

ANXIOLYTIC EFFECT OF THE HERBAL REMEDY “KY CUC DIA HOANG THANG GIA VI” IN EXPERIMENTAL ANIMALS

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The present study evaluated the anxiolytic effect of the herbal remedy “Ky cuc dia hoang thang gia vi” in experimental animals. Swiss mice were treated with “Ky cuc dia hoang thang gia vi” remedy at 31.2 and 62.4 g/kg per day for seven consecutive days. The anxiolytic effect of “Ky cuc dia hoang thang gia vi” was assessed in mice using elevated plus maze, spontaneous locomotor activity, rotarod performance and grip strength tests. The results showed that “Ky cuc dia hoang thang gia vi” remedy increased the frequency and time in the open arms and decreased the index of open arm avoidance. Moreover, “Ky cuc dia hoang thang gia vi” remedy inhibited spontaneous locomotion, shortened the stay time on the rotating cylinder in rotarod performance test, and reduced the force in the grip strength test. No statistically significant difference in the anxiolytic effect was found between two doses after three hours of the last administration. Our findings demonstrated that “Ky cuc dia hoang thang gia vi” remedy exerted anxiolytic effect. The rationale for the ethnomedicinal use of “Ky cuc dia hoang thang gia vi” remedy for the management of anxiety was confirmed scientifically in this study.

Keywords: Ky cuc dia hoang thang gia vi, anxiolytic, experimental animals.

I. INTRODUCTION

Anxiety disorders are the most prevalent mental disorders and are characterized by excessive or irrational fear associated with a real or anticipated stimulus.¹ Anxiety is often accompanied by phobic avoidance and a constellation of somatic symptoms. Moreover, according to large population-based surveys, up to 33.7% of the population are affected by an anxiety disorder during their lifetime.^{2,3} Thus, anxiety disorders attempts to find new remedies, especially with herbs are steps in the right direction.

Nowadays, phytotherapy has never stopped gaining in popularity. The use of complementary traditional medicine in treating various diseases has expanded rapidly in both developed and developing countries.^{2,4} The use of herbal medicines to treat anxiety has attracted much interest recently. The herbal remedy “Ky cuc dia hoang thang gia vi” is a traditional medicinal formula for treating anxiety. It consists of 12 different medicinal herbs. To our knowledge, this is the first study to investigate the anxiolytic effect of this herbal remedy in experimental animals. The investigations sought scientific evidence for the ethnomedicinal use of this remedy to manage anxiety. Therefore, we aimed to investigate the anxiolytic effect of the herbal remedy “Ky cuc dia hoang thang gia vi” in experimental animals.

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Received: 03/08/2022

Accepted: 26/08/2022

II. MATERIALS AND METHODS

1. Preparation of “Ky cuc dia hoang thang gia vi”

Herbal remedy “Ky cuc dia hoang thang gia vi” consists of 12 different medicinal herbs: *Rehmania glutinosa* 12g, *Dioscorea persimilis* 12g, *Cornus officinalis* 8g, *Cortex Moutan* 8g, *Alisma plantago* 12g, *Poria cocos* 12g, *Lycium chinense* 12g, *Chrysanthemum morifolium* 8g, *Semen Zizyphus jujube* 12g, *Polygala tenuifolia* 6g, *Semen Nelumbinis* 16g, *Poria* 12g. The expected dosage in clinical is 130g herbs per day. The remedy is prepared in the form of a decocted solution according to traditional medicine methods by a semi-automatic decoction machine. This extractive solution was stored in the refrigerator.

2. Experimental animals

Male and female Swiss mice 8-12 weeks old weighing 18-22 grams were obtained from the National Institute of Hygiene and Epidemiology, Hanoi, Vietnam. Mice were housed in the laboratory animal room ($25 \pm 1^\circ\text{C}$ under $65 \pm 5\%$ humidity and 12h dark-light cycle (from 7:00 - 19:00). Commercial laboratory food and tap water were given *ad libitum*. Mice were kept for one week to acclimatize before starting the experiments.

