

# Anti-depression Effects of *Lithospermum erythrorhizon* Extract by regulation of inflammation

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## Abstract

*Lithospermum erythrorhizon* is the purple root of a perennial herb included in the Boraginaceae family and it has been generally used as dye or medicine in Korea. In precedent studies, it was reported that shikonin, one of ingredients of *Lithospermum erythrorhizon*, regulated an oxidation-reduction reaction and inhibited TNF- $\alpha$  as anti-inflammatory effects. Depression is a very common mental disease observed in 5-10% of males and, particularly, 10-20% in females in the ordinary living environment. A continuous stimulus of mental stress may cause hormone disorders in the hypothalamic-pituitary-adrenal axis. We induced mental stress through the Forced Swimming Test (FST) and made a depression animal model in order to investigate effects of *Lithospermum erythrorhizon* extract on stress-induced depression. Rats were trained by FST for 15 minutes the day before drug administration and orally administered with 25 mg/kg and 100 mg/kg *Lithospermum erythrorhizon* extract and 10 mg/kg tianeptine sodium consecutively for five days. Acute effects were investigated by behaviour response test at day 1 of drug administration and chronic effects were investigated at day 5. Dead time was measured in the FST and total entries and total travel were determined in the Y-maze test. Then, anti-stress effects were investigated and the inflammation-regulating effect of iNOS and Nrf2, inflammation-related signaling protein in the pons and adrenal, by regulating hormone secretion was identified by western assay. In the result, dead time and both iNOS and Nrf2 expression decreased, and total entries and total travel increased in the *Lithospermum erythrorhizon* extract administration group compared to the FST group. It suggests that *Lithospermum erythrorhizon* extract orally administered regulates inflammation by inhibiting iNOS and Nrf2 expression and has anti-stress effects.

**Keywords:** *Lithospermum erythrorhizon* extract, Depression, Forced swimming test, Y-maze test, iNOS, Nrf2

## 1. Introduction

Stress-induced depression is not temporary depressed feeling, but pathological and it is caused by external and internal stress factors. Stress-induced depression is classified into depressive disorder (monopolar disorders) and bipolar disorder according to DSM-IV. Depressive disorders include acute and severe depressive (major) disorder, and chronic dysthymic (minor) disorder depending on the degree or period. Bipolar disorder is so-called manic-depressive insanity and it is accompanied by mania and depression [1]. In general, bipolar disorder, rather than monopolar disorder, is hard to treat and it was reported that combined treatment of mood stabilizer and antidepressant was not very effective for bipolar disorder and 30% of depression patients were not responding well to treatment and became chronically ill [2]. Thus, the development of new antidepressants is still being studied [3,4]. General symptoms include continuous depressed feeling, anorexia, inertia and fatigue and those cause inconvenience. In addition, the life prevalence is 5-12% in males and 10-25% in females which are high. The recurrence rate is 50% or higher and therefore depression is a very common mental disease [5]. Most drugs inhibit reuptake of monoamine hormones to improve depression symptoms based on the monoamine hypothesis. However, single administration of antidepressants has not shown significant effects compared to a placebo [6], and the effect of combined treatment of antidepressants and stabilizers varies individually. In addition, several side effects can arise. In this way, the relation between depression and inflammation or oxidative stress is actively being studied emerged from monoamine hypothesis.

Lithospermum erythrorhizon is the root of gromwell, a perennial herb, and it is called ji-cho, ja-dan and ja-geun in Korea. An outer cover of the root contains red pigment, so it has been used as food dye and medicine. In Korean medicine, it is used as burn, frostbite, bleb and eczema as well as antidote, antipyretic drug, contraceptive and diuretic [7]. Shikonin and acetylshikonin, which are naphthoquinone derivatives and active ingredients of Lithospermum erythrorhizon, are used for functional food, cosmetics and mordanting of silk fabrics, and massive cultivation of them is not easy, so shikonin is produced by cell culture [8,9]. It has been reported that shikonin has an effect on obesity and pancreatitis and also have anticancer effects, antibiotic effects and antioxidative activity [10,11,12,13,14], but Lithospermum erythrorhizon is naturally toxic. However, it was reported that it was not toxic up to 100 mg/kg in the result of precedent studies which performed weight measurement, electrocardiogram, urine test, hematologic test, biochemical test and toxicity test after oral administration of Lithospermum erythrorhizon extract at various concentrations [15].

