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Original Article

# Anti-depressant Effect of *Chilbokum* under the Forced Swimming Test in Rats

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**Objectives :** The aim of this study was to evaluate the anti-depressant effect of *Chilbokum* on rats under the forced swimming test (FST)

**Methods :** The rats were treated with the herbal extract, *Chilbokum*. In order to induce depression-like symptoms, the FST was conducted afterwards. The immobility time was measured during a 5-min experimental session. The alterations of the 5-HT level in the hypothalamus and hippocampus and the change of corticosterone level in the blood induced by FST were determined in the rats.

**Results :** The results were as follows:

1. The immobility time during 5 min of FST in the drug administration group showed significant decreases compared with the control group ($p<0.05$).
2. The FST+ *Chilbokum* group had significantly increased 5-HT levels of the hypothalamus and hippocampus, compared with the control group ($p<0.05$, respectively).
3. The FST+ *Chilbokum* group had significantly decreased corticosterone levels, compared with the control group ($p<0.05$).

**Conclusions :** These results demonstrate that the reduced immobility time by *Chilbokum* may be mediated by the increase in 5-HT level in the hypothalamus and hippocampus, suggesting that *Chilbokum* has a potential therapeutic efficacy for human depression.

***Key Words :*** *Chilbokum*, anti-depressant effect, forced swimming test (FST), 5-HT, corticosterone

## Introduction

Depression is nowadays a prominent health problem. Functional monoamine neurotransmitter system deficiency in the brain plays a role in the pathogenesis of depression[1]. Basic and clinical studies have demonstrated that alterations in the hypothalamic-pituitary-adrenal (HPA) axis system are characteristic of depression as evidenced by increased release of corticotropin-releasing factor[2,3,4]. Reductions in the HPA axis activity may contribute to antidepressant actions of some treatments, at least partly, by reducing CRF and cortisol levels [5,6]. There is considerable clinical evidence that serotonin receptor (5-HT) containing pathways in the CNS play a significant role in the pathological development of major depression [7]. Stress is thought to impair the hippocampus, leading to a deficiency of 5-HT in the hippocampus and the outbreak of depression[8].

Chilbokum has been described in traditional oriental medicine. According to traditional oriental medical theory, the clinical condition of depression

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could be mainly classified into liver qi stagnation, the symptoms of which can be described as mental stress, hypochondriac distensive pain, or lumps in the breasts, hernial pain and irregular menstruation. We found some studies about efficacy of Chilbokum on brain tissue and cerebral cortex neuron[9,10]. However, there was no study indicating neurochemical and neuroendocrine mechanisms of Chilbokum by the forced swimming test of animals, which is widely used to predict antidepressant efficacy. We reported the effects of Chilbokum on the concomitant biochemical parameters, 5-HT and corticosterone as pathophysiological indicators involved in the rat FST model of depression.

## Materials and Methods

### 1. Preparation of drug

Chilbokum was prepared by mixing the 7 crude drugs in a uniform ratio (Table 1). It was provided by the department of Oriental Medicine, Graduate School of Dongguk University. This herbal mixture was soaked in distilled water (10 times as much as the volume of the mixture). After boiling at 100℃, the decoction was filtered through filter paper, and was concentrated by rotary evaporator.

**Table 1.** Composition of Chilbokum used in this study.

| Herbs | Amount(g) |
|---|---|
| 人蔘 Ginseng Radix | 10g |
| 熟地黃 Rehmanniae Radix Preparat | 10g |
| 當歸 Angelicae Gigantis Radix | 8g |
| 白朮 Atractylodis Macrocephalae Rhizoma | 4g |
| 甘草 Glycypphizae Radix | 4g |
| 酸棗仁 Zizyphi Spinosae Semen | 8g |
| 遠志 Polygalae Radix | 2g |
| Total amount(g) | 46g |

1) Animals and experimental design

Male Sprague Dawley rats of 250~280g (Samtaco, Inc. Korea) were used. Animals were housed under controlled temperature (22~24℃) on a 12h light/dark cycle. Lights were on from 8:00 to 20:00. Food and water were given ad libitum. They were allowed at least 1 week to adapt to their environment before use for experiments.

