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Anxiolytic-like Effects of Sanjoin-Tang Extracts and its Ingredients in the Elevated Plus-Maze in Mice

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Abstract – This study was carried out to evaluate the putative anxiolytic-like effects of the aqueous extracts of Sanjoin-tang (SJIT) and its ingredients using the elevated plus maze (EPM) test in mice. SJIT consists of five herbs, namely, Zizyphi Spinosi Semen (roasted), Glycyrrhizae Radix, Cnidii Rhizoma, Anemarrhenae Rhizoma, and Hoelen. The aqueous extracts of SJIT and each herbal drug were orally administered to ICR mice, 1 hr before evaluating behavioral activity in the EPM test, respectively. Repeated treatments (for 3 days) of the aqueous extract of SJIT (400 mg/kg) significantly increased time-spent in the open arms and arm entries into the open arms in the EPM test. Zizyphi Spinosi Semen (400 mg/kg), an ingredient of SJIT, significantly increased time-spent in the open arms and arm entries into the open arms ($P < 0.05$). However, the other ingredient of SJIT did not show any anxiolytic-like behaviors. In addition, the anxiolytic-like effects of Zizyphi Spinosi Semen were blocked by pindolol (10 mg/kg), a 5-HT_{1A} receptor antagonist. These results suggest that Zizyphi Spinosi Semen (roasted) as an ingredient of SJIT plays a crucial anxiolytic role, and it acts via the serotonergic nervous system.

Keywords □ anxiety, Sanjoin-tang, Elevated Plus-Maze, Zizyphi Spinosi Semen, Pindolol, Flumazenil

INTRODUCTION

From the 1960s, benzodiazepines have been the most commonly prescribed for treating anxiety. Although these compounds remain the mainstay of drug treatment in anxiety disorders, their side-effects are prominent, such as sedation, myorelaxation, ataxia, amnesia, and pharmacological dependence (Lader and Morton, 1991). Recently, research has been conducted to investigate safer and more specific, and perhaps lower cost therapies. Natural anxiolytic agents feature in such research because herbs have been used to treat psychiatric disorders and generally have fewer harmful effects (Carlini, 2003).

Sanjoin-tang (SJIT, Suanzaoren-tang in Chinese) consists of five herbs, namely, Zizyphi Spinosi Semen (roasted), Glycyrrhizae Radix, Cnidii Rhizoma, Anemarrhenae Rhizoma, and Hoelen. In the clinical practice, SJIT is widely used to alleviate weakness, irritability and insomnia (Chen *et al.*, 1986). The aqueous extract of Zizyphi Spinosi Semen exhibited sedative and hypnotic effects in mice examined by pentobarbital sodium-induced sleeping time and spontaneous motion (Li *et al.*, 2001). Chen *et al.* (1986) also reported that SJIT was effective in treating anxiety patients. However, it is not known yet which ingredients primarily contribute to the anxiolytic-like effects of SJIT or which nervous system is involved in those activities. These observations persuaded us to study the effects of SJIT and its ingredients on the anxiolytic activity.

The purpose of the present study is to evaluate the putative anxiolytic-like activity of the aqueous extracts of SJIT and its ingredients. Elevated plus-maze (EPM) and horizontal wire test were used to examine anxiolytic and myorelaxant effects.

MATERIALS AND METHODS

Materials

Diazepam, pindolol, and flumazenil were obtained from Sigma Chemical Co. (USA). Each component of SJIT was obtained from a herbalist supplier in Seoul, Korea, and voucher specimens (No. KHOPS-03-20; Zizyphi Spinosi Semen (roasted),

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No. KHOPS-03-22; Glycyrrhizae Radix, No. KHOPS-03-24; Cnidii Rhizoma, No. KHOPS-03-17; Anemarrhenae Rhizoma, No. KHOPS-03-25; Hoelen) were maintained. The material was authenticated by Prof. C.S. Yook of the Department of Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University (Seoul, Korea). The rest of materials were obtained from standard commercial sources.

Animals

Male ICR mice, weighing 25-30 g, were purchased from the Orient Co., Ltd. of Charles River branch (Seoul, Korea). Animals were housed 10 per cage under a standard 12-h light/dark cycle (lights on 07:30 19:30), in a room maintained at constant temperature (23±1°C) and humidity (60±10%) with free access to food and water. Animal treatment and maintenance were conducted in accordance with the animal care guidelines of Kyung Hee University, Korea.