Based on the results mentioned above, we focused on antidepressive effects due to regulation of inflammation and antioxidative activity to investigate effects of oral administration of Lithospermum erythrorhizon extract. There are three depression models(stress model, pharmacological model, genetic model). We selected Forced Swimming Test (FST), one of very common and easy stress models, and evaluated antidepressive effects of Lithospermum erythrorhizon extract [16]. In addition, we also assessed motility recovery by Y-maze test after depression was induced. The expression of iNOS and Nrf2, which are signaling protein related to inflammation and antioxidative activity, from the pons and the adrenal extracted from laboratory animals was quantified and compared to investigate inflammation-regulating effects.

## 2. Method

### 2.1 Subjects

We purchased fifteen five-week-old Sprague-Dawley female rats(100~130g) from Orient bio(Busan). Rats were adapted in the animal laboratory for a week and freely given the same amount of feed and water. Cage

temperature of the facility was 25°C, humidity was 60% and 12-hour night-day rotation was kept.

### 2.2 Study model

A cylindrical glass tube, 25 cm in diameter and 50 cm in height, was filled with water up to 30 cm and rats which were adapted to a cage for a week were dropped in water the day before the test. They were forced to swim for 15 minutes to adapt to water. Lithospermum erythrorhizon extract and Tianeptine Sodium were orally administered by jondae in 24 hours in order to investigate acute effects of Lithospermum erythrorhizon extract. After 30-minute absorption, the Forced Swimming Test(FST) was performed for five minutes. The Y-maze test was conducted for three minutes immediately after the FST. Lithospermum erythrorhizon extract and Tianeptine Sodium were orally administered as above five times for five days and the behaviour response test was performed in order to investigate chronic effects. After the behaviour response test, rats were anesthetized by ether inhalation and then their pons and adrenal were immediately extracted and stored at -70°C. Test groups were classified as follows: Group I : normal group(n=3), Group II : forced swimming group(control group, n=3), GroupIII: forced swimming + 100mg/kg Lithospermum erythrorhizon extract group(n=3), GroupIV: forced swimming + 25mg/kg Lithospermum erythrorhizon extract group(n=3), GroupV: forced swimming + 10mg/kg Tianeptine Sodium group(n=3).

### 2.3 Analysis

#### 2.3.1 Measurement of dead time in the Forced Swimming Test

Rat's behavior observed for five minutes was classified into immobility, climbing and swimming and time of immobility except climbing and swimming was measured. To determine acute effects and chronic effects, respectively, it was performed at day 1 and day 5 of drug administration.

### 2.3.2 Measurement of total entries and total travel in the Y maze

The Forced Swimming Test was performed and then the Y-maze test was immediately conducted for three minutes, in order to evaluate motility of rats where depression was created from FST-induced stress. Total entries in Y-maze A, B and C for each group were measured, added and compared. The average of total travel in each group was calculated and then compared after statistical analysis.

### 2.3.3 Protein quantitative analysis

Rats were sacrificed immediately after the behaviour response test and their pons and adrenal were extracted and stored at -70°C. The samples were chopped and lysis buffer (PRO-Prep™, protein Extraction solution) was added to extract protein. After protein quantification of each sample, electrophoresis and protein transfer to nitrocellulose membrane were performed. iNOS and Nrf2 were reacted with primary antibodies at 4°C for 24 hours and then with secondary antibodies for two hours. ECL prime (Amersham Pharmacia Biotech, Buckinghamshire, UK) was used to develop an X-ray film in the darkroom. The degree of expression was quantified.

### 2.3.4 Statistical analysis

The statistical package used was SPSS version 18. Dead time in the FST and total travel in the Y-maze were analyzed by ANOVA. Vision Works Image Software was used to compare the result of protein quantitative analysis and the degree of expression.

## 3. Result

### 3.1 The effects of Lithospermum erythrorhizon Extract with FST

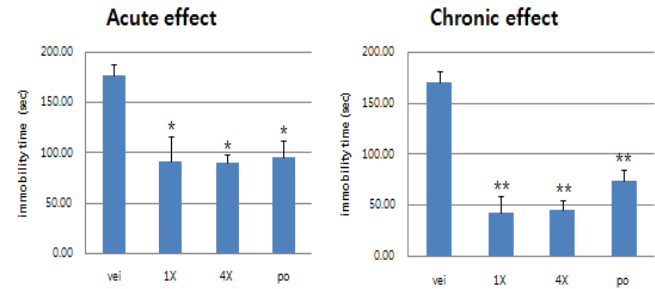


Fig. 1 Acute and chronic effects of GU Extract in FST induced depression rat model. Vei: FST induced Group, 1X: FST + Gu Extract 100mg/kg treated Group, Po: FST + Sodium Tianeptine 10mg/kg treated Group. \*, p<0.05 (compare with vehicle); \*\*, p<0.01 (compare with vehicle).

