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Traditional Herbal Medicine Yukmijihwang-won Alleviates Reserpine-Induced Pain and Depression-Like Behavior in Mice

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Objectives: Yukmijihwang-won (Liuweidihuang-wan in Chinese) is a frequently used medicinal herbal formula. It is used as Yin tonic in Korea and China to recover patients from Yin deficiency. However, the scientific evidence on this drug has not revealed the beneficial effect or mechanism of its effects on the neurological disorder. We designed this study to examine the antidepressive and analgesic effects of Yukmijihwang-won (YJ-01) and the minor modification of YJ-01, YJ-06 on the reserpine-induced pain-depression dyad mice model.
Methods: Reserpine (1 mg/kg) was administered subcutaneously once a day for three consecutive days to induce pain and depression-like behavior. The oral administration of YJ-01 and YJ-06 (100, 200, or 300 mg/kg) was performed once daily from three days after the reserpine injection.
Results: Repeated administration of the YJs significantly reduced the immobility time in a forced swimming test and increased the moved distance and number of crossings in the open field test. In the von-Frey filament test, the oral administration of YJs remarkably suppressed the increase in paw withdrawal frequency.
Conclusions: The results of this study suggest that YJ-01 and 06 may be good candidates to treat the pain-depression dyad.

Key Words: Yukmijihwang-won, Liuweidihuang-wan, Pain, Depression, Reserpine, Mice.

I. INTRODUCTION

Chronic pain and depression are one of the most frequent reasons for people to seek medical assistance and these patients are much more emotionally suffered, poor quality of life and, even life-threatened[1,2]. Not only that, frequent combinations of pain and depression, proven in some epidemiological studies, are more problematic. Various types of pain act as a factor for depression and about 35% of chronic pain sufferers suffer from depression[3,4]. In addition, depression is also accompanied by progressive pain in 43.4% of patients and causes one or more painful physical symptoms. These relationships between chronic pain and depression called depression pain syndrome or pain-depression dyad[5].

Unfortunately, preclinical and clinical studies of the pain-depression dyad are still insufficient. Hence, the animal models of pain-depression should include definitely pain and depressive symptoms. Reserpine (RSP) is well known to produce an irreversible depletion of monoamines such as serotonin, dopamine, and norepinephrine in the brain by inhibiting the vesicular monoamine transporter[6]. Previous studies demonstrated that repetitive administration of reserpine induces mechanical allodynia and depression-like behaviors[7,8]. These researches suggest that the reserpine-treated animal model may be suitable for the study of pain-depression dyad symptoms.

Traditional Chinese medicine (TCM) has been used therapeutics for various diseases in Asia, as they have shown the advantages of multi-components and multi-targets[9]. Yukmijihwang-won (YJ; Liuweidihuang-wan in Chinese, Rokumigan in Japanese) is a classic herbal formula that has been used as a cure for diseases including renal disorder, cognitive vitality, and diabetes mellitus in China[10]. YJ was created to treat the "Yin deficiency pattern" in TCM[11]. The YJ formula includes following herbs: Rehmanniae Radix, Preparata, Corni Fructus, Dioscoreae Rhizoma, Moutan Radicis Cortex, Poria Sclerotium, Alismatis Rhizoma. YJ and its ingredients have been investigated in modern research to have a wide range of pharmacological activities, such as improvement of learning and memory function, enhanced rheological properties of blood, osteoporosis and, etc[12-15]. Moreover, YJ protected the animals from depressive-like behaviors in the MSG+PH-induced liver regeneration neonatal rat model[16]. In this study, YJ was minor modified into two types, YJ-01 and YJ-06, to evaluate their effects on pain-depression dyad. While YJ-01 follows its original formula, YJ-06 removes 'Bo (activate and restore a decreased function to normal)' herbs and maintains 'Sa (expel pathogenic factors)' herbs, and add Dan-sam (Dan-shen in Chinese).

The YJ formula and its ingredients have various effects that have been proven via various researches, but scientific studies related to pain and depression are still insufficient. Therefore, this study was designed to evaluate the effects of YJ-01 and minor modified YJ-06 on symptomatic behaviors in reserpine-induced pain-depression dyad animal models.