3. Methods

Experimental groups

The mice were randomly divided into four groups of ten animals (five males and five females) as follows:

- Group 1 (control group): received 20 ml/kg per day sterile distilled water;
- Group 2: orally administered diazepam (Seduxen®, Gedeon Richter) at dose 2.4 mg/kg per day;
- Group 3: orally administered “Ky cuc dia hoang thang gia vi” remedy at dose 31.2 g/kg per day;

- Group 4: orally administered “Ky cuc dia hoang thang gia vi” remedy at dose 62.4 g/kg per day.

Carrier solution, diazepam and “Ky cuc dia hoang thang gia vi” were administered daily for seven days to all animals. On the last drug administration day, the anxiolytic effect of “Ky cuc dia hoang thang gia vi” remedy was assessed in mice using elevated plus maze, spontaneous locomotor activity, rotarod performance test and grip strength test.⁵

Elevated plus maze

The elevated plus maze apparatus consisted of two open arms (30cm x 5cm), two closed arms (30cm x 5cm x 15cm) and a central platform arranged so that the open and closed arms were directly opposite each other. The elevated plus maze apparatus was elevated to a height of 50 cm above the floor. The sample trial on the 6th day, mice were individually placed in the center of the maze and allowed to move freely for five minutes. On the 7th day, one hour after the drug administration, mice were placed in the center of the maze and its head facing an open arm. The following parameters were recorded during the 5 min test period: number of open-arm entries, number of closed-arm entries, time spent in open arms, and time spent in closed arms. After 5 min of free exploration, the mouse may be moved out of the maze and back into its home cage. Between each trial, two arms were cleaned using 70% ethanol to avoid olfactory cues. The index of open arm avoidance is calculated as $[100 - (\% \text{ time spent on open arm} + \% \text{ entries into open arms}/2)]$.

Spontaneous locomotor activity

The activity cage (Ugo Basile, Italy) measures horizontal and vertical activity in mice using infrared beams. Movements are

recorded unobtrusively and saved to the electronic unit (stand-alone controller).⁶ On the 7th day of treatment, one hour and three hours after the last administration, mice were placed individually in each cage. The total activity was recorded for two minutes as the summation of all individual activities of the animal. Between each trial, the cage was cleaned using 70% ethanol to avoid olfactory cues.

Rotarod test

An accelerating rotarod (Ugo Basile, Italy) was used to test the motor function of mice. Mice were placed on the rungs (diameter 29 mm) of the rotarod, which accelerated in speed from 4 rpm to 40 rpm. A trial ended if the mouse fell off the rungs or gripped and spun around for one complete revolution.⁷ Mice were trained for two days before the experiment. On the last drug administration day, after one hour and three hours, mice were carefully placed in the rotarod. The data are expressed as the duration spent on the rotarod cylinder.

Grip strength test

A grip strength meter (Ugo Basile, Italy) was used to measure the forelimb grip strength of mice. As a mouse grasped the bar, the peak pull force in grams was recorded on a digital force

transducer. The gauge was reset to 0g after stabilization, and the mouse’s tail was slowly pulled back by an inspector. After one hour and three hours of the last drug administration on the 7th day, the mouse was removed from its home cage and gripped the base of the tail between the thumb and the forefinger. Then, gently pull the mouse back by its tail ensuring the mouse grips the top portion of the grid and the torso remains horizontal and record the maximal grip strength value of the mouse that is displayed on the screen.

This study was conducted at the Department of Pharmacology - Hanoi Medical University.

4. Statistical analysis

SigmaPlot 12.0 (SYSTA Software Inc, Richmond, CA, USA) was used for statistical analysis. Values were presented as mean ± S.D Data obtained were analyzed by a one way ANOVA followed by post hoc Student-Newman-Keuls test for multiple comparisons. p values < 0.05 were considered statistically significant.

