J. Trad. Med. **26**, 11－17, 2009

Medical and
Pharmaceutical Society
for WAKAN-YAKU

An anxiolytic-like effect of kososan is different from the effect of hangekobokuto on two anxiety models in mice

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Abstract

Benzodiazepines (BDZs) and selective serotonin reuptake inhibitors (SSRIs) are used mainly to treat anxiety disorders, but the side effects of these agents often limit their use in the management of anxiety disorders. We aimed to show that treatment of anxiety disorders using Kampo medicine was useful. Although hangekobokuto and kososan are primarily used to treat anxiety-related disorders, little experimental work has been done on its anxiolytic effects. We carried out this study to reveal the difference in anxiolytic-like effects between hangekobokuto and kososan using two anxiety models in mice: marble-burying test and the elevated plus maze. Oral administration of hangekobokuto (1.0 g/kg/body weight, 7 days) significantly inhibited marble-burying behavior; increased the number of entries into the open arms; and the total time spent in the open arm. Oral administration of kososan (1.0 g/kg/body weight, 7 days) significantly inhibited marble-burying behavior, but did not increase the number of entries into the open arms and the total time spent in the open arm. Hangekobokuto and kososan show anxiolytic-like effects in two behavioral anxiety models in mice, and that the mode of action of kososan is different from that of hangekobokuto.

Key words Kampo medicine, hangekobokuto, kososan, anxiolytic effect, marble-burying behavior, elevated plus maze.

Introduction

Anxiety is one of the most frequent symptoms encountered in various diseases. According to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition text revision, anxiety disorders (i.e., mental disorders that can be characterized by anxiety) include panic disorder, agoraphobia, social phobia, obsessive-compulsive disorder or generalized anxiety disorder.[1] Benzodiazepines (BDZs) and selective serotonin reuptake inhibitors (SSRIs) are used mainly to treat anxiety disorders. SSRIs are clinically effective in the treatment of all anxiety disorders, whereas BDZs are limited to generalized anxiety disorder or acute panic attacks.[2] The side effects of SSRIs (e.g., insomnia, agitation, gastrointestinal distress, sexual dysfunction[2]) often limit their use in the management of the anxiety disorders. Using Kampo (Japanese herbal) medicine for the treatment of anxiety disorders may be useful to circumvent this issue.[3]

Several Kampo medicines have been used in the treatment of anxiety.[4,5] Empirically, Kampo medicine has a higher therapeutic effect and fewer side effects in some patients. Hangekobokuto and kososan are primarily used to treat anxiety-related disorders, but the clinical indication for treatment using these Kampo medicines is different. Hanawa *et al.* reported that

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hangekobokuto is used for confirmed peripheral symptoms, whereas kososan is used for poorly localized symptoms.[6] Kuribara and Maruyama reported that hangekobokuto shows anxiolytic effects in an animal model,[7] but there has been no experimental study on the anxiolytic effect of kososan, regardless of clinical efficacy.

In the present study, we used two animal models of anxiety, marble-burying behavior and the elevated plus maze, to evaluate the anxiolytic effect of kososan and hangekobokuto.

Marble-burying behavior is recognized as an animal model for obsessive-compulsive disorder.[8] Harmless objects such as marbles may reflect the formation of compulsive behavior.[9] It has been reported that marble-burying behavior is inhibited by BDZs and SSRIs.[10] The elevated plus maze uses the natural fear of rodents to avoid open and elevated places.[10] Treatment with BDZs significantly increases open-arm exploration;[11] results with SSRIs in the elevated plus maze are inconsistent.[10]

Firstly, we verified the effect of kososan on anxiety-like behaviors, and then examined the difference in the anxiolytic effect between hangekobokuto and kososan using two animal models of anxiety.

Materials and Methods

Animals: Animal experiments were carried out according to the Guidelines for Care and Use of Laboratory Animals at the Kitasato Institute and Kitasato University.

Naive 6-week-old male ICR mice (Japan SLC, Shizuoka, Japan) were employed in each experiment. Mice were housed in groups of six per cage at least seven days before behavioral tests. The room was maintained at constant temperature (23 ± 2℃) and humidity (55% ± 10%) with a regular 12-h light/dark cycle (light on from 08:00 to 20:00). Water and food were available ad libitum.