Sample preparation

An extract of the SJIT and each herbal drug were prepared by boiling with 10 volume of water for 2 h. SJIT consists of Zizyphi Spinosi Semen (roasted), Glycyrrhizae Radix, Cnidii Rhizoma, Anemarrhenae Rhizoma, and Hoelen, in the ratio of 6:1:2:3:3. Then the aqueous solution obtained was filtered, concentrated on a water bath under vacuo, frozen and lyophilized (Eyela, model FDU-2000, Japan) to yield water extracts, which were then stored at -20°C until required. The yields were as follows: SJIT; 12.1%, Zizyphi Spinosi Semen (roasted); 10.0%, Glycyrrhizae Radix; 31.6%, Cnidii Rhizoma; 29.3%, Anemarrhenae Rhizoma; 55.3%, Hoelen; 2.1% .

Spontaneous behavior in the open field test

Testing was conducted in clear black Plexiglass boxes (41.5 × 41.5 × 41.5 cm) equipped with a video-base Ethovision System (Nodulus, Wageningen, The Netherlands). Mice were placed in the center of the apparatus to evaluate horizontal locomotor activity 1 h after last treatment with SJIT or each ingredient and behavioral activities were recorded for 5 min. Horizontal locomotor activity was expressed as the total ambulatory distance.

Elevated plus-maze test

The EPM consists of two open arms (30 × 7 cm) and two enclosed arms (30 × 7 cm) with 20 cm high walls, extending from the central platform (7 × 7 cm). The arms were connected with a central square (7 × 7 cm), to give the apparatus a plus sign appearance. The maze was raised to a height of 50 cm above floor level in a dimly lit room (20 Lux) and the video camera was suspended above the maze to record movements for analysis. The maze floor and walls were constructed from dark opaque polyvinyl plastic. Each mouse was placed on the center of the platform facing an enclosed arm. Animals were tested individually and only once for 5 min. The maze was cleaned following each trial to remove any residue or odors. The following activity parameters were recorded and analyzed using the video-based Ethovision System: the number of entries into open and closed arms, the time spent in each arm, and the total distance moved in the EPM.

The test materials were freshly resuspended in saline to be administered per os (po). One hour after the last treatment of SJIT or each herbal drug (400 mg/kg, po), mice were placed in the EPM. Animals were tested individually for 5 min. Mice in the control group were treated vehicle solvent only. SJIT and each herbal drug were treated for 3 days, once a day at 400 mg/kg (po). For the dose-dependent study, SJIT and Zizyphi Spinosi Semen (100, 200, 400, and 800 mg/kg, po) were treated for 3 days.

In a separate experiment, in which mice were subjected to the co-administration of Zizyphi Spinosi Semen and pindolol or flumazenil as the antagonist, mice were administered Zizyphi Spinosi Semen extract (400 mg/kg) orally and received an intraperitoneal (i.p.) injection of pindolol (10 mg/kg) or flumazenil (10 mg/kg), 1 h and 30 min prior to testing. Mice treated with diazepam (1 mg/kg, i.p.) were used as a positive control.

Horizontal wire test

Immediately after the EPM test, a horizontal wire test was carried out, according to the method described by Hui *et al.* with minor modifications (Hui *et al.*, 2002). Mice were lifted by the tail and allowed to grasp a horizontally strung wire (1 mm diameter, 40 cm long, and placed 60 cm above the table) with their forepaws, and then released. The number of mice from each treatment group that did not grasp the wire with the forepaws or actively grasped the wire with at least one hind paw within 10 sec was recorded. A myorelaxant will impair the ability of mice to grasp the wire.

Statistics

Values are expressed as mean±S.E.M. Data were analyzed by a one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test for multiple comparisons. Statistical significance was set at $P < 0.05$.

RESULTS

Effect on the locomotor activity test

To differentiate between the possible stimulatory effects of the tested drugs on the modulation of exploratory behavior, a locomotor activity test was performed. SJIT and Zizyphi Spinosi Semen (roasted) (800 mg/kg, as maximum dosage in this study) produced no significant changes in the total ambulatory distances compared with the saline control group (SJIT; 1959.1 ±124.2 cm, Zizyphi Spinosi Semen (roasted); 1927.4±75.0 cm, Control; 1837.5±91.2 cm). There was also no significant change of the spontaneous locomotor activity in the diazepam-treated group as positive control (1731.4 ± 99.7 cm).