III. RESULTS

1. Effect of “Ky cuc dia hoang thang gia vi” remedy on the elevated plus maze

Table 1. Effect of “Ky cuc dia hoang thang gia vi” remedy on the elevated plus maze

Treatment	Number of open-arm entries	Time spent in open arms (secs)	Number of closed-arm entries	Time spent in closed arms (secs)
Control	2.80 ± 0.79	72.20 ± 22.88	5.30 ± 1.16	217.40 ± 22.13
Diazepam (2.4 mg/kg)	5.10 ± 1.20***	109.70 ± 27.62*	3.30 ± 1.06**	184.20 ± 22.23*
“Ky cuc dia hoang thang gia vi” (31.2 g/kg)	2.90 ± 0.99	92.60 ± 24.71	4.30 ± 1.42	201.80 ± 23.80
“Ky cuc dia hoang thang gia vi” (62.4 g/kg)	4.20 ± 1.23*	104.80 ± 25.90*	3.70 ± 0.95*	190.40 ± 26.51*

*p < 0.05; **p < 0.01, ***p < 0.001 compared to the control group

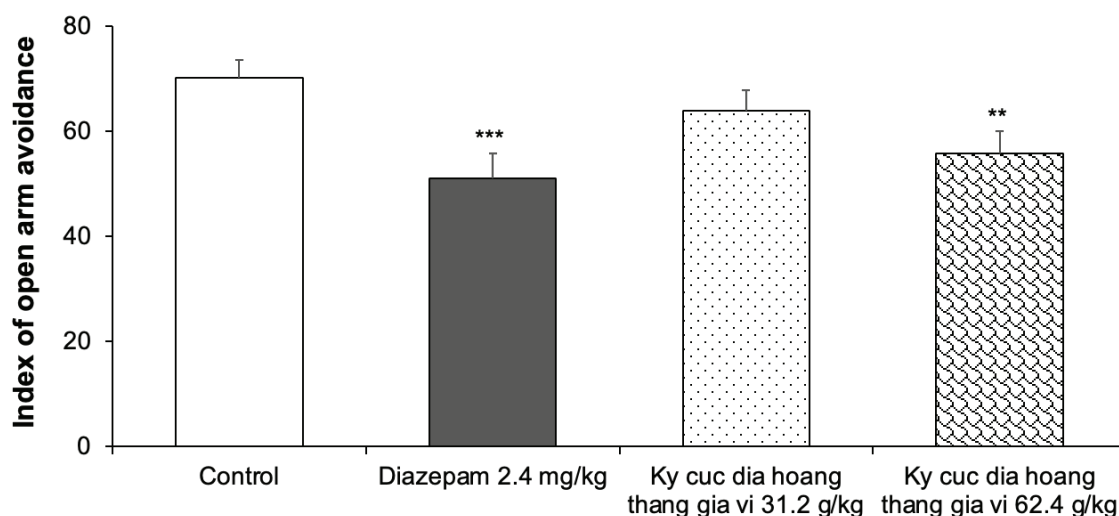


Figure 1. Effect of “Ky cuc dia hoang thang gia vi” remedy on the index of open arm avoidance (n=10). Each data column represents the mean±SD. * $p<0.05$; ** $p<0.01$, *** $p<0.001$ compared to the control group

Table 1 showed that, on the 7th day, one after the drug administration, diazepam at the dose of 2.4 mg/kg and “Ky cuc dia hoang thang gia vi” remedy at the dose of 62.4 g/kg increased the number of entries and amount of time spent in the open arms compared to the control group ($p<0.05$). Treatment of diazepam 2.4 mg/kg and “Ky cuc dia hoang thang gia vi” 62.4 g/kg significantly reduced the number of closed-arm entries and amount of time in the closed arms

of the maze ($p<0.05$). In contrast, “Ky cuc dia hoang thang gia vi” at the dose of 31.2 g/kg insignificantly increased the frequency of open arm entries and duration in open arms ($p>0.05$). In addition, as shown in Figure 1, treatment with diazepam at the dose of 2.4 mg/kg and “Ky cuc dia hoang thang gia vi” at the dose of 62.4 g/kg resulted in a decrease in the index of open arm avoidance in comparison with the control group ($p<0.01$).