The male rats were randomly divided into four groups (n=8 per group): the nonstressed normal group was given saline, the control group was given saline and the two experimental groups were given diluted Chilbokum by 2ml/kg in concentrations of 200mg/kg (A group) or 400mg/kg (B group) 24h, 5h, and 1h before getting stressed by forced swimming.

2) Forced swimming test

This procedure is similar to that previously described[11]. The FST was conducted in Plexiglas cylinders (height: 40 cm, diameter: 20 cm) filled with 20 cm of 25 °C water. On the first day all rats except the normal group were stressed by immobility during a 15-min forced swim. After forced swimming, the control group was administered saline, and the two experimental groups were administered Chilbokum diluted by saline by 2ml/kg at uniform concentration. Administration was executed at 24h, 5h and 1h before the next day's FST. On the next day during the 5-min FST, immobility was defined as the rat making only those movements necessary to keep its head above the water (i.e., absence of vigorous activity such that the forepaws did not break the surface of the water).

3) Blood and tissue collection

Four groups of rats were sacrificed immediately

after exposure to the FST. Blood was sampled into tubes and separated in a refrigerated centrifuge at 4 ℃. The serum was stored at -80℃ until assayed. Following blood collection, their hippocampi were quickly removed, frozen in liquid nitrogen and stored at -80℃ until assayed.

4) Serum level of corticosterone

Corticosterone was measured by competitive enzyme immunoassay using a rabbit polyclonal corticosterone antibody (Octeia Corticosterone; Alpco Diagnostics, American Laboratory Products, Windham, NH).

5) Serotonin level of hypothalamus and hippocampus

*Sample preparation*

The animals were sacrificed by decapitation immediately after the FST. The brain was then rapidly removed and the hippocampus and hypothalamus were dissected out and placed onto an ice cold plate. All the tissue samples were quickly frozen and stored in a deep freezer at -80 C until assayed. The samples were weighed and then homogenized with a ultrasonic disruptor (Sonics Materials, INC, USA) in an ice cold 0.1 M perchloric acid (PCA) solution (600 ㎕) containing 0.1% sodium metabisulfate and 40 ng/ml of dihydroxybenzylamine (DHBA) was used as an internal standard. After homogenization, the solution was centrifuged at 15,000 rpm in a micro 17R centrifuge (Micro 17R, Hanil Co., Korea) for 30 min at 4 C.

*Determination of tissue level of 5-HT*

The levels of 5-HT were determined by performing HPLC coupled with ECD. A 20 ㎕ sample of the supernatant was injected into a Bondapak C18 reverse-phase column (Waters Co., with a 3003.9 mm internal diameter and a particle size of 5 m) for the separation of 5-HT (flow rate of 1 mL/min). Determination of 5-HT was done with an ECD (ESA, Coulochem II, Model 5200A), and a pump (ESA, Model 580). A guard cell (ESA, Model 5020) was set at +400 mV, the first and second electrodes of the analytical cell (ESA, Model 5011) were set at -40 and +200 mV, and the output of the second electrode was recorded as a chromatograph using an HP 3395B printer (Hewlett Packard, Germany). The composition of the mobile phase was 150 mM sodium phosphate monobasic, 0.7 mM sodium octane sulfonate, 0.1 mM EDTA and 10% acetonitrile, and this was adjusted to pH 3.2 using 0.1 M phosphoric acid. The tissue level of the 5-HT was determined by performing a linear regression analysis for the peak heights obtained from a range of standard curves, and expressed as ng of 5-HT per g of fresh tissue weight.

*Statistical analysis*

FST immobility data were analyzed by one-way analysis of variance (ANOVA) with drug administration as the between-subject factor. Post hoc comparisons were performed using the LSD test. Neurochemical data were analyzed by one-way ANOVA with drug administration, and this was followed by post hoc LSD test. All data were considered statistical significant at $p<0.05$. SPSS 10.0 for Windows was used for analysis of statistics.