Effect of repeated SJIT and its ingredients treatments in the elevated plus-maze

As shown in the saline-treated group, mice typically avoided spending time in the open arm and entering into open arms. Vehicle-treated mice remained for 31.28±5.74 sec (33.04± 2.30 %) in open arms, whereas SJIT-treated mice (400 mg/kg) spent significantly more time in open arms (Fig. 1; $P < 0.05$). In addition, SJIT-treated mice (400 mg/kg) showed significantly more entries into the open arms than the saline-treated mice (Figs. 1 and 2; $P < 0.05$). Among the ingredients of SJIT, only Zizyphi Spinosi Semen (400 mg/kg) significantly increased the time spent in open arms and arm entries into open arms ($P < 0.05$). However, no significant change was observed in terms of the time spent in or entries into the open arms at doses of 100, 200 and 800 mg/kg of SJIT or Zizyphi Spinosi Semen (Figs. 2 and 3). The

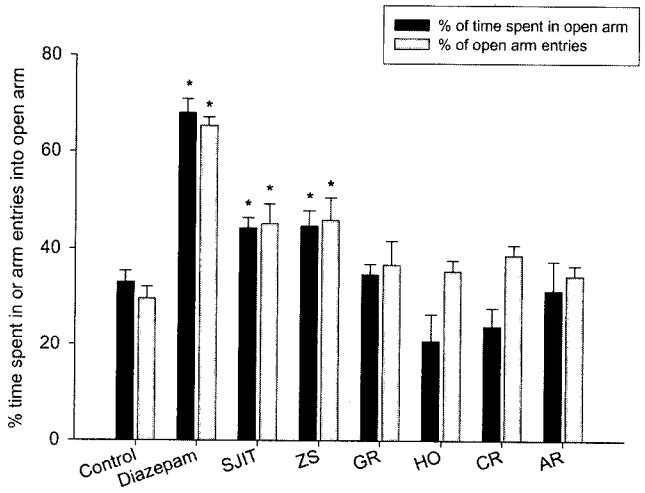

Fig. 1. Effect of the water extract of Sanjoin-tang (SJIT) and its ingredients on the percentage of the time spent in and the number of entries into the open arms of the elevated plus-maze over a 5 min test period in the mice. Each bar represents the mean±S.E.M. obtained from 10-12 mice. P values for group comparisons were obtained by one way ANOVA followed by Student-Newman-Keuls test ($^*P<0.05$ as compared with the saline treated control group). SJIT; Sanjoin-tang, ZS; Zizyphi Spinosi Semen, GR; Glycyrrhizae Radix, HO; Hoelen, CR; *Cnidii Rhizoma*, AR; Anemarrhenae Rhizoma.

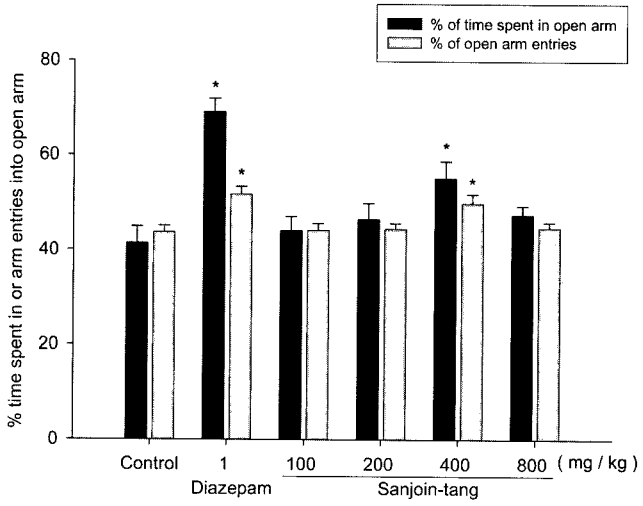

Fig. 2. Effect of the water extract of Sanjoin-tang in dose dependent manner. Each bar represents the mean±S.E.M. of percentage of the number of open arm entries and the time spent in open arm of the elevated plus-maze over 5 minutes in 10-12mice. P values for group comparisons were obtained by one way ANOVA followed by Student-Newman-Keuls test ($^*P < 0.05$ as compared with the saline treated control group).