II. MATERIALS AND METHODS

1. Animals

Adult male ICR mice (DBL, Eumseong, South Korea) weighing 20~25 g were used in this study. All animal experimentation adheres to the policy of the Chungnam National University regarding the use and care of animals and this study was conducted with the approval of the Animal Experiment Ethics Committee of Chungnam National University (approval number: 201906A - CNU - 083). Mice were housed in under a 12 hr light/dark cycle, a constant room temperature (maintained between 20 and 25°C) and 40~60% humidity, with food and water *ad libitum*. Mice were acclimatized at least 1 week prior to the experi-

ment.

2. Drug administration

YJ-01 and YJ-06 were frozen and dried after extraction of heat water, and each composition is shown in Table 1. YJ-01 and YJ-06 were dissolved in physiological saline and orally administered 100 μl at a dose of 100, 200, or 300 mg/kg. Fluoxetine (FLU, Sigma) was dissolved in 100 μl of saline at a dose of 10 mg/kg, gabapentin (GBP; Sigma) at a dose of 50 mg/kg and administered intraperitoneally as a positive control for depression and pain, respectively. The doses used in the present study were selected based on dosages previously used in literature. Additionally, to investigate the synergistic effect, 100 mg/kg of YJ (01, 06) and 10 mg/kg of Fluoxetine were administered simultaneously by oral and intraperitoneal administration, respectively. All experimental drugs were administered once a day from 3 days after reserpine injection.

3. Reserpine-induced depression and pain

Reserpine (RSP, Sigma) was dissolved in 5% dimethyl sulfoxide (DMSO, Sigma) with physiological saline and subcutaneously injected into the abdominal area once a day for 3 consecutive days to induce depression and pain. Mechanical allodynia was measured at 2, 4, and 6 days after injection. On the 7th day after RSP injection, forced swimming test and open field test were performed after each drug administration. Mice were randomly assigned to the experimental and control groups, and the codes were used as animal numbers to keep the treatment from being known in all behavioral tests.

4. Mechanical allodynia assay

To assess nociceptive responses to innocuous mechanical stimuli (mechanical allodynia), we measured paw withdrawal response frequency (PWF) by using von Frey filament (2.0 g, North Coast Medical, Morgan Hill, CA, USA) as described previously. A von Frey filament was applied from underneath the metal mesh flooring to each plantar of the hind paw. The filament was applied 10 times to each paw, with 10 sec of the time intervals. The number of foot withdrawal reactions after each filament stimulus was counted and the results were expressed as a percentage of the paw withdrawal response frequency (PWF, %).

5. Rota-rod test

Rota-rod test is a commonly used screening procedure to examine motor incoordination and/or ataxia in rodents. In addition, this test can differentiate the antinociceptive effect of the drug from adverse side effects such as sedation. Following the method described previously, mice were placed on a cylindrical platform (12 cm wide; 3 cm diameter) suspended 33 cm above the bottom of the apparatus (SciTech Korea Inc., Seoul, Korea). Falls were cushioned by wood shaved bedding. After 3 days of the

Table 1. Composition of Administered YJ-01 and YJ-06 Extract Formula per a Day

Name of herb		Pharmacognostic name	Weight (g)	
Korean	Chinese		YJ-01	YJ-06
Suk-Ji-Hwang	Shu-Di-Huang	Rehmanniae Radix Preparata	16	-
San-Yak	Shan-Yao	Dioscoreae Rhizoma	8	-
San-Su-Yu	Shan-Zhu-Yu	Corni Fructus	6	-
Taek-Sa	Ze-Xie	Alismatis Rhizoma	6	6
Mok-Dan-Pi	Mu-Dan-Pi	Moutan Radicis Cortex	6	6
Baek-Bok-Ryung	Fu-Ling	Poria Sclerotium	6	6
Dan-Sam	Dan-Shen	Salviae Miltiorrhizae Radix	-	6

acclimation period, the rota-rod test was measured before the administration and at every 20, 40, 60, 80, 100, and 120 minutes after the administration of drugs or vehicles. Time spent on a rotating rod (constant speed of 5~6 revolutions per min) was measured at each time point and cut-off time was 2 minutes.

