Honokiol reverses depressive-like behavior and decrease in brain BDNF levels induced by chronic corticosterone injections in mice

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ABSTRACT

Background: Honokiol, an active component isolated and purified from Chinese traditional herb Magnolia officinalis. It is known to have a wide range of biological activities including antidepressant-like effects which have been observed in stress-induced depression models. This study was designed to investigate the antidepressant potential of honokiol in corticosteroid induced model of depression.

Method: Adult Swiss albino mice were injected with 40 mg/kg of corticosterone (CORT) chronically for 21 days. Behavioral and biochemical parameters were estimated. Moreover, since brain derived neurotrophic factor (BDNF) has been implicated in antidepressant effects of many drugs, we also evaluated the effects of honokiol on BDNF in the hippocampus.

Results: The results showed that the 3-week CORT injections caused the significant elevation in serum CORT levels in mice. Repeated CORT injections also caused depression-like behavior in mice, as indicated by the significant decrease in sucrose consumption (P < 0.01) and increase in immobility time in the forced swim test (P < 0.001). Moreover, it was found that BDNF levels in the hippocampus were significantly decreased (P < 0.001) in CORT-treated mice. Treatment of the mice with honokiol significantly suppressed the depression-like behavior and increased brain BDNF levels (P < 0.01) in CORT-treated mice.

Conclusion: These results conclude that honokiol produces an antidepressant-like effect in CORT-induced depression, which is possibly mediated by increasing BDNF expression in the hippocampus.

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1. Introduction

Major depression (MD) is a highly prevalent, debilitating and life-threatening psychiatric disorder that affects about 21% of the world population. It is characterized by a persistent low mood, diminished ability to experience pleasure and a variety of other features including anergia, changes in sleep, appetite and suicidal tendency. World Health Organization predicts that depression will be leading cause of disability worldwide by the year 2030. Psychological stress is a common risk factor involved in the development of major depression. Stress associated dysregulation of HPA axis functionality is one of the characteristic features of MD, and is demonstrated by altered feedback inhibition, as seen by increased circulatory cortisol and non-suppression of cortisol following administration of dexamethasone. Studies of chronic intense stress and associated glucocorticoid elevation have been performed extensively in adult rodents. Glucocorticoids have been proven to induce depressive-like behavior in rodents, as indicated by the significant changes in behavioral traits, neurochemistry and brain anatomy. These findings suggest that a glucocorticoid-induced depression model in rodents is suitable for evaluating the efficacy of potential antidepressants and explore the mechanism of action of antidepressants.

At present, there are several types of conventional antidepressants in clinical practice, including tricyclic antidepressants, monoamine oxidase inhibitors, serotonin and noradrenaline re-uptake inhibitors. Most of these drugs, however, have undesirable side effects. Thus, there is an unmet need for safe and powerful antidepressants. This necessitates the development of safe, better tolerated and effective pharmacotherapeutics, and one such promising class of drugs is plant based natural products.
Honokiol, a major active component of *Magnolia officinalis* (Magnoliaceae), possesses antioxidant, anti-cancer, anxiolytic, anti-thrombotic, neuroprotective, anti-inflammatory, anti-diabetic, anti-emetic and antibacterial activities (Fig. 1). Previous studies also established that honokiol produces an antidepressant-like effect in chronic stress-induced depression in mice. In this study, the antidepressant-like effect of honokiol treatment was further assessed in a mice model of CORT induced depression. Various other studies have shown that down regulated expression of BDNF is observed in depression, and upon treatment with antidepressants there is an upregulated expression of BDNF in the brain indicating a positive response to therapy. We also explored whether the antidepressant-like effect of honokiol was associated with the upregulation of BDNF expression by estimating BDNF levels in hippocampus of mice exposed to CORT.

2. Materials and methods

2.1. Drugs and chemical reagents

Honokiol, fluoxetine hydrochloride, corticosterone and DMSO were purchased from Sigma Aldrich (USA). BDNF ELISA kit purchased from Promega Corporation (Madison, WI) and corticosterone ELISA kit was purchased from Abnova Corporation (Walnut, CA). All the other reagents and chemicals used were of analytical grade.

2.2. Animals

Male Swiss albino mice weighing 20–25 g were obtained from the Pasteur Institute, Shillong, India. The animals were maintained on a 12-hour (h) light/dark cycle under regulated temperature (22 ± 2 °C), humidity (45 ± 10%) and fed with standard diet and water *ad libitum*. They were allowed to acclimatize seven days before use. The animal experiments have been approved by the Institutional Animal Ethics Committee of the Gauhati Medical College, Assam, India. All procedures were conducted in accordance with the CPCSEA guidelines for the care and use of laboratory animals.