Drugs: Depromel® (fluvoxamine maleate; Meiji Seika Kaisha, Limited, Tokyo, Japan) and Cellkam® (diazepam; Tsuruhara Pharmaceutical Company Limited, Osaka, Japan) were used.

Hangekobokuto is composed of Pinelliae Tuber (6.0 g), Hoelen (5.0 g), Magnoliae Cortex (3.0 g), Perilla Herba (2.0 g), and Zingiberis Rhizoma (0.5 g). Kososan is composed of Cyperi Rhizoma (4.0 g), Aurantii Nobilis Pericarpium (3.0 g), Perilla Herba (2.0 g), Glycyrrhizae Radix (2.0 g), and Zihgiberis Rhizoma (0.5 g). The Kampo formula was decocted with 600 ml of distilled water until the volume was reduced by half. The extract was filtered immediately through paper *in vacuo*. The filtrate was lyophilized, and the yield of hangekobokuto and kososan extracts were approximately 22% and 28%, respectively, from the herbal mixture based on dry weight.

Drugs were suspended in distilled water. Diazepam (10 mg/kg/body weight), fluvoxamine (60 mg/kg/body weight), hangekobokuto (0.5 and 1.0 g /kg/body weight) and kososan (0.5 and 1.0 g/kg/body weight) were administered (p.o.) by intragastric gavage at 0.5 ml/mouse once daily for seven consecutive days.

Behavioral testing: Behavioral testing was carried out between 13:00 and 19:00 in a behavioral testing space under the same environmental conditions as the animal rooms. Behavioral experiments were conducted 24 h after the last administration of drugs.

Open field test: Spontaneous locomotor activities of mice were measured in the open field test. Just before assessment of marble-burying behavior, mice were placed individually in an opaque plastic box (40 × 40 × 40 cm) and allowed to move freely. The total distance of movement for a duration of 5 min was measured automatically using a video tracking system (EthoVision; Noldus, Netherlands).

Marble-burying test: The marble-burying test was conducted as previously described.[9] A clear cubic plastic box (30 × 30 × 30 cm) was used, and 25 glass marbles (20 mm in diameter) were evenly spaced on 5 cm deep wood bedding (Japan Laboratory Animals Incorporated, Tokyo, Japan). Immediately after assessment of spontaneous locomotor activities, individual mice were placed in the box for 30 min and the number of marbles buried at least two-thirds deep counted.

The elevated plus maze: The elevated plus maze is described elsewhere.[12] Briefly, the apparatus was

comprised of two open arms (30 × 6 cm) and two closed arms (30 × 6 × 10 cm) that extended from a common central platform (6 × 6 cm). The floor and walls of each arm were made from black plastic. The entire maze was elevated to a height of 40 cm above floor level. Mice were placed individually in the center of the maze, facing an open arm. They were allowed free access to the arms for 5 min. The total distance and duration of movement on the maze was measured automatically by using a video tracking system (Etho Vision).

Statistical analysis: Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's test with the exception of the marble-burying test. The Steel multiple range test was applied to the marble-burying test. Differences were considered significant at $p<0.05$.

Results

Effects of diazepam, fluvoxamine, hangekobokuto and kososan on locomotor activity in the open field: The total distance of movement on the open field was not affected by administration of any drug as compared with the water-administered control (Fig. 1).

Effects of diazepam, fluvoxamine, hangekobokuto and kososan on marble-burying behavior: Marble-burying behavior was significantly suppressed by administration of diazepam and fluvoxamine ($p<0.01$) (Fig. 2). Hangekobokuto significantly suppressed marble-burying behavior at 0.5 g/kg and 1.0 g/kg ($p<0.05$ and $p<0.01$, respectively). Although administration of 1.0 g/kg kososan significantly suppressed marble-burying behavior ($p<0.01$), administration of 0.5 g/kg kososan did not suppress such behavior ($p=0.5$).