### 3.2 Total entered of Y maze

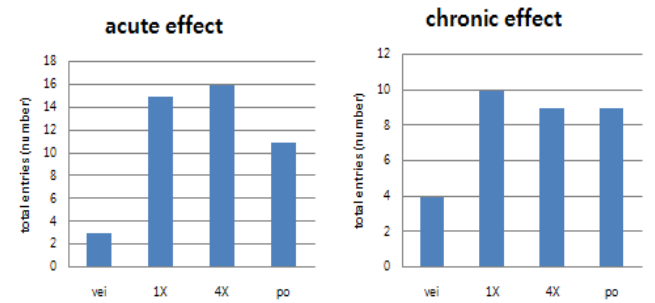


Fig. 2 Total entries in Y-maze test of FST induce depression rat model. Vei: FST induced Group, 1X: FST + Gu Extract 100mg/kg treated Group, Po: FST + Sodium Tianeptine 10mg/kg treated Group.

### 3.3 Movement of Y maze

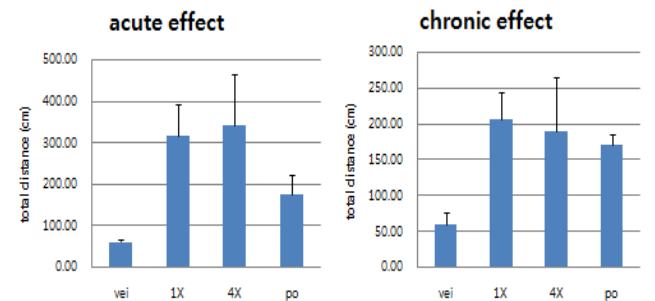


Fig. 3 Total distance in Y-maze test of FST induce depression rat model.

Vei: FST induced Group, 1X: FST + Gu Extract 100mg/kg treated Group, Po: FST + Sodium Tianeptine 10mg/kg treated Group.

### 3.4 Protein quantitative analysis

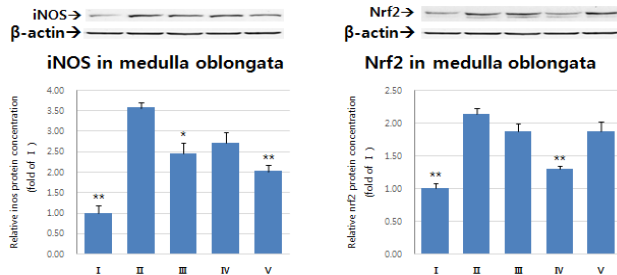


Fig. 4 iNOS, Nrf2 MAPK protein Expression in the medulla oblongata I . Control; II . FST induced Rat; III,IV. FST + FFE 100, 25mg/kg treated Rat; V . FST + sodium tianeptine 10mg/kg treated Rat. \*, p<0.05 (compare with vehicle); \*\*, p<0.01 (compare with vehicle).

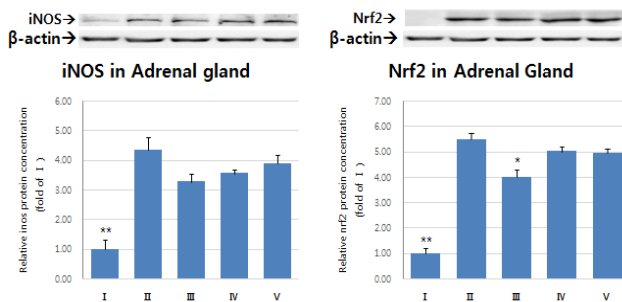


Fig. 5 iNOS, Nrf2 MAPK protein Expression in the adrenal gland I . Control; II . FST induced Rat; III,IV. FST + FFE 100, 25mg/kg treated Rat; V . FST + sodium tianeptine 10mg/kg treated Rat. \*, p<0.05 (compare with vehicle); \*\*, p<0.01 (compare with vehicle).