2.3. Experimental design

Animals are divided into four groups of eight animals in each group, includes; vehicle control group (saline containing 0.1% dimethyl sulfoxide and 0.1% Tween-80), CORT plus vehicle (positive control) group, CORT plus honokiol (20 mg/kg) group and CORT plus fluoxetine (15 mg/kg) group. CORT (40 mg/kg, dissolved in saline containing 0.1% dimethyl sulfoxide and 0.1% Tween-80) was administrated subcutaneously (s.c.) for 21 days. Honokiol and fluoxetine were administered intragastrically (p.o.) 30 minutes (min) prior to the CORT injection for 21 days. The doses of CORT, honokiol and fluoxetine were selected based on earlier studies. The experiments were conducted in a noise-free, illumination controlled room. Efforts were made to minimize the number and suffering of the animals.

![Chemical structure of honokiol](image)

**Fig. 1.** Chemical structure of honokiol.

2.4. Behavioral tests

After 21-day treatment with respective drugs, mice were randomly assigned to a series of behavioral tests for depression like behavioral characterization. To avoid having compound effect due to sequential behavioral measurements, 24 h later after last CORT treatment sucrose preference test was performed, forced swim test started with a recovery period of 48 h in between.

2.5. Sucrose preference test

The test was performed as described previously, with minor modifications. In this test, mice were given a free choice between two bottles for 24 h, one with 1% sucrose solution and another with regular water. To avoid possible effects of side preference in drinking behavior, the location of the bottles was switched after 12 h. No previous food or water deprivation was applied before the test. The consumption of water and sucrose solution was estimated simultaneously in control and experimental groups by measuring the amount of solution (ml) in each bottle. The sucrose preference was calculated by the following formula:

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\text{Sucrose preference} = \frac{\text{sucrose consumption (ml)} - \text{water consumption (ml)}}{\text{water consumption (ml)}} \times 100.
\]

2.6. Forced swim test

The test was carried out 48 h after the sucrose preference test, according to the method of Porsolt et al. Briefly, mice were forced to swim in a transparent glass vessel (25 cm high, 14 cm in diameter) filled with 10 cm of water at 25 ± 1 °C. The total duration of immobility in seconds (s) was measured during the last 4 min of a single 6-min test session. Immobility period was regarded as the time spent by the mouse floating in the water without struggling and making only those movements necessary to keep its head above the water.

2.7. Biochemical parameters

After 24 h of conducting forced swim test, blood samples were collected by retro orbital method. Serum was separated by centrifugation at 4000 × g for 10 min and stored at −80 °C until assay. Whole brains were rapidly removed and placed on an ice-chilled glass plate. The hippocampus of animals was dissected on a cold plate and placed in an isolation medium containing 230 mM mannitol, 70 mM sucrose, 1 mM EDTA, 10 mM Tris—HCl, pH 7.4, and homogenized in the same medium. Homogenates were centrifuged at 4000 × g at 4 °C for 10 min to obtain the supernatant. Aliquots of the supernatant were used for the determination of BDNF levels.

2.8. Estimation of serum CORT levels

Serum corticosterone level was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Abnova Corporation, Walnut, CA), according to the manufacturer’s protocol. Briefly, 25 μl of standard, sample solutions were added to the already precoated antibody plate provided with the kit and then immediately 25 μl of biotinylated corticosterone was added to each well and incubate for 2 h. After washing 50 μl of Streptavidin– Peroxidase Conjugate was added to each well and incubated for 30 min. After incubation, plate washed properly and 50 μl of chromogen substrate was added and incubated for 12 min. The reaction was stopped with 50 μl of stop solution and absorbance was read at 450 nm immediately. The detection limit of the assay is ~0.3 ng/ml.
2.9. Estimation of BDNF levels

The BDNF content in the hippocampus homogenate measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit from Promega Corporation (Madison, WI) according to the manufacturer’s protocol. All samples and standards were added in duplicate into 96-well plate precoated with Anti-BDNF mAb, which were incubated overnight without shaking at 4 °C. After washing, biotinylated anti-Human BDNF pAb were added and the plate was incubated for 2 h at room temperature. After washing, anti-IgY HRP Conjugate solution was added and the plate was incubated for 2 h at room temperature. The reaction was stopped with 100 µl of 1 N hydrochloric acid and then immediately absorbance was read using a microplate reader recorded at 450 nm. The detection limit of the assay is 15.6 pg/ml of BDNF.

2.10. Statistical analysis

All the data were expressed as mean ± S.E.M. Multiple group comparisons were performed using one-way analysis of variance (ANOVA) followed by Dunnett’s test in order to detect inter-group differences. Differences were considered statistically significant when the P < 0.05.