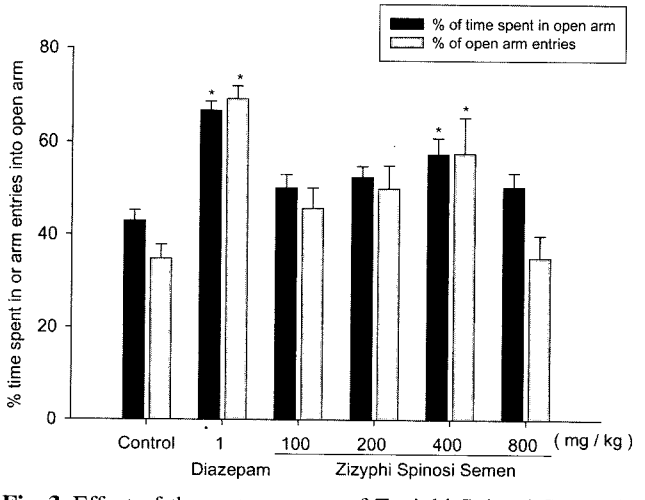

Fig. 3. Effect of the water extract of Zyziphi Spinosi Semen in dose dependent manner. Each bar represents the mean±S.E.M. of percentage of the number of open arm entries and the time spent in open arm of the elevated plus-maze over 5 minutes in 10-12 mice. P values for group comparisons were obtained by one way ANOVA followed by Student-Newman-Keuls test ($^*P < 0.05$ as compared with the saline treated control group).

diazepam-treated (1 mg/kg) group spent more time in open arms than the saline-treated group ($P < 0.05$).

Effect of pindolol and flumazenil antagonism on the anxiolytic-like activity of Zizyphi Spinosi Semen

To investigate whether the anxiolytic effect of Zizyphi Spinosi Semen is exerted via the serotonergic or GABAergic nervous system, Zizyphi Spinosi Semen (400 mg/kg) treated mice were subjected to the co-treated with pindolol, a 5-HT_{1A} receptor antagonist, or flumazenil, a benzodiazepine antagonist. As shown in Fig. 4, the anxiolytic-like effects of Zizyphi Spinosi Semen were antagonized by pindolol (10 mg/kg). However, those effects were not antagonized by flumazenil (10 mg/kg).

Horizontal wire test

By the treatment of SJIT and Zizyphi Spinosi Semen (800 mg/kg) for 3 days, the percentage of mice grasping the wire did not show any significant effects compared with the saline control group (Fig. 5).

DISCUSSION

The main findings of this study were that treatments with the crude extract of SJIT and Zizyphi Spinosi Semen, one of its ingredients, significantly increased the time spent in the open arms and the frequency of open arm entries in the EPM test. Moreover, these effects were antagonized by pindolol, demonstrating the involvement of the 5-HT nervous system. Therefore, we suggest that the anxiolytic-like activity of SJIT is due to Zizyphi Spinosi Semen, and that the anxiolytic action of Zizyphi Spinosi Semen is due to 5-HT_{1A} receptor activation.

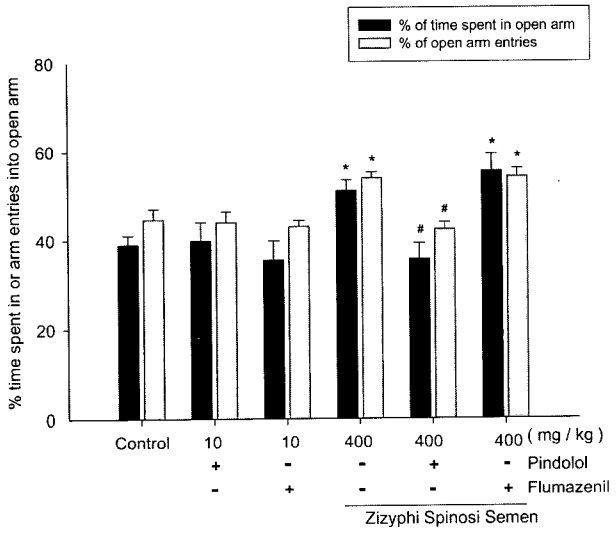

Fig. 4. Anxiolytic-like effects of Zyziphi Spinosi Semen were blocked by pindolol not by flumazenil. Data are expressed as the means ± S.E.M. of percentage of the number of open arm entries or the time spent in open arms in mice given 5-min test. 30 min after the oral administration of water extract of Zyziphi Spinosi Semen (400 mg/kg), pindolol (10 mg/kg) or flumazenil (10 mg/kg) was administered intraperitoneally (i.p.) injection; N =10-12 mice per group. *P* values for group comparisons were obtained by one way ANOVA followed by Student Newman-Keuls test ($^{*}P<0.05$ versus the saline treated controls, $^{\#}P<0.05$ as compared with the Zizyphi Spinosi Semen treated group).