2. Effect of “Ky cuc dia hoang thang gia vi” remedy on spontaneous locomotor activity

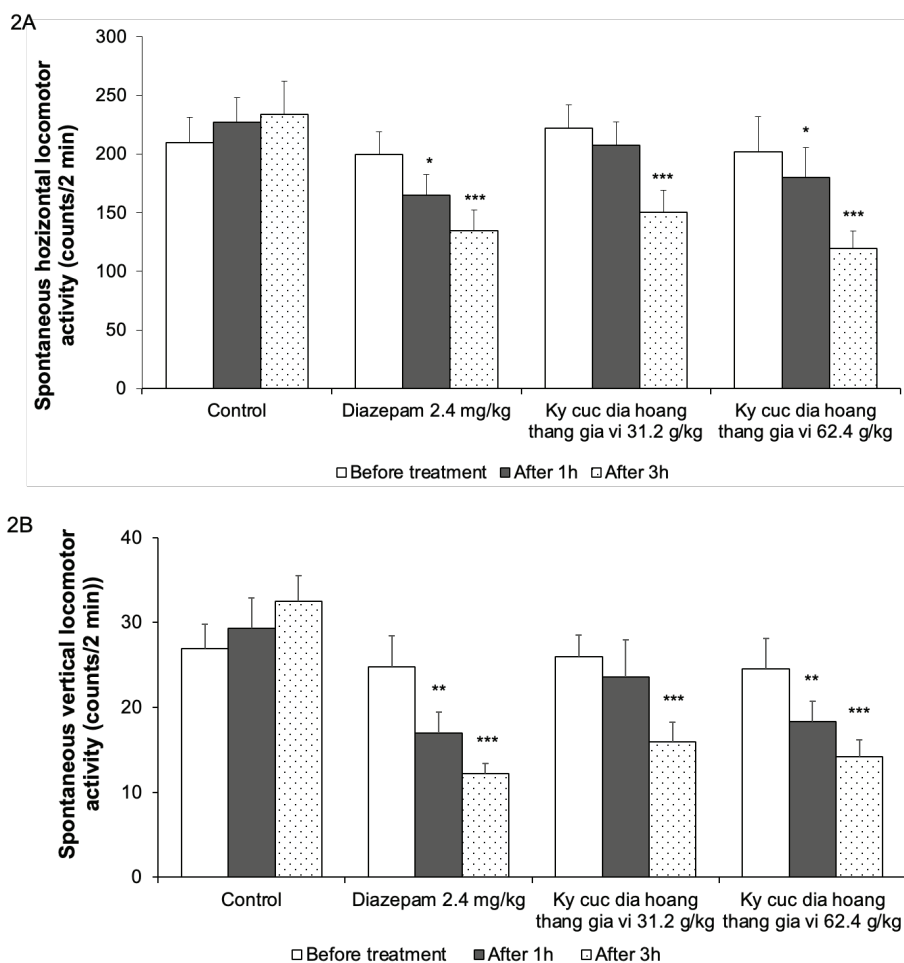


Figure 2. Effect of “Ky cuc dia hoang thang gia vi” remedy on spontaneous locomotor activity in mice (n=10). 2A. Spontaneous horizontal locomotor activity. 2B. Spontaneous vertical locomotor activity. Each data column represents the mean±SD. *p<0.05; **p<0.01, *p<0.001 compared to the control group**

The results showed that after one hour and three hours after the last administration on the 7th day, diazepam at the dose of 2.4 mg/kg and “Ky cuc dia hoang thang gia vi” at doses of 62.4 g/kg markedly inhibited spontaneous horizontal and vertical locomotor activity in mice compared to the control group (p<0.05) (shown in Figure 2A, 2B). In contrast, there was no difference in the spontaneous locomotor activity between “Ky cuc dia hoang thang gia vi” remedy at the

dose of 31.2 g/kg-treated group and the control group (p>0.05). However, after three hours, administration of “Ky cuc dia hoang thang gia vi” at doses of 31.2 g/kg induced inhibition of spontaneous locomotor activity compared to the control group (p<0.001). After three hours of the last administration, no statistically significant difference in the anxiolytic effect was found between two doses.