Effects of diazepam, fluvoxamine, hangekobokuto and kososan on the elevated plus maze: The total distance of movement on the elevated plus maze was not affected by administration of any drug as compared with the water-administered control (Fig. 3). Administration of diazepam exhibited a significant increase in the number of entries into the open arms ($p<0.01$) as well as a significant increase in the time spent in the open arms ($p<0.01$). Although administration of 1.0 g/kg hangekobokuto significantly increased the number of entries into the open arms ($p<0.01$) and the time spent in the open arms ($p<0.01$), administration of 0.5 g/kg hangekobokuto increased the number of entries only into the open arms ($p<0.05$). Fluvoxamine and kososan had no effect on the number of entries into the open arms and the time spent in the open arms. The effect of drugs on the number of

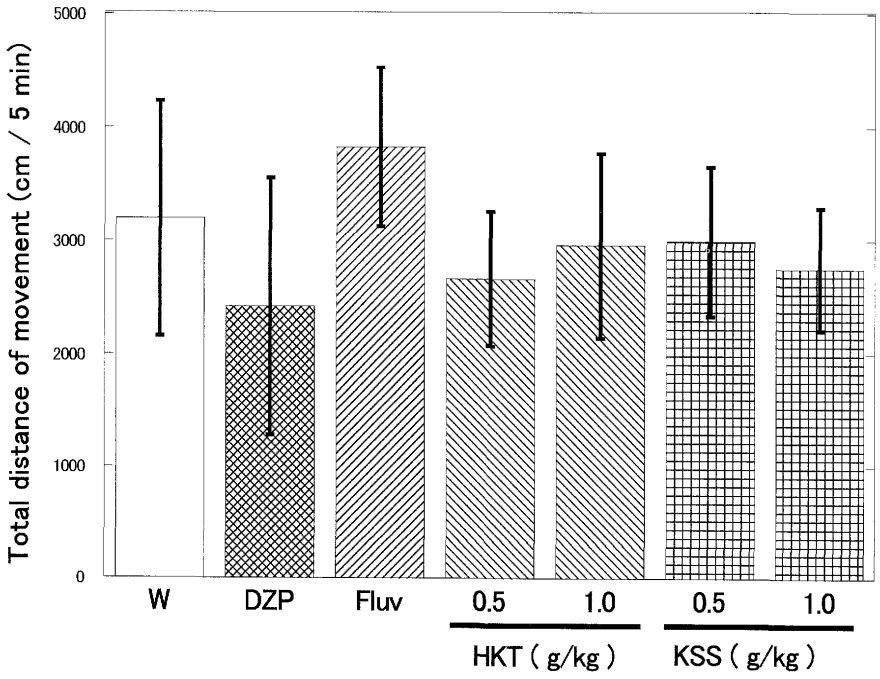

Fig. 1 Effects of diazepam, fluvoxamine, hangekobokuto and kososan on locomotor activity in the open field. The total distance of movement was measured during 5 min. Each column represents the mean ± S.D. (n=10). W, Water ; DZP, Diazepam; Fluv, Fluvoxamine; HKT, Hangekobokuto; KSS, Kososan.

entries into the open arms is shown in Fig. 4, and the time spent in the open arms is shown in Fig. 5.