## Results

### 1. Forced swimming test

Fig. 1 shows effect of *Chilbokum* on the total duration of immobility in the forced swimming test (FST) in rats. The immobility time was measured during a 5-min experimental session.

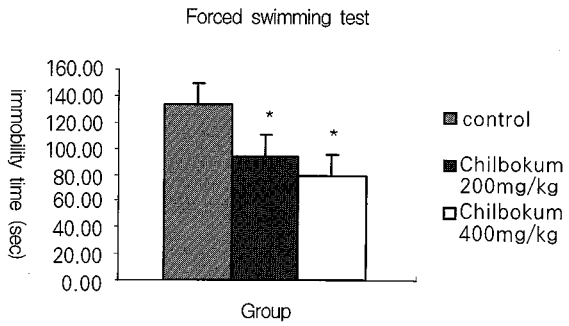


**Fig. 1.** The total duration of immobility in the forced swimming test (FST)
*: Statistically significant as compared with the control group (*: P<0.05)

The total duration of immobility in the forced swimming test (FST) of the control group was 133.5 ± 16.6 sec, that of the A group (*Chilbokum* 200mg/kg) was 95.0 ± 15.4 sec, and that of the B group (*Chilbokum* 200mg/kg) was 79.0 ± 16.7 sec. respectively. There was a statistically significant effect on the experimental groups as compared with the control group (P<0.05) (Fig. 1).

1) Serotonin level of Hypothalamus and Hippocampus

Fig. 2 showed effects of *Chilbokum* on the alteration of the 5-HT level induced by the forced swimming test in the rat hypothalamus. The level of 5-HT in the hypothalamus of the normal group was 1300 ± 68 ng/mg, that of the control group was 1450.00 ± 76.00ng/mg, that of the A group (*Chilbokum* 200mg/kg) was 1647.00 ± 78.00 ng/mg, and that of the B group (*Chilbokum* 400mg/kg) was 1766.00 ± 85.00 ng/mg. Levels of 5-HT were significantly increased in the *Chilbokum*-treated A and B groups, compared with the control group (P<0.05, respectively) (Fig. 2).

Fig. 3 shows effects of *Chilbokum* on the alteration of the 5-HT level induced by the forced swimming test in the rat hippocampus. The level of 5-HT in the hippocampus of the

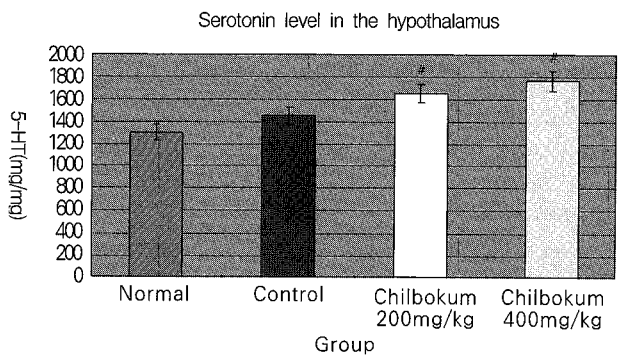


**Fig. 2.** Hypothalamus 5-HT level
#: Statistically significant as compared with the control group (#: P<0.05)

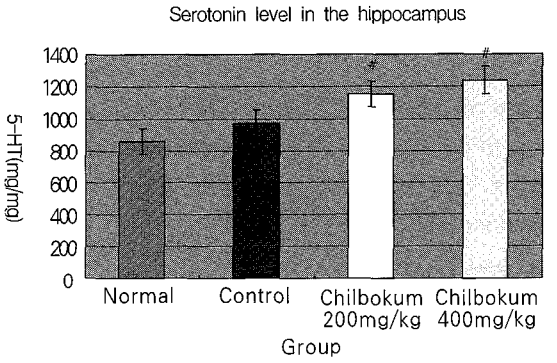


**Fig. 3.** Hippocampus 5-HT level
#: Statistically significant as compared with the control group (#: P<0.05)

normal group was 858 ± 77 ng/mg, that of the control group was 965.00 ± 86.00ng/mg, that of the A group (*Chilbokum* 200mg/kg) was 1152.00 ± 78.00 ng/mg, and that of the B group (*Chilbokum* 400mg/kg) was 1235.00 ± 85.00 ng/mg. Levels of 5-HT were significantly increased in the *Chilbokum*-treated A and B groups, compared with the control group (P<0.05, respectively) (Fig. 3).