6. Forced swimming test

Forced swimming test was performed according to the method described by Porsolt with minor modification. One day before the test, mice were placed in a clear plexiglass cylinder (10 cm×25 cm) containing 15 cm of water (24±0.5°C) for 15 minutes. For the test period, the mice were placed in the same system for 6 minutes after 24 hours from the pre-test. After the initial 1 minute of vigorous activity, the mice showed a period of immobility by stop climbing or swimming and remained floating motionless in the water, making only small forelimb movements to keep head above water. The duration that the mice remain immobile during the last 5 minutes of the testing period was recorded as immobility time.

7. Open field test

The Square black acrylic open field box (40 cm× 40 cm×40 cm) to provide the best contrast to the white mouse was placed in a testing soundproof room equipped with a video camera. Before the test, all mice were placed in the testing room for 30 min for adaptation. At the beginning of the test trial, the mouse was placed in the center of open field box for 20 sec and the next 5 min of behavior was recorded by the EthoVision-XT video tracking system (Noldus Information Technology, Netherlands). The EthoVision-XT software analyzes automatically various parameters such as moved distance, moving direction and number of turning or crossing so on. In this study, we examined moved distance to determine the effects of YJ on reserpine-induced depression mice by anti-anxiety based depressive behaviors.

8. Statistical Analysis

Data values were expressed as mean±SEM. The level of statistical significance was determined by unpaired Student's t-test for comparisons between two means, and by analysis of variance (ANOVA) followed by a Dunnett's test for multiple comparisons. Graph pad Prism 6 (Graph Pad Software, Inc., CA, and USA) was used to perform the statistical analysis and a value of $p<0.05$ was considered statistically significant.

III. RESULTS

1. Effects of YJ administration on RSP-induced mechanical allodynia

To investigate the antinociceptive roles of YJ on RSP-induced pain, we examined the effect of YJ administration on RSP-induced MA in mice. Before RSP injection, the baseline of PWF (%) was tested. As shown in Fig. 1A, C, MA was gradually developed in the hind paws for 6 days after RSP injection. The repeated s.c. administration of the RSP gradually increased PWF (%) to innocuous mechanical stimuli as compared with those of the vehicle-treated group. Repetitive oral administration with all used doses of YJ-01 suppressed the RSP-induced increase in PWF (Fig. 1A; $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ as compared with Reserpine group). Additionally, YJ-06 showed antinociceptive effects at doses of 200 and 300 mg/kg (Fig. 1B; $^{*}p<0.05$ and $^{***}p<0.001$ as compared with Reserpine group). GBP was used as a positive drug (Fig. 1A, B; $^{***}p<0.001$ as compared with the Reserpine group). FLU did not show the antinociceptive effect on RSP-induced MA and simultaneous administration of low doses YJ (01 or 06) with FLU showed no synergistic effect.