3. Effect of “Ky cuc dia hoang thang gia vi” remedy on Rotarod test

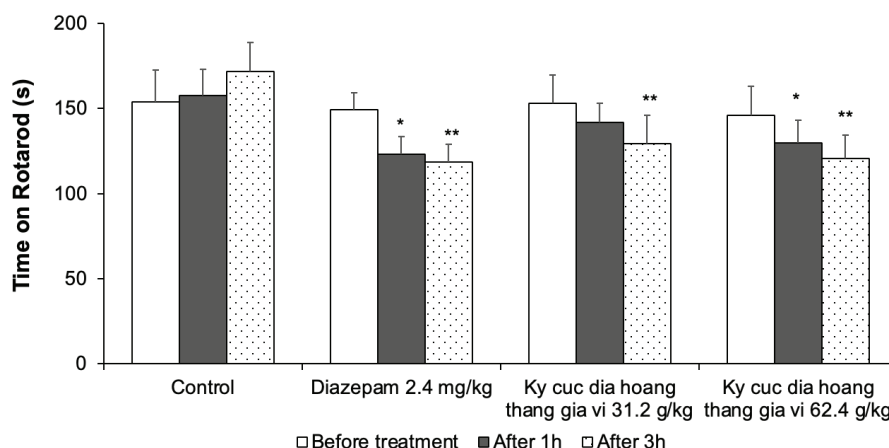


Figure 3. Effect of “Ky cuc dia hoang thang gia vi” on motor function of mice in rotarod test (n=10). Each data column represents the mean±SD (n=10). *p<0.05; **p<0.01, ***p<0.001 vs control group

As seen in Fig 3, one hour after the drug administration on the 7th day, diazepam at the dose of 2.4 mg/kg and “Ky cuc dia hoang thang gia vi” remedy at the dose of 62.4 g/kg showed a significant reduction on time spent on rotarod compared to control group (p<0.05). Treatment with “Ky cuc dia hoang thang gia vi” remedy at the dose of 31.2 g/kg insignificantly reduced time on the rotarod (p>0.05). After three hours

of the last administration, diazepam and “Ky cuc dia hoang thang gia vi” at both doses markedly shortened the stay time on the rotating cylinder in the rotarod performance test (p<0.01). There was no significant difference in the anxiolytic effect between two doses after three hours of the last administration (p>0.05).

4. Effect of “Ky cuc dia hoang thang gia vi” remedy on grip strength test

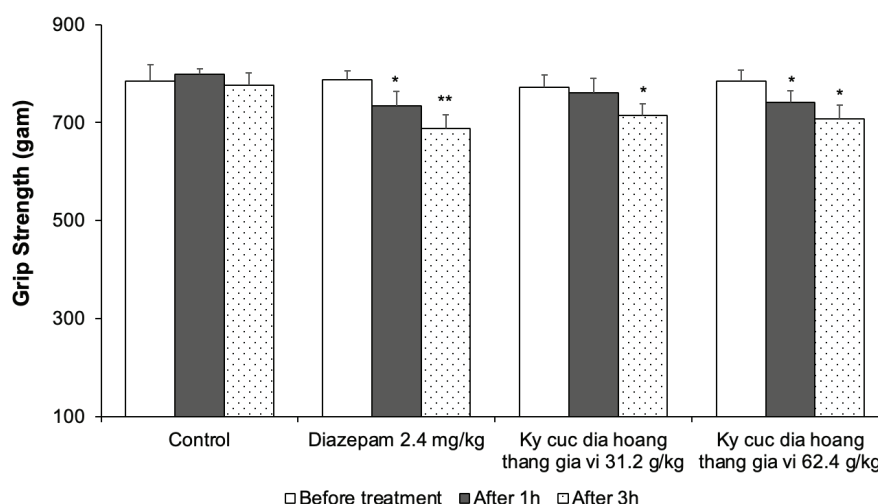


Figure 4. Effect of “Ky cuc dia hoang thang gia vi” remedy on forelimb grip strength test (n=10). Each data column represents the mean±SD. *p<0.05; **p<0.01, ***p<0.001 vs control group

As summarized in Figure 4, one hour after the drug administration on the 7th day, “Ky cuc dia hoang thang gia vi” at the dose of 31.2 g/kg insignificantly reduced the force ($p>0.05$). However, treatment with diazepam and “Ky cuc dia hoang thang gia vi” at the dose of 62.4 g/kg significantly decreased grip strength ($p<0.05$). Three hours after the last administration, the forelimb grip strength of diazepam and “Ky cuc dia hoang thang gia vi” - treated groups were markedly lower compared to the control group ($p<0.05$). No statistically significant difference in the anxiolytic effect was found between two doses after three hours of the last administration.