3. Results

3.1. The effect of honokiol on sucrose consumption

The effect of honokiol on the percentage of sucrose consumption in CORT-only treated mice is given in Fig. 2. The CORT injections resulted in a significant reduction in the percentage of sucrose consumption in the animals (P < 0.01) compared with the controls. Treatment with honokiol and fluoxetine significantly increased the percentage of sucrose consumption in the CORT-treated mice (P < 0.05 and P < 0.01, respectively) compared with the CORT-only treated mice.

3.2. The effect of honokiol on immobility time in forced swim test

In this test, animals treated with CORT (40 mg/kg) showed increase in immobility time, which was significant (P < 0.001) when compared with vehicle control [Table 1]. Animals treated with fluoxetine (15 mg/kg) showed a significant decrease in the immobility time (P < 0.001) as compared to CORT control. Similarly animals treated with honokiol also showed significant decrease in immobility time (P < 0.01) and thus effective antidepressant activity.

3.3. The effect of honokiol on serum CORT levels

The serum CORT levels in different groups are shown in Fig. 3. The CORT injections resulted in a significant increase in the level of serum CORT in the animals (P < 0.001) when compared with the vehicle controls. Treatment with honokiol (20 mg/kg) and fluoxetine (15 mg/kg) significantly decreased serum CORT levels (P < 0.01 and P < 0.01, respectively) when compared with the CORT-only treated mice.

3.4. The effect of honokiol on BDNF levels in the hippocampus

The effect of honokiol on BDNF levels in the hippocampus of CORT-treated mice is given in Fig. 4. Exposure to CORT significantly decreased BDNF protein levels in the hippocampus (P < 0.001) compared with the vehicle controls. Treatment with honokiol (20 mg/kg) and fluoxetine (15 mg/kg) significantly increased BDNF protein levels in the hippocampus (P < 0.01 and P < 0.001, respectively) when compared with the CORT-only treated mice.

4. Discussion

The corticosteroid induced model of depression is received as a suitable method for predicting the antidepressant action of compounds rodents. In this study, the antidepressant-like effects of
honokiol were investigated in the corticosterone induced depression model. Honokiol showed antidepressant-like effect by reversing the depressive-like behavior, decrease in brain BDNF level and increase in corticosterone levels induced by chronic CORT injections in mice.

The sucrose preference test is an indicator of anhedonia-like behavioral change. Anhedonia, a core symptom of major depression, is modeled by inducing a decrease in responsiveness to rewards, as reflected by the reduced consumption of and/or preference for sweetened solutions. In the present study, our data is in line with other findings showing that repeated CORT injections results in significant decreased in the percentage of sucrose consumption of mice,8 whereas treatment with honokiol (20 mg/kg) significantly suppressed the percentage of CORT injection-induced decrease in sucrose consumption by the mice.

The forced swim has been widely used for assessing the effectiveness of antidepressant candidates. In line with earlier findings,8,18 in this study, the 3-week CORT injections dramatically increased the immobility time of the mice in the forced swim test, indicating behavioral despair in these animals. Treatment with honokiol (20 mg/kg) significantly reversed the CORT-induced increase in the immobility time in mice. Taken together, the results obtained from the behavioral studies indicate that honokiol treatment produced an antidepressant-like action in the CORT-treated mice.

Dysregulated HPA associated increase in plasma/serum CORT is an indicator of ongoing depression physiology.4 Consistent with previous studies,14,18 in the present study also CORT injections to the mice resulted in a significant increase in the serum CORT levels, which were decreased by treatment with honokiol and standard drug fluoxetine at 20 mg/kg and 15 mg/kg respectively.

BDNF is one of the most common neurotrophic factors in the central nervous system. The role of BDNF in the pathogenesis of depression and in the mechanism of action of antidepressants is well appreciated. Clinical studies have found decreased BDNF levels in the blood of depressive patients,21,22 whereas antidepressant treatment seems able to normalize the BDNF levels.23 Furthermore, BDNF might play an important role in CORT induced depression.24 Several rodent studies have reported that treatment with exogenous CORT causes a significant decrease in BDNF expression in the brain regions which are critically involved in the regulation of emotion, motivation, learning and memory.13 Consistent with these findings, in the present study, the 3-week CORT injections significantly decreased BDNF protein levels in the hippocampus of the mice, whereas honokiol treatment reversed the CORT-induced changes in BDNF expression.

5. Conclusion

In conclusion, the current study demonstrates honokiol produces an antidepressant-like effect in CORT-treated mice, which is may be mediated by increasing BDNF expression in the hippocampus.

Conflicts of interest

All authors have none to declare.

References

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