SJIT is widely prescribed by traditional Chinese medicine practitioners for alleviating the weakness, irritability and insomnia (Chen *et al.*, 1986). Hsieh and Chen reported that SJIT might be a promising anxiolytic remedy, without apparent adverse effects on the cardiovascular system, and appearing not to interact with other drugs (Hsieh and Chen, 1986). The aqueous extract of Zizyphi Spinosi Semen showed sedative and hypnotic effect in mice determined by pentobarbital sodium-induced sleeping time and spontaneous motion (Li *et al.*, 2001). Chen *et al.* also reported that SJIT was effective to the anxiety patients (Chen *et al.*, 1985). However, to our knowledge no one study has investigated the anxiolytic mechanism of SJIT and determined which ingredient primarily contributes to the anxiolytic effect of SJIT.

The EPM is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli, such as a fear of a new, brightly-lit open space and the fear of balancing on a relatively narrow raised (Dawson and Tricklebank, 1995). An anxiolytic agent increases the frequency of entries into open arms and increased the time spent in open arms of the EPM. In the present study, repeated administrations of SJIT prolonged the time spent in the open arms and the number of entries into open arms (Figs. 1 and 2). Based on the results of our behavioral experiments, SJIT did not alter spontaneous behavior and exerted no significant myorelaxant effect at the chosen dosage regimen (Fig. 5). Total distances of movement on the EPM were unchanged by SJIT treatment versus the saline controls (data not shown). These observations indicate that the anxiolytic effect of SJIT is selective, and not simply the result of either a general stimulation of locomotor activity or of exploratory behavior consequent to exposure to a novel environment. Hsieh *et al.* reported that SJIT might decrease the serotonergic activity but have no significant effect on GABAergic activity (Hsieh *et al.*, 1986). However, we also observed that the anxiolytic-like behaviors were abolished by the 5-HT_{1A} receptor antagonist not by flumazenil, a $GABA_A$ receptor antagonist (data not shown). The discrepancy between two studies may be resulted from the characteristics of 5-HT_{1A} receptors. There are

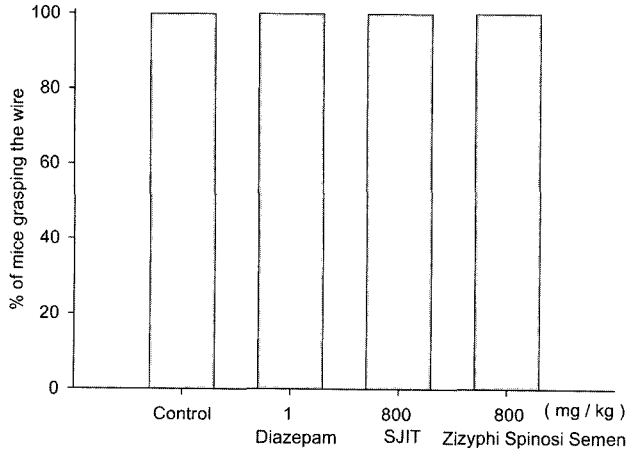

Fig. 5. Performance of mice in the horizontal wire test was carried out immediately after the elevated plus-maze test in the repeated treatment of water extract of Sanjoin-tang (SJIT) and Zyziphi Spinosi Semen (800 mg/kg, as maximum dosage in this study). A single diazepam (1 mg/kg, i.p.) treatment was used as a positive control. Each bar represents the mean± S.E.M. of 10-12 mice.

at least two kinds of 5-HT_{1A} receptors (Blier 1993). One is a somatodendritic autoreceptor that, when activated by systemic stimulation, is believed to exert anxiolytic-like effects and reduces 5-HT release both in the cell body and in terminal regions of serotonergic neurons. The other 5-HT_{1A} receptor is localized postsynaptically to serotonergic neurons in the hippocampus, septum, amygdala, and cortex, where it increases signal transfer leading to inhibitory effects on firing activity. Chronic treatment of 5-HT_{1A} receptor agonist affects presynaptic and postsynaptic 5-HT_{1A} receptors in a different manner: the somatodendritic 5-HT_{1A} autoreceptors are desensitized, whereas postsynaptic 5-HT_{1A} receptors in the hippocampus remain normosensitive (Blier and Montigny, 1987). Therefore, it is likely that the decrease of the serotonergic activity in the terminal regions, such as hippocampus, cortex, and amygdala results from the reduction of serotonin release.

