

Sogeunjang-tang improves depressive-like behavior decreased by forced-swimming test

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ABSTRACT

Sogeunjang-tang (S-tang) is a traditional Korean medicine that has been used for the therapy of anxiety disorder in a variety of clinical situations. However, no study has investigated this effect. The aim of this study was to investigate the effectiveness of S-tang for reducing depression in animal model. The anti-depressant effect of S-tang was investigated in ICR mice by using forced swimming test (FST). After FST, we analyzed the blood biochemical parameters from blood. The immobility time was significantly decreased in the S-tang oral administration or pharmaceutical acupuncture group compared with the control group at 14 days ($p < 0.05$). The anti-depressant effect of S-tang was higher than that of fluoxetine (a classical anti-depressant). S-tang or fluoxetine significantly decreased the creatine kinase and lactate dehydrogenase level compared with the control group ($p < 0.05$) but not glucose, protein, and blood urea nitrogen level. Further investigation of S-tang as a potential pharmacologic agent in the management of depression related anxiety is warranted.

Keywords Sogeunjang-tang, forced swimming test, immobility time, depression

INTRODUCTION

Depression is one of the world's leading disabilities, and as measured over the years, large numbers of people are getting afflicted by this disabling condition (Chang and Fava, 2010; McKenna et al., 2005). The forced swimming test (FST) is commonly used as a screening test for antidepressant properties of drugs and also as an endurance test to examine antifatigue effects of candidate agents (De-Mello et al., 1992; Kim et al., 2008; Porsolt et al., 1978). Glucose, blood urea nitrogen (BUN), lactate dehydrogenase (LDH), creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total protein are blood biochemical parameters related to depression and fatigue. Energy for exercise is derived initially from the breakdown of glycogen and, later, from circulating glucose released by the liver and from nonesterified fatty acids (Dorchy, 2002). As is commonly known, glucose levels are decreased immediately after exercise. The BUN test is a routine test used primarily to evaluate renal function. Serum LDH and CK are known to be accurate indicators of muscle damage. Total protein is a rough measure of serum protein. Protein measurements can reflect nutritional state, kidney disease, liver disease, and many other conditions (Dorchy, 2002). FST exposure produces a variety of time-dependent neurochemical, endocrine, and immune alterations in the rat (Connor et al., 1997). Following related animal studies, repeated

administration of drugs daily for 14 days resulted in the demonstration of antidepressant activity at doses lower than those which were previously necessary to demonstrate activity when the drugs were administered over a period of 24 h (Lifschytz et al., 2006). Antidepressants such as monoamine oxidase inhibitors, selective serotonin reuptake inhibitors, and tricyclic antidepressants have been used in the clinic for several decades (Kim et al., 2002). Sogeunjang-tang (S-tang) is a traditional Korean medicine that has been used for the treatment of anxiety disorder. However, its mechanism of action remains unknown. In the current study, we examined the immobility time and blood biochemical parameters after FST to investigate the anti-depressant effect of S-tang.

MATERIALS AND METHODS

Animals

The original stocks of male ICR mice (4-week-old) weighing 13 - 15 g were purchased from the Dae-Han Experimental Animal Center (Daejeon, Korea), and were maintained at the Department of Food & Nutrition, Hallym University. The mice were housed in a laminar-airflow room maintained at a temperature of $22 \pm 1^\circ\text{C}$ and relative humidity of $55 \pm 10\%$ throughout the study, respectively. No animal was used more than once. Food and water were available *ad libitum*. All protocols were approved by the institutional animal care and use committee of Kyung Hee University (KHUASP(SE)-10-032).

Preparation of S-tang

The S-tang was obtained from Noa drug store (Seoul, Korea). S-tang is composed of 6 oriental herbs containing of

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Table 2. Value of blood biochemical parameters

	CON	Fluoxetine	ST 10 mg/kg	ST 100 mg/kg
Glucose (mg/dL)	218.00 ± 3.06	232.67 ± 25.44	227.33 ± 42.90	226.67 ± 42.90
Total protein (g/dL)	5.57 ± 0.15	5.73 ± 0.12	5.57 ± 0.07	5.43 ± 0.07
LDH (IU/L)	1781.00 ± 412.35	779.00 ± 106.31*	1657.67 ± 249.76	818.67 ± 108.69*
BUN (mg/dL)	15.73 ± 0.44	18.70 ± 0.47	16.07 ± 0.84	15.90 ± 0.65
CK (mg/dL)	1428.67 ± 577.75	749.67 ± 113.59*	1549.67 ± 707.47	461.00 ± 88.04*

CON; saline administered control group, ST; S-tang orally administered group.

Values represent the mean ± SEM. * $p < 0.05$: significantly different from the control group.