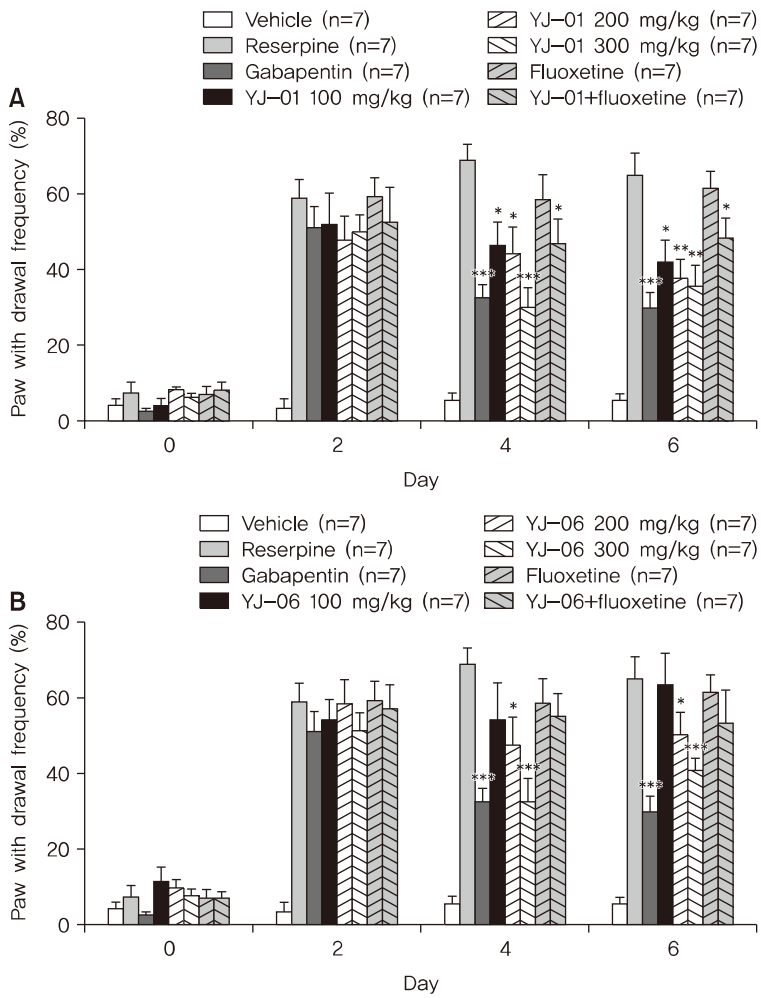

Fig. 1. Antinociceptive effect of oral administration with YJ on reserpine-induced mechanical allodynia. (A) YJ-01 100, 200 and 300 mg/kg administered mice were significantly reduced on the paw withdrawal frequency (%) in the von-Frey test as compared with only reserpine injected mice. (B) YJ-06 200 and 300 mg/kg treated mice were reduced paw withdrawal frequency. High dose of YJ-01 and 06 (300 mg/kg) were similar to those of the positive control gabapentin.
*$p<0.05$, **$p<0.01$ and ***$p<0.001$ as compared with those of the reserpine group.

2. Effects of YJ administration on rota-rod test

The effect of YJ on normal motor function was assessed using the rota-rod test. Normal mice remained on the rota-rod apparatus for 120 sec without fall from the cylinder. After test drug administration, each animal was tested on the rota-rod apparatus and the running time for which the mouse was able to remain on the cylinder was recorded. All doses of YJ-01 and 06 did not affect the normal motor function of mice during 120 min after administration and produced no significant change of spent time in the rota-rod (Fig. 2A, B). 2, 2, 2-tribromoethanol is an anesthetic, used as a positive control in this test (Fig. 2A, B; ***$p<0.001$ as compared with all group). This result indicated that the antinociceptive effect of YJ is not induced by motor impairment.

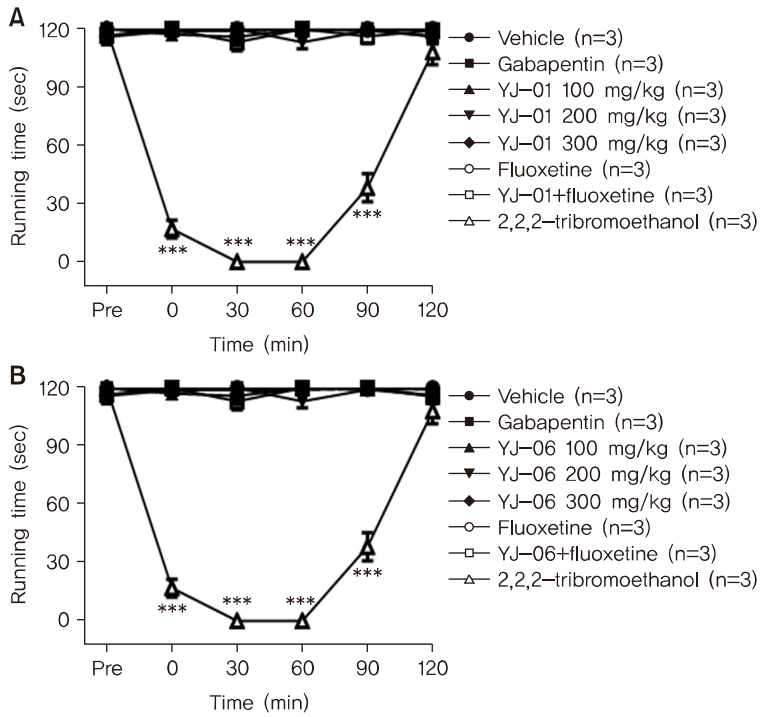

Fig. 2. Antidepressant effect of YJ on forced swimming test. Rota-rod test revealed that the treatment of (A) YJ-01 and (B) YJ-06 did not affect normal motor function on 120 min after treatment compared with vehicle treated group. 2, 2, 2-tribromoethanol is an anesthetic, used as a positive control.
***$p<0.001$ as compared with those of the vehicle group.