Of the SJIT ingredients, Zizyphi Spinosi Semen was found to have anxiolytic-like activity ($P < 0.05$). In the case of another ingredients, Glycyrrhizae Radix and Cnidii Rhizoma, a slight increase of the time spent in the open arms was observed but was not significant. However, the other ingredient, Anemarrhenae Rhizoma and Hoelen, had no observable effect. Recently, it was reported that Glycyrrhizae Radix showed the anxiolytic activity and that glabridine and its related compounds purified from the *Glycyrrhiza glabra* showed anti-depressive activity (Ofir *et al.*, 2003). However, it is likely that those compounds did not dissolved in water decoction of Glycyrrhizae Radix, because no significant anxiolytic effects were observed in the EPM study. However, further studies are needed to evaluate the anxiolytic activities of Glycyrrhizae Radix varying the decoction solvent.

Zizyphi Spinosi Semen is one of the important medicinal herbs, and is widely used for the insomnia (Namba, 1993). Until now, it is not known which constituent of Zizyphi Spinosi Semen exerts this anxiolytic effect. The plant has been shown to contain a large number of triterpenes and other constituents, such as, flavonoids and cyclopeptide alkaloids (Su *et al.*, 2002; Tripathi *et al.*, 2001). Of these constituents, spinosin, a flavone derivatives, is known to play a role on the effect of sedation and hypnosis (Kawashima *et al.*, 1997; Yuan *et al.*, 1987). Previously, various synthetic flavone derivatives have anxiolytic-like activities in the EPM, which was attributed to benzodiazepine receptor activation (Griebel *et al.*, 1999). Liao *et al.* also reported that baicalein and baicalin isolated from the *Scutellaria baicalensis* activated the benzodiazepine binding site of the $GABA_A$ receptors (Liao *et al.*, 2003). Thus, if the active principle in Zizyphi Spinosi Semen is a flavone derivative, the anxiolytic-like effects of Zizyphi Spinosi Semen may be mediated by the activation of benzodiazepine binding sites. In our study, however, the anxiolytic effect of Zizyphi Spinosi Semen (400 mg/kg) was not antagonized by flumazenil. Instead, the Zizyphi Spinosi Semen effect was significantly reversed by pindolol (Fig. 3). Therefore, we think that the anxiolytic-like effect of Zizyphi Spinosi Semen is mediated primarily by the 5-HT_{1A} receptor. Although speculative, the main contributory compounds might not be flavones, but more likely to be triterpenes or other species in the EPM study. However, we can not exclude other compounds as contributors to the anxiolytic-like effects of Zizyphi Spinosi Semen and can not be concluded that the serotonergic nervous system is the only system involved in the anxiolytic-like effects of Zizyphi Spinosi Semen because receptors or binding sites predominantly acted by Zizyphi Spinosi Semen extract were also 5-HT_{1A}, 5-HT_2, and GABA (Liao *et al.*, 1995). We are currently investigating the major component(s) of Zizyphi Spinosi Semen involved in the anxiolytic-like effect and the exact receptor systems associated with the effects.

In summary, the present study demonstrates that SJIT has an anxiolytic-like effect. Zizyphi Spinosi Semen appeared to the main ingredient of SJIT responsible for its anxiolytic property mediated by the serotonergic nervous system. Further studies are required to elucidate the full mechanism of action and a major compound of SJIT and Zizyphi Spinosi Semen.

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REFERENCES

Blier, P., Lista, A., de Montigny, C. (1993). Differential properties of pre- and postsynaptic 5-hydroxytryptamine1A receptors in the dorsal raphe and hippocampus: II. Effect of pertussis and cholera toxins. *J. Pharmacol. Exp. Ther.*, **265**, 16-23.