Saccharum Granorum 32 g, *Paeonia lactiflora* Pall. 12 g, *Cinnamomum cassia* Blume 6 g, *Glycyrrhiza uralensis* Fisch 6 g, *Zingiber officinale* Roscoe 6 g, and *Zizyphus jujuba var. inermis* (Bunge) Rehder 6 g. A voucher specimen (number 1-01-60) was deposited at the Herbarium of the Department of Food & Nutrition, Hallym University. An extract of S-tang was prepared by decocting the dried prescription of herbs with boiling distilled water (68 g/l). The duration of decoction was about 3 h. The decoction was filtered, lyophilized and kept at 4°C. The yield of dried extract from starting crude materials was about 10%. The samples were dissolved in saline and then filtered through 0.22 µm syringe filter. The daily recommended doses of Korean medicine for adult Korean are around 100 - 150 g. The yield of freeze drying is commonly about 5 per cent (w/w). Therefore, an adult Korean (average body weight 60 kg) takes 5-7.5 g at one time. From this, the dose of S-tang for an adult person can be 100 mg/kg. For oral administration and pharmaceutical acupuncture, 10 mg/kg and 100 mg/kg S-tang were used. Fluoxetine was used as a positive control (Kim et al., 2008).

Pharmaceutical acupuncture

S-tang (20 µl) was subcutaneously injected into the right Taiyuan acupoint (LU-9) located on the anterolateral aspect of the wrist, between the radial styloid process and the scaphoid bone, in the depression ulnar to the abductor pollicis longus tendo (WHO Regional office for the Western Pacific, 2008). LU-9 is one of the most commonly used acupuncture points to tonify the vital energy and regulate the immune system (Zhou et al., 2012). The anatomical location of the LU-9 in mice is equivalent to that described in humans and the location of this acupoint was identified based on the description provided by Stux and Pomeranz (Stux and Pomeranz, 1987). Animals in the control group received an injection of saline into the same site.

FST

After the first measurement of the immobility time, the mice ($n = 5$ /group) were divided into the control group, fluoxetine group, S-tang oral administration (10 and 100 mg/kg) group, and S-tang pharmaceutical acupuncture (10 and 100 mg/kg) group to match the swimming time in each group. These drugs were administered into mice once per day for 2 weeks. Fluoxetine, known as a classical anti-depressant, was used as a reference agent. During a 6 min FST, the duration of immobility was measured as previously described by Porsolt *et al.* (1977). The apparatus used for this test consisted of two Plexiglas cylinders (height 25 cm, diameter 10 cm) placed side by side in a Makrolon cage filled with water (10 cm height) at 23 - 25 °C. Two mice were tested simultaneously for 6 min inside vertical Plexiglas cylinders; a non-transparent screen placed between the two cylinders prevented the mice from seeing each other. The total duration of immobility, after a delay of 2 min, was measured during a period of 4 min. Each mouse was considered to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water.

Blood biochemistry analysis

After the last FST, the mice were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (4 mg/kg) and blood (1 ml) was withdrawn from the heart of the mice into syringes. Serum was then prepared by centrifugation at $1500 \times g$ at a temperature of 4 °C for 10 min. The BUN, CK, LDH, glucose, and total protein contents were determined by an autoanalyzer (Hitachi 747, Hitachi, Japan).

Statistical analysis

The results were expressed as mean ± S.E.M. for the number of experiments. Statistical significance was compared among each group and by the ANOVA with post-hoc multiple comparison test. Results with $p < 0.05$ were considered statistically significant.

RESULTS

Effect of S-tang oral administration or pharmaceutical acupuncture on the FST

To estimate the anti-depressant effect of S-tang, we measured immobility time on FST. The immobility time of the S-tang oral administration or pharmaceutical acupuncture group at 14 days was significantly decreased in comparison with the control group (Figure 1). The anti-depressant effect of S-tang was higher than that of fluoxetine (a classical antidepressant).

Effect of S-tang oral administration on blood biochemical parameters

In Figure 1, S-tang oral administration and pharmaceutical acupuncture showed the anti-depressant effect. Therefore, we analyzed the glucose, total protein, LDH, BUN, and CK level in serum. As shown in Table 1, the LDH and CK content of the serum in the fluoxetine and S-tang100 mg/kg treated groups was decreased significantly in comparison with that of the control group ($p < 0.05$). But S-tang or fluoxetine did not affect the glucose, total protein, and BUN levels compared with the control group.