3. Effects of YJ administration on RSP-induced immobility time in forced swimming test

To demonstrate the potential antidepressant-like effect of YJ on RSP-induced depression, we performed the forced swimming test on RSP injected mice. As shown in Fig. 3, repeated s.c administration of RSP was significantly increased on immobility time as compared with those of the control group at 7 days after injection. Repetitive oral administration with 200 mg/kg doses of YJ-01 and 200, 300 mg/kg doses of YJ-06 have suppressed the RSP-induced increase in the immobility time (Fig. 3A, B; *$p<0.05$ and ***$p<0.001$ as compared with reserpine group). However, administration with 100, 300 mg/kg doses of YJ-01 and 100 mg/kg dose of YJ-06 not shown the significance but has reducible tendency on RSP-induced increased immobility time. FLU was used as a positive drug (Fig. 3A, B; ***$p<0.001$ as compared with the Reserpine group). Additionally, simultaneous administration of low doses YJ (01 or 06) with FLU showed no synergistic effect. Interestingly, positive control GBP in the pain group also had a significant effect on the increase of reserpine-induced decrease of immobility time.

4. Effects of YJ administration on RSP-induced moved distance in open field test

As part of the depressed behavioral test to demonstrate the potential antidepressant-like effect of YJ, we also performed an open field test on RSP injected mice. As shown in Fig. 4, repeated s.c administration of RSP was significantly suppressed on moved distances and the number of crossings in the field at 7 days after injection (Fig. 4, 5). Repetitive oral administration with 200, 300 mg/kg doses of YJ-06 and FLU

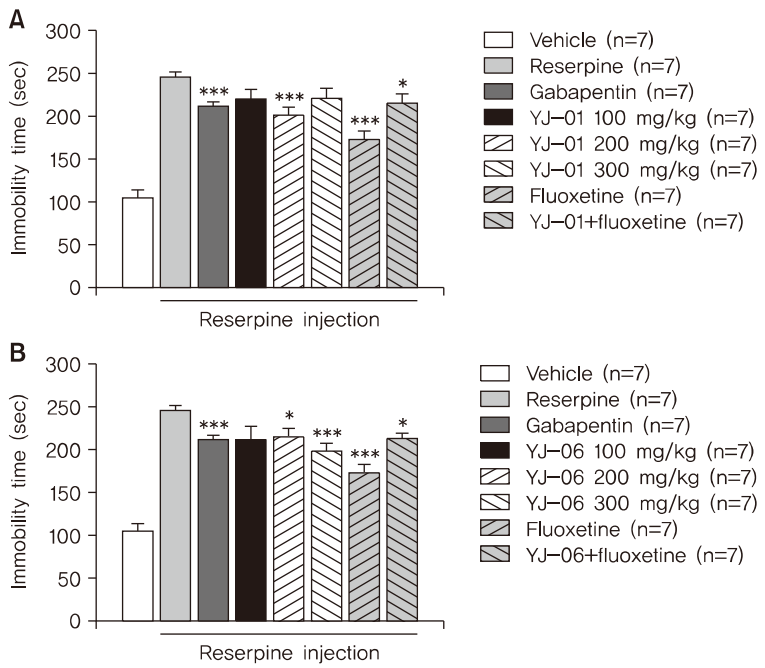

Fig. 3. Antidepressant effect of YJ on forced swimming test. As compared with vehicle injected group, reserpine remarkably increased the immobility time. Treatment of (A) YJ-01, (B) YJ-06, (A, B) fluoxetine and gabapentin significantly reduced the immobility time in forced swimming test.
*$p<0.05$ and ***$p<0.001$ as compared with those of the reserpine group.