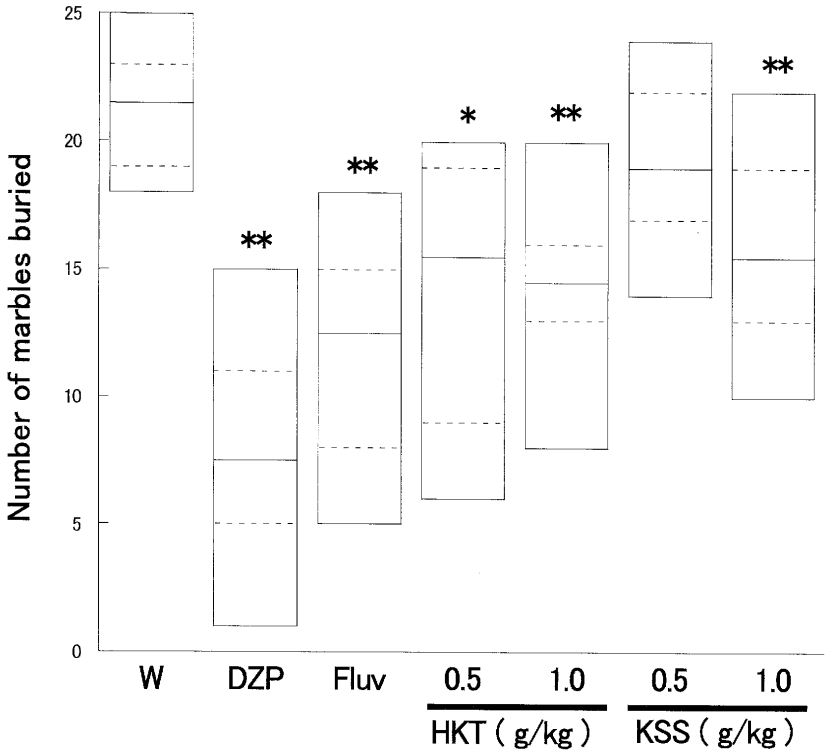

Fig. 2 Effects of diazepam, fluvoxamine, hangekobokuto and kososan on the marble-burying behavior. A column indicates the range of the number of marbles buried after 30 min in burying test (n=10). The bold horizontal line in the column indicates the median value of each group. The dotted line in the column indicates the quartile values. Asterisks indicate a significant difference (**p<0.01, *p<0.05) from water-administered control group with Steel multiple range test. W, Water ; DZP, Diazepam; Fluv, Fluvoxamine; HKT, Hangekobokuto; KSS, Kososan.

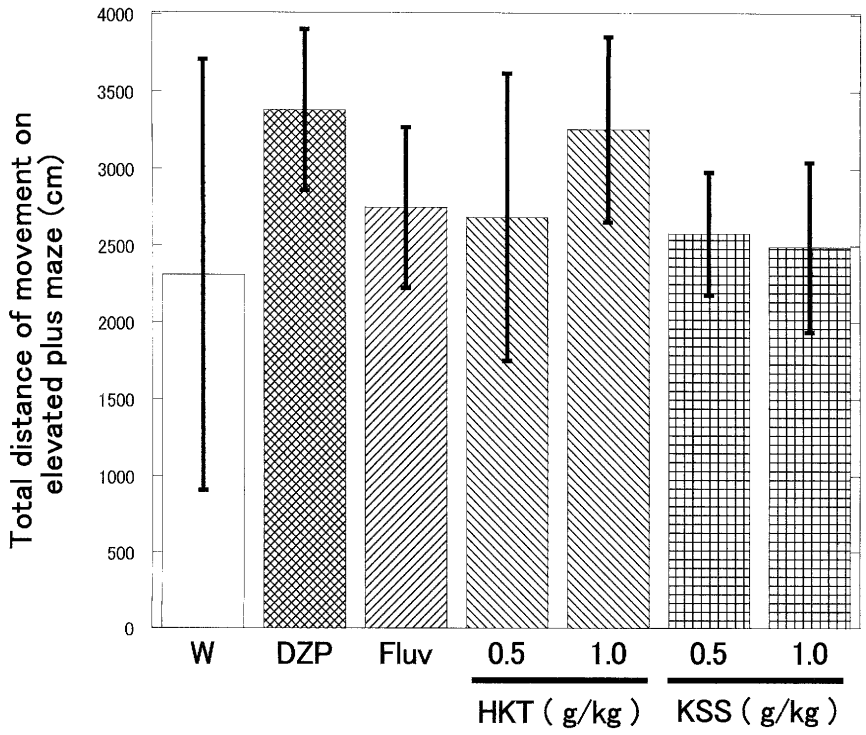

Fig. 3 Effects of diazepam, fluvoxamine, hangekobokuto and kososan on locomotor activity in the elevated plus maze. The total distance of movement was measured during 5 min. Each column represents the mean ± S.D. (n=6). W, Water; DZP, Diazepam; Fluv, Fluvoxamine; HKT, Hangekobokuto; KSS, Kososan.

