behavioral testing was done in independent groups of mice on the 22nd day.

Laboratory models employed for evaluation of antidepressant-like activity

Forced swim test

This test was carried out on mice according to the method of Porsolt et al. [29] and as followed earlier in our laboratory [8]. Briefly, mice were individually forced to swim in an open glass chamber (25 cm × 15 cm × 25 cm) containing fresh water to a height of 15 cm and maintained at 26 ± 1 °C. Water in the chamber was changed after subjecting each animal to FST. Mice placed in the chamber for the first time were initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2 min, activity began to subside and to be interspersed with phases of immobility or floating of increasing length. The duration of immobility was manually recorded during the next 4 min of the total 6 min testing period. Mice were considered to be immobile when they ceased struggling and remained floating in water, making only those movements necessary to keep their head above water. Following the swimming session, mice were towel dried and returned to their housing conditions.

Tail suspension test

It is a commonly employed behavioral model for screening antidepressant-like activity in mice [33]. For the test, the mouse was individually suspended on the edge of a table, 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Each animal under test was both acoustically and visually isolated from other animals during test. The total period of immobility was recorded manually for 6 min. Animal was considered to be immobile when it did not show any body movement, hung passively and completely motionless. The test was conducted in a quiet room to avoid disturbances to animals.

Sucrose preference test

The sucrose preference test [37] was employed herein to determine anhedonia, one of the core symptoms of major depression in human. The procedure was composed of training and testing courses. After 1 week of acclimatization, mice were trained to consume 1% (w/v) sucrose solution before the start of the CUMS protocol. In training course, mice were deprived of food and water for 24 h and only exposed to 1% (w/v) sucrose solution. Three days later, after 23-h food and water deprivation, 1-h baseline test was performed, in which mice were housed in individual cages and were free to access two pre-weighted bottles, one with 1% (w/v) sucrose solution and the other with tap water. Then, the sucrose preference was calculated according to the following formula:

$$\text{Sucrose preference} = \frac{\text{sucrose solution intake (g)}}{\text{sucrose solution intake (g)} + \text{water intake (g)}} \times 100$$

The test was again performed on the 21st day to evaluate the effect of stress as well as drug treatment.

Measurement of locomotor activity

To rule out the effects of various drug treatments on locomotor activity, horizontal locomotor activities of control and test animals were recorded for a period of 5 min using photoactometer (INCO, Ambala, India). The locomotor activity was assessed 2 h after drug administration on day 22.