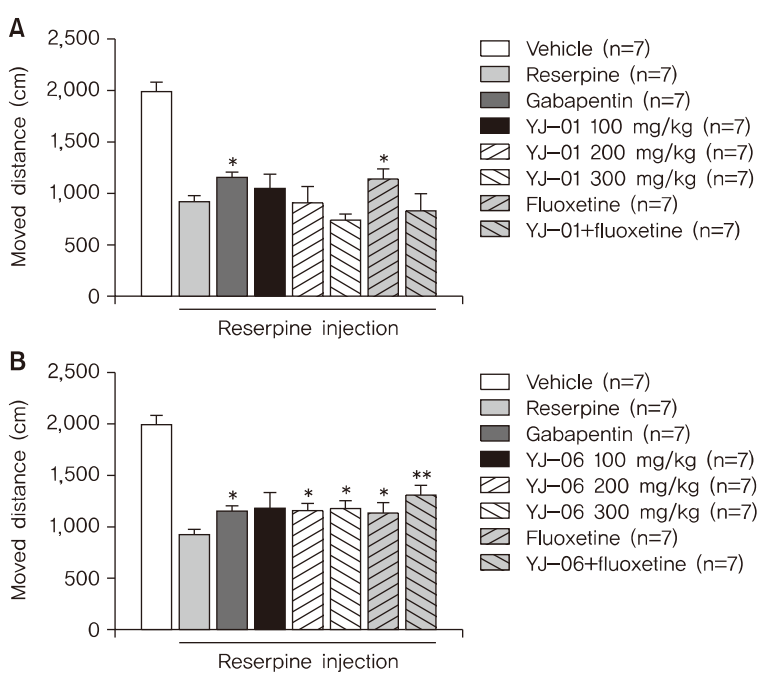

Fig. 4. Antidepressant effect of YJ on open field test. As compared with vehicle injected group, reserpine remarkably decreased the moved distance. (A) Treatment of YJ-01 had no effect in open field test. On the other hand, (B) Treatment of YJ-06, (A, B) fluoxetine and gabapentin significantly recovered the moved distance in open field test.
*$p<0.05$ and **$p<0.01$ as compared with those of the reserpine group.

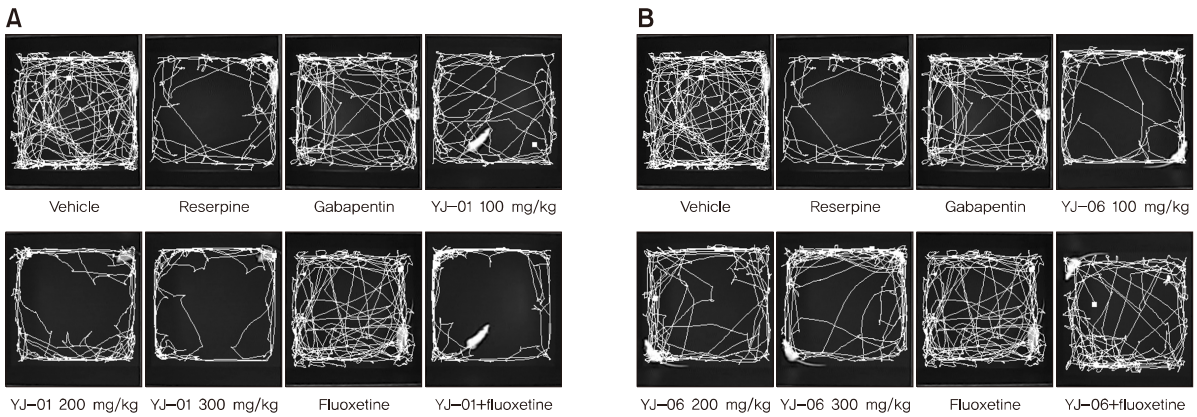

Fig. 5. Visualized data of the open field test. Visualized moved distance and number of crossings by tracking mouse movements in open field box. (A) YJ-01 had no recovery effect on moved distance and number of crossings. On the other hand, (B) Treatment of YJ-06, (A, B) fluoxetine and gabapentin remarkably recovered the moved distance and number of crossings in open field box.

have increased the RSP-induced decrease in the moved distances and number of crossings. (Fig. 4A; $^{*}p<0.05$ and $^{**}p<0.01$ as compared with reserpine group). However, repetitive oral administration of all YJ-01 doses did not show the recovery effect and simultaneous administration of low doses YJ (01 or 06) with FLU showed no synergistic effect. Similar to the FST test, GBP also had a significant effect on the recovery of reserpine-induced decrease moved distance and number of crossings.