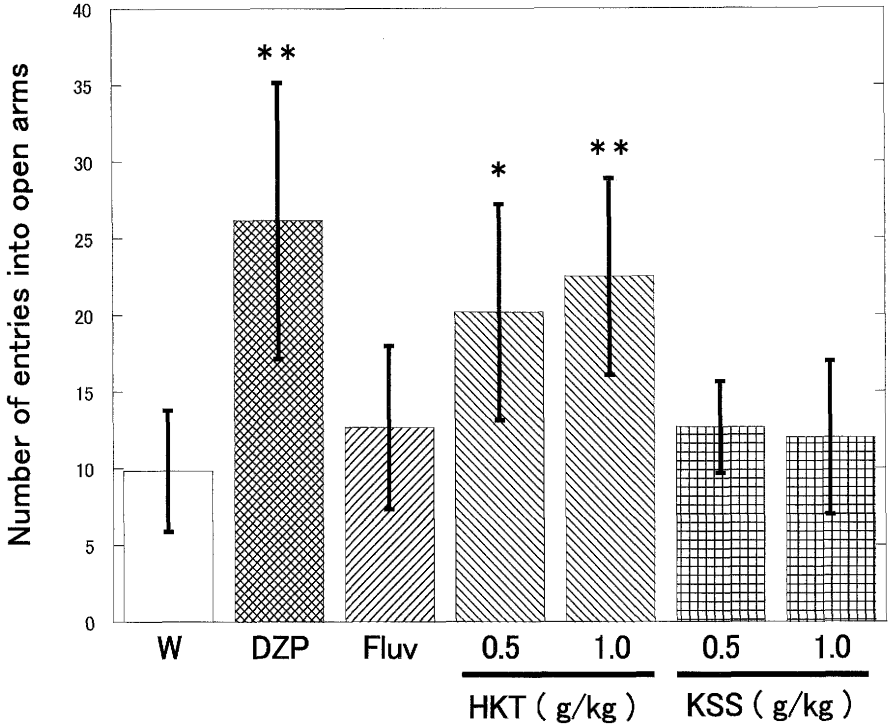

Fig. 4 Effects of diazepam, fluvoxamine, hangekobokuto and kososan on the number of entries into the open arms. The number of entries into the open arms was measured during 5 min. Each column represents the mean ± S.D. (n=6). $^{**}p<0.01$ and $^{*}p<0.05$ v.s. water-administered control group with Dunnett's test. W, Water ; DZP, Diazepam; Fluv, Fluvoxamine; HKT, Hangekobokuto; KSS, Kososan.

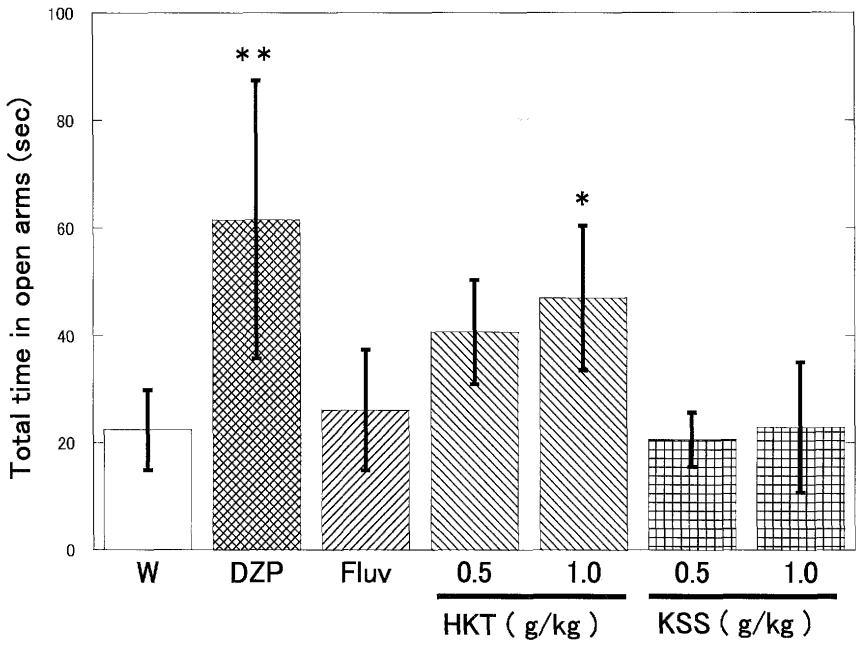

Fig. 5 Effects of diazepam, fluvoxamine, hangekobokuto and kososan on the time spent in open arms. The number of entries into the open arms was measured during 5 min. Each column represents the mean ± S.D. (n=6). $^{**}p<0.01$ and $^{*}p<0.05$ v.s. water-administered control group with Dunnett's test. W, Water ; DZP, Diazepam; Fluv, Fluvoxamine; HKT, Hangekobokuto; KSS, Kososan.

Discussion

Oral administration of hangekobokuto and kososan for seven days produced an anxiolytic-like effect. The marble-burying test and the elevated plus maze test revealed, in part, the difference in anxiolytic-like effect between hangekobokuto and kososan.