## 2. Serum level of corticosterone

Fig. 4 shows the effect of *Chilbokum* on the change of corticosterone level induced by FST. *Chilbokum* was administered 1hr prior to FST. The level of corticosterone in the serum of control group was 67.00 ± 12.00 ug/dL, significantly increased as compared with that of the normal group (35 ± 8 ug/dL) (p<0.01). Serum level of the A group (*Chilbokum* 200mg/kg) was 54.00 ± 8.00 ug/dL, and that of the B group (*Chilbokum* 400mg/kg) was 43.00 ± 9.00 ug/dL. The level of corticosterone in the serum of the *Chilbokum* groups significantly decreased as compared with that of the control group (P<0.05) (Fig. 4).

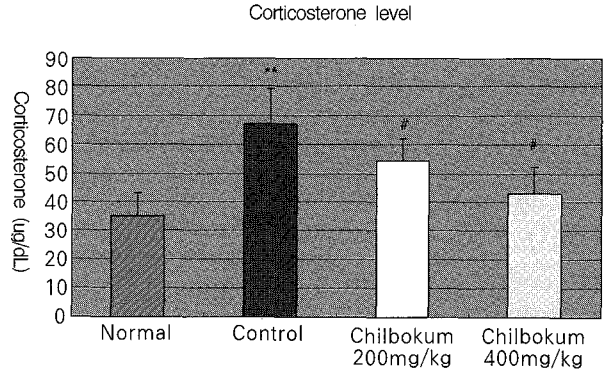


**Fig. 4.** Serum level of corticosterone
**: p< 0.01 vs. normal, #: p< 0.05 vs. control.

## Discussion

The forced swimming test is one of the most widely accepted behavioral models for assessing pharmacological antidepressant activity[11,12]. The characteristic behavior scored in this test is termed immobility, reflecting behavioral despair as seen in human depression, and it is well known that antidepressant drugs are able to reduce this immobility time in rodents[11]. The present study provides behavioral and cellular evidence for the antidepressant-like activities of *Chilbokum*.

The significant percent reduction by *Chilbokum* in immobility time in the rat was 29% to 41%. Although the efficacy of the A group (*Chilbokum* 200mg/kg) was lower, the treatment effect was statistically significant compared with the control group in FST in rats.

The hippocampus is a brain structure that has been extensively studied with regard to stress, depression, and antidepressant actions. There is now substantial evidence to show that major depression is associated with reduced hippocampal and striatal volumes[13].

Monoamine neurotransmitters including 5-HT play important roles in depression and in mediating behavioral effects of antidepressant drugs. Swim stress produced reductions in brain 5-HT in male rats. Some studies have shown that swim stress increased the levels of brain 5-HT, DA and their metabolites in male BALB/cA mice and Wistar rats[14,15]. Furthermore, swim stress significantly elevated the ratio of 5-HIAA/5-HT, a usual indicator of serotonergic activity. Such an increase was in accordance with some results of Connor et al., 1997 and Connor et al., 2000 obtained in the rat FST[16,17].

## Conclusion

1. The immobility time during 5 min of FST in the *Chilbokum* administration groups showed significant decreases compared with the control group ($p<0.05$).

2. 5-HT levels of the hypothalamus and hypothalamus in the groups administered *Chilbokum* significantly increased compared with that of the control group ($p<0.05$).

3. Blood corticosterone levels in the groups administered *Chilbokum* significantly decreased compared with the control group ($p<0.05$).

These results demonstrate that the immobility time reduction by *Chilbokum* may be mediated by the increase in 5-HT level in the hypothalamus and hippocampus, suggesting that *Chilbokum* has a potential therapeutic efficacy for human depression.

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