IV. DISCUSSION

In this study, we demonstrated both antinociceptive and antidepressant effects of YJs in the pain-depression dyad mice model. Repetitive oral administration of YJ-01 and 06 significantly reduced reserpine-induced nociceptive behavior in the mechanical allodynia test and shown to decrease the immobility time which is despair-based behavior induced by consecutive reserpine injection in the forced swimming test. Moreover, oral administration of YJ-06 also shown to recover moved distance and number of crossings which are anxiety-based behavior induced by reserpine injection in the open field test. In addition, the changes in general motor function by administration of YJs were measured through the rota-rod test and as a result, the administration of YJs showed no side effects such as sedation or motor impairment.

Reserpine has been used clinically as an antihypertensive and antipsychotic drug for the control of high blood pressure and for the relief of psychotic symptoms. However, consecutive administration of reserpine to rodent also causes chronic muscular pain accompanied by depression through the decrease of central nervous system regional biogenic amines (dopamine, serotonin, and norepinephrine) which are related to pain signal processing[7]. In addition to this, it has been suggested that the reserpine can be used as an inducer of pain depression in rodents[8,17,18].

YJ compound contains several medicinal herbs and has been traditionally used to treat various diseases and maintain normal body function in China and Korea. In the concept of traditional medicine, YJ has both function of 'Bo (보, 補)' and 'Sa (사, 瀉)'. It is known that 'Bo' herb reinforce the vital energy, and the 'Sa' herb eliminate the pathogenic factors[19]. In YJ, the herbs that play the role of 'Bo' are Rehmanniae Radix Preparata, Corni Fructus, and Dioscoreae Rhizoma, which refill 'Yin' of liver and

kidney and make new blood. Herbs for 'Sa' are Moutan Radicis Cortex, Poria Sclerotium, and Alismatis Rhizoma. These herbs cool down the fever and make blood flow more smoothly in the body, therefore sedate the Heat syndrome and let the inappropriate energy out. Additionally, Salviae Miltiorrhizae Radix (Dan-sam), which originated from "Shen Nong's Herbal Classic", is one of the major traditional herbs and has been widely used in TCM, and is also called Dan-sam in Korean traditional medicine. Known to promote blood flow, it is widely used to treat cardiovascular, brain, thrombus and other diseases[20-23]. In other words, the action of YJ is to recover where circulation is not proper due to blockage, and supply nutrition to areas where nutrition is not properly supplied due to lack of yin in body parts. Especially, it seems to improve the overall symptoms such as pain and depression caused by the lack of yin through the action of supplementing the yin and blood.

The present study showed that YJ-01 has more effective analgesic efficacy against mechanical allodynia induced by reserpine than that of YJ-06. On the other hand, YJ-06 was more effective in depression-related behaviors induced by reserpine, determined by FST and OF experiments. Summarizing these results, YJ-06 may be a formula that exhibits more suitable efficacy for pain-depression dyad than YJ-01. In other words, 'Sa' herbs and Dan-sam is more effective in pain-depression dyad than 'Bo' herbs. One of the 'Sa' group herb, Alismatis Rhizoma is the rhizome of *Alisma orientale Juzepczuk*, which has been used as diuretics for accelerating water metabolism and removing moisture according to the TCM for centuries[24]. The other one, Moutan Radicis Cortex is the root cortex of *Paeonia suffruticosa Andrews*, has been widely applied as an analgesic, antipyretic and anti-inflammatory agent[25,26]. Another material, Poria Sclerotium is one of the most common materials from *Poria cocos Wolf.*, in TCM and has been used for nearly two thousand years[24,27]. It has been widely used alone or in combination with other medicinal substances to treat various symptoms such as immune dysfunction, urination disorders, diarrhea, vomiting, etc., and modern studies have demonstrated its efficacy[28-30]. By removing 'Bo' herbs and concentrating 'Sa' herbs and Dan-sam, YJ-06 could focus on letting out inappropriate energies circulated through the body and promoting the blood flow effects. There is a high possibility that its difference has an effect on improving the pain-depression dyad.

In conclusion, the present study shows that repetitive oral administration of YJ compound may produce outstanding antinociceptive and antidepressant effects without side effects in reserpine-induced pain and depression mice model, suggesting that YJ compounds including YJ-01 and -06 may be good candidates for the treatment of pain-depression dyad.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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