In the marble-burying test, fluvoxamine and diazepam significantly suppressed marble-burying behavior without changing spontaneous locomotor activities. These data were consistent with another study.[13] Although hangekobokuto (0.5 g/kg and 1.0 g/kg) and kososan (1.0 g/kg) suppressed marble-burying behavior, kososan (0.5 g/kg) did not suppress such behavior. Imanishi *et al.* reported that the anxiolytic-like effect of diazepam was weakened by administration for 14 days.[14] Our study showed that administration of diazepam for seven days suppressed marble-burying behavior.

The disparity in results may be due to the duration of administration of diazepam.

In the elevated plus maze, diazepam significantly increased the number of entries into the open arms and the total time spent in the open arm. This result is in good agreement with that of Pellow *et al.*[11] The result that fluvoxamine in the elevated plus maze had no effect was in agreement with another study.[15] In the present study, hangekobokuto significantly increased the time spent in the open arm in a dose-dependent manner. The result from the present study suggests that hangekobokuto modulates gamma-aminobutyric acid (GABA) as well as diazepam. Mantani *et al.* reported that kamishoyosan and hangekobokuto might be useful as additional or alternative treatments for panic disorder.[16] Mizowaki *et al.* suggested kamishoyosan showed the anxiolytic effect through the neurosteroid synthesis followed by GABA (A) / BDZ receptor stimulations.[17] Hangekobokuto may have similar mechanisms. Our data showed a similar result to that of Kuribara and Maruyama,[7]who showed that hangekobokuto (2.0 g/kg) significantly increased the time spent in the open arm. This discrepancy of effective dosage may be due to the phylogenetic difference between ICR mice and ddY mice. Kososan did not induce effects in the elevated plus maze. Considering this result, it may be presumed that the mechanism of action of kososan is analogous to SSRIs. Ito *et al.* indicated that administration of kososan or milnacipran hydrochloride, a serotonin and noradrenaline reuptake inhibitor (SNRI), increased protein expression of the glucocorticoid receptor in the hypothalamus of depression-like model mice.[18] Their data support this hypothesis. The discrepancy between marble-burying behavior and the elevated plus maze is probably due to differences in the type of anxiety measured by the different tests.[19] Although Kaneko *et al.* suggested that hangekobokuto mediated reduction of serotonin turnover might be related to a clinical efficacy,[20] no link between hangekobokuto and serotonin could be demonstrated in our study. Other anxiety models, such as Geller-Seifter conflict test, hole-board test, and social interaction test, or depression model may establish causal connections between hangekobokuto and serotonin.

Differences between hangekobokuto and kososan have been reported in clinical studies. Wakasugi *et al.* indicated that hangekobokuto administration inhibited sympathetic nerve activity in patients belonging to the sympathetic nerve domination type, whereas kososan administration stimulated sympathetic nerve activity in patients belonging to the non-sympathetic nerve domination type on pupillary dynamics.[21] Perillae herba is an important component herb of hangekobokuto and kososan. Rosmarinic acid, one of the major polyphenolic ingredients of Perillae herba, produces an antidepressant-like effect in animal models.[22,23] The difference between hangekobokuto and kososan on pupillary dynamics and anxiolytic-like effects cannot be explained by the action of rosmarinic acid. Another Kampo medicine such as kamishoyosan showed the anxiolytic-like effect,[17] hence, the anxiolytic-like effect is not fully explained in terms of the effect of a herb. Further research on Kampo medicines as formulations is therefore needed. Anxiety assessment is affected by experimental conditions, including times of administration, and time after administration. It is reasonable to suppose that hangekobokuto and kososan would lose their effects by the 14th day of administration.

In conclusion, the present study is the first report that kososan induces an anxiolytic-like effect in marble-burying behavior in mice. The difference in anxiolytic-like effect between hangekobokuto and kososan was revealed. Through this experiment, we considered that hangekobokuto modulate GABA as well as BDZs, whereas the action of kososan is, in a sense, similar to SSRIs. With regard to experimental conditions, further studies are needed to clarify the difference in effect between hangekobokuto and kososan. The findings of our study provide further insight about the anxiolytic-like effect of Kampo medicine.

Acknowledgments

This research was supported by a grant from the Uehara Memorial Foundation.

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