DISCUSSION

As is already described in the materials and methods section, S-tang consists of 6 different herbs. Mao et al. (2008a) reported anti-depressant-like effect of ethanol extract from *Paeonia lactiflora* in mice. They also reported that administration of total glycosides of peony (paeoniflorin and albiflorin) caused a significant reduction of immobility time in both the FST and tail suspension tests (Mao et al., 2008b). Wang et al. reported that liquiritin and isoliquiritin, isolated from *Glycyrrhiza uralensis* have an anti-depressant-like effect in the FST and tail

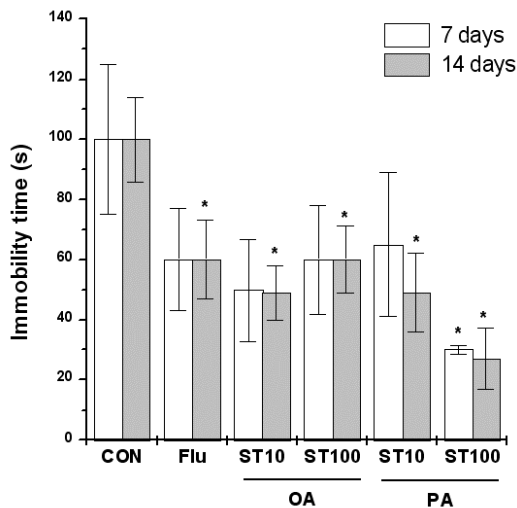


Fig.1. Effect of S-tang oral administration or pharmaceutical acupuncture on the FST. The S-tang oral administration or pharmaceutical acupuncture continued for 14 days at the same time every morning. During the FST, S-tang oral administration or pharmaceutical acupuncture was conducted 1 h before the test. Values are the means \pm SEM. * $p < 0.05$ vs. a saline-treated control group. CON, saline-administered control group; Flu, Fluoxetine (10 mg/kg)-administered group; ST10, S-tang (10 mg/kg)-administered group; ST100, S-tang (100 mg/kg)-administered group; OA, oral administration; PA, pharmaceutical acupuncture.

suspension test in mice (Wang et al., 2008). Liquiritin, isolated from *Glycyrrhiza uralensis* has also an anti-depressant-like effect in chronic variable stress induced depression model rats (Zhao et al., 2008). In our study, S-tang decreased the immobility time and blood biochemical parameters related to depression and fatigue. From this, we postulated that active components of S-tang are paeoniflorin, albiflorin, liquiritin, and isoliquiritin. However, other active components of S-tang should be isolated in further studies to clarify whether the components may also be effective in depression.

The FST is one of the most commonly used behavioral tests for rodents and it has been used as a pre-clinical diagnostic tool for predicting the antidepressant properties of candidate agents (Naitoh et al., 1992; Porsolt et al., 1977). In general, the swimming exercise is known to induce the biochemical changes in blood (De-Mello, 1992). Thus, the BUN, CK, LDH, glucose, total protein, and albumin contents in the blood of the mice were examined after the FST. BUN and serum creatinine are standard metrics used to diagnose and monitor kidney injury (Ferguson et al., 2008). Urea is formed in the liver and excreted in the kidney as the end product of protein metabolism. During digestion, protein is broken down into amino acids. Amino acids contain nitrogen, which is removed as NH_4^+ (an ammonium ion), while the rest of the molecule is used to produce energy or other substances needed by the cell. Previous research showed in an increased BUN value due to exposure to a FST in mice (Shin et al., 2004). Marar et al. showed that the administration of fluoxetine to laboratory rats does not significantly affect BUN and creatinine (Marar et al., 1998). Serum CK and LDH are generally known as accurate indicators of muscle damage (Coombes and McNaughton, 2000). CK is present in both skeletal and cardiac muscle and it is released into the blood when myocyte necrosis occurs (Bessman and Carpenter, 1985; Van der Veen and Willebrands, 1966). LDH catalyzes the interconversion of pyruvate and lactate (Vasudevan et al., 1978). As is commonly known, exercise or

stress increases the serum concentrations of the intramuscular enzymes CK and LDH. Glucose plays an important role as the main source of energy during exercise or in the case of starvation (Rose and Sampson, 1982). When exercising or in the case of starvation, liver glycogen reserves and gluconeogenesis appears to maintain blood glucose levels in the body. Moreover, the glucose level generally decreases immediately after the FST. Total protein measurements can reflect the nutritional state, kidney disease and chronic liver disease and it can also indicate the condition between the extracellular and intracellular fluids (Costill and Fink, 1974). The result of this study indicates that the serum concentrations of the intramuscular enzymes such as CK and LDH, which were increased by the FST, decrease significantly with S-tang treatment. Therefore, these results suggest that S-tang regulate the blood biochemical parameters related to depression in blood.

Conclusively, it was found for the first time that S-tang decreases the immobility time after FST in mice. Moreover, this treatment tends to improve the levels of several blood biochemical parameters such as LDH and CK after FST. These results indicate that S-tang may have beneficial applicability as a potential anti-depressant agent.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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