Blier, P., de Montigny, C. (1987). Modification of 5-HT neuron properties by sustained administration of the 5-HT1A agonist gepirone: electrophysiological studies in the rat brain. *Synapse* **1**, 470-480.

Carlini, E.A. (2003). Plants and the central nervous system. *Pharmacol. Biochem. Behav.*, **75**, 501-512.

Chen, H.C., Hsieh, M.T., Lai, E. (1985). Studies on the suanzaorentang in the treatment of anxiety. *Psychopharmachology* **85**, 486-487.

Chen, H.C., Hsieh, M.T., Shibuya, T.K. (1986). Suanzaorentang versus diazepam: a controlled double-blind study in anxiety. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **24,** 646-650.

Dawson G.R., Tricklebank M.D. (1995). Use of the elevated plus maze in the search for novel anxiolytic agents. *Trends Pharmacol. Sci.*, **16**, 33-36.

Griebel, G., Perrault, G., Tan, S., Schoemaker, H., Sanger, D.J. (1999). Pharmacological studies on synthetic flavonoids: comparison with diazepam. *Neuropharmacology* **38**, 965-977.

Hsieh, M.T., Chen, H.C. (1986). Suanzaorentang in cardiac patients with anxiety. *Eur. J. Clin. Pharmacol.*, **30**, 481-484.

Hsieh, M.T., Chen, H.C., Kao, H.C., Shibuya, T. (1986). Suanzaorentang, and anxiolytic Chinese medicine, affects the central adrenergic and serotonergic systems in rats. *Proc. Natl. Sci. Counc. Repub. China B* **10,** 263-268.

Hui, K.M., Huen, M.S., Wang, H.Y., Zheng, H., Sigel, E., Baur, R., Ren, H., Li, Z.W., Wong, J.T., Xue, H. (2002). Anxiolytic effect of wogonin, a benzodiazepine receptor ligand isolated from Scutellaria baicalensis Georgi. *Biochem. Pharmacol.*, **64**, 1415-1424.

Kawashima, K., Saito, K., Yamada, A., Obara, S., Ozaki, T., Kano, Y. (1997). Pharmacological properties of traditional medicines. XXIII. Searching for active compounds in the blood and bile of rats after oral administrations of extracts of sansohnin. *Biol. Pharm. Bull.*, **20**, 1171-1174.

Lader, M., Morton, S. (1991). Benzodiazepine problems. *Br. J. Addict.*, **86,** 823-828.

Li, Y., Wang, R., Chen, M., Wang, Z., Bi, K. (2001). Pharmacodynamics study of suanzaoren decoction extracted by different technological process. *Zhong Yao Cai* **24**, 884-885.

Liao J.F., Hung W.Y., Chen C.F. (2003). Anxiolytic-like effects of baicalein and baicalin in the Vogel conflict test in mice. *Eur. J. Pharmacol.*, **464**, 141-146.

Liao, J.F., Jan, Y.M., Huang, S.Y., Wang, H.H., Yu, L.L., Chen, C.F. (1995). Evaluation with receptor binding assay on the water extracts of ten CNS-active Chinese herbal drugs. *Proc. Natl. Sci. Counc. Repub. China B* **19**, 151-158.

Namba, T., (1993). *The encyclopedia of Wakan-Yaku with Color Picture.* **1**. Osaka, Japan: Hoikusha Co., 142-144,.

Ofir, R., Tamir, S., Khatib, S., Vaya, J. (2003). Inhibition of serotonin re-uptake by licorice constituents. *J. Mol. Neurosci.*, **20**, 135-40.

Su, B.N., Cuendet, M., Farnsworth, N.R., Fong, H.H., Pezzuto, J.M., Kinghorn, A.D. (2002). Activity-guided fractionation of the seeds of Ziziphus jujuba using a cyclooxygenase-2 inhibitory assay. *Planta Med.*, **68,** 1125-1128.

Tripathi, M., Pandey, M.B., Jha, R.N., Pandey, V.B., Tripathi, P.N., Singh, J.P. (2001). Cyclopeptide alkaloids from Zizyphus jujuba. *Fitoterapia* **72,** 507-510.

Yuan, C.L., Wang, Z.B., Jiao. Y., Cao, A.M., Huo, Y.L., Cui, C.X. (1987). Sedative and hypnotic constituents of flavonoids in the seeds of Ziziphus spinosae. *Zhong Yao Tong Bao* **12,** 34-36, 62-63.