## 4. Conclusions

From old times, the mechanism of stress-induced depression has been controversial. This controversy is still being continued. The psychiatric association has two contrary opinions. Antidepressants were actively developed in 1950s and 1960s, so the monoamine hypothesis and the serotonin hypothesis were the most powerful mechanism of depression [17]. Monoamine is a neurotransmitter or hormone. Catecholamine, serotonin and melatonin are also included in this category. Monoamine hormones play a great role in emotion regulation, cognitive function, a feeling of happiness and homeostasis due to excitement of sympathetic nerves and

parasympathetic nerves and the level of monoamine hormones is low in depression [18]. Antidepressants based on the monoamine hypothesis inhibit reuptake of monoamine hormones to maintain excitatory of the synapse much longer and therefore a decline in monoamine hormones observed in depression patients is supplemented to show therapeutic effects [19]. Thus, tricyclic antidepressants were first developed, but pharmacological specificity was low and there were side effects, such as hepatorrhagia and gastrointestinal hemorrhage. In addition, overdose had a risk of death due to its toxicity. Therefore, selective serotonin reuptake inhibitors (SSRIs) were developed, but it also had digestive side effects, anxiety and insomnia. After that, NDRIs (Norepinephrine dopamine reuptake inhibitors) and SNRIs (Serotonin norepinephrine reuptake inhibitors) combining dopamine, serotonin and norepinephrine were developed and are being used now [20]. However, the monoamine hypothesis is facing unsolved methodological issues and limitations due to non-consistent result. Due to this situation, the macrophage theory about depression was first reported in 1991 and the relation between depression and inflammation or oxidative stress is frequently being studied [17,21,22].

The Forced Swimming Test and Y-maze test are included in a behaviour response test often used for evaluation of antidepressive effects [23]. In depressive animals, dead time increased and motility decreased. In the result of Forced Swimming Test, dead time of both 25 and 100 mg/kg Lithospermum erythrorhizon extract group was significantly lower than that of the control group in acute and chronic effects. The result was comparable when it was compared to the positive control group. In the Y-maze test, total entries showed that motility was higher than that of the control group. However, although total travel tended to increase compared to that of the control group, the difference was not significant because of a big individual error.

According to the inflammation hypothesis mentioned above, sharp stress induces overload in the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis regulates the immune system, homeostasis, emotion and body's response to stress. The abnormal and excessive expression of the HPA axis due to stress has been known as a direct cause of depression [24]. External stress promotes secretion of adrenocorticotrophic hormone corticotropin-

releasing hormone (CRH) from the hypothalamus and the activated HPA axis secretes catecholamine to induce the response of macrophages and immune cells [25].

The adrenal gland stimulated by CRH secretes cortisol and glucocorticoid. However, continuous stress causes the exhaustion of adrenal hormones and the reduction in receptor sensitivity, so inflammation is not regulated properly but up-regulated [26]. Cytokines including IL-1, IL-2, IL-6, IFN- $\gamma$  and TNF- $\alpha$  are secreted from activated macrophage and the signaling system of NF $\kappa$ B and Nrf2 is activated by up-regulated inflammation and oxidative stress (by ROS) to induce protein transcription of COX2 or iNOS [27].

In the previous study, shikonin and acetylshikonin, active ingredients of *Lithospermum erythrorhizon* extract, inhibited the activation of NF- $\kappa$ B to repress the production of nitric oxide (NO) and TNF- $\alpha$ , which are produced by LPS/rIFN- $\gamma$ , and therefore the antioxidative activity was confirmed [28]. Furthermore, in LPS-induced Raw 264.7 macrophage, shikonin derivatives inhibited the activation of iNOS through the MAPK/NF- $\kappa$ B mechanism [29]. In the present study like the precedent studies, the expression of iNOS and Nrf2 in the adrenal gland and medulla was significantly reduced in the *Lithospermum erythrorhizon* extract administration group compared to the control group. Therefore, the regulation of inflammation and antioxidative activity of *Lithospermum erythrorhizon* extract were confirmed. It suggests that *Lithospermum erythrorhizon* extract orally administered regulates resistance to stress-induced depression, motility recovery and the protein expression of iNOS and Nrf2, and therefore has anti-stress effects by decreasing inflammation and increasing the antioxidative activity.

## 5. References

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