IV. DISCUSSION

In this study, the anxiolytic property of “Ky cuc dia hoang thang gia vi” remedy at doses of 31.2 and 62.4 g/kg have been investigated. This is the first study demonstrating the anxiolytic effect of “Ky cuc dia hoang thang gia vi” remedy in an the experimental animal. The anxiolytic effect was assessed in mice using an elevated plus maze, spontaneous locomotor activity, rotarod performance test and grip strength test.

The elevated plus maze is a widely used behavior to assess anxiety-like behaviors in rodents. The task is based on an approach-avoidance conflict, meaning that the animal is faced with a struggle between a propensity to explore a novel environment and an unconditioned fear of high and open spaces. Consequently, an anxiety-like state is characterized by a reduced number of entries and amount of time spent in the open arms of the maze, and thus, an increased amount of time in the closed arms of the maze, increased open arm avoidance, compared to control animals. This assay essentially determines a preference between a comparatively safe and comfortable environment (the closed arms) and

a risky environment (elevated open spaces).^{6,9,10} In addition, evaluation of muscle strength is an essential step for researching neuromuscular disorders by using rodent models. Multiple methods have been developed to assess muscle strength essential for researching neuromuscular disorders using rodents models. Among them, the rotarod test is widely used to evaluate motor coordination, and is especially sensitive in detecting cerebellar dysfunction.^{1,7} Furthermore, the grip strength test is also used to measure neuromuscular function. This test is a commonly used method in which an inspector horizontally pulls the rodent's tail that grips a bar connected to a monitoring device, and the maximal value is recorded as the forelimb grip strength.¹¹ Thus, we used these tests to evaluate the anxiolytic effect of the herbal remedy “Ky cuc dia hoang thang gia vi” in experimental animals. Our findings suggested that “Ky cuc dia hoang thang gia vi” remedy possessed the anxiolytic property in mice.

Our study was consistent with the result from the previous reports about the anxiolytic effects of each component in “Ky cuc dia hoang thang gia vi” remedy. Recently, a study by Rui Shi et al. revealed that loganin, one of the major constituents derived from *Cornus fructus* (*Cornus officinalis*) exerted a sedative effect in normal mice. It was found that loganin (20-50 mg/kg) produced beneficial sedative and hypnotic activity, which might be mainly mediated by modification of the serotonergic system and GABAergic neurons.¹² Furthermore, *Poria cocos* is historically used as a complementary therapy to treat depression and anxiety. *Poria cocos* exhibited these effects via reducing inflammation and modulating neurotransmitters and it can be used as a functional food to prevent depression and anxiety.¹³ In addition, *Semen Ziziphi jujuba* is one ingredient of

“Ky cuc dia hoang thang gia vi” remedy. The ethanolic extract of *Semen Ziziphi jujuba* possessed an anxiolytic effect at a dose of 0.5-2.0 g/kg through increasing the percentage of time-spent and the percentage of arm entries in the open arms of the elevated plus maze and decreasing the percentage of time-spent and the percentage of arm entries in the closed arms.¹⁴ In the literature, the findings of Bombi Lee et al. demonstrate that the administration of crude extract of *Polygala tenuifolia* prior to repeated restraint stress significantly reduced anxiety-like behaviors in mice. The daily administration of this extract at the dose of 250 mg/kg increased open-arm exploration in an elevated plus maze and the total number of line crossings in an open-field test.¹⁵ In another study, the anxiolytic and sedative-hypnotic activities of polygalasaponins extracted from *Polygala tenuifolia* (40, 80, 160 mg/kg) were determined in mice using hole-board, elevated plus maze, open field, and sodium pentobarbital-induced hypnosis tests. These results suggest that polygalasaponin possesses evident anxiolytic and sedative-hypnotic activities, which supports the use of *Polygala tenuifolia* root as an anxiolytic and sedative-hypnotic drug in folk medicine.¹⁶ Moreover, lotus seeds (*Nelumbinis semen*) at doses of 100 and 200 mg/kg showed significant anti-anxiety effects as demonstrated through an elevated plus maze and the light and dark test. Thus, lotus seeds proved their usage as a therapeutic agent against anxiety and depression.¹⁷ *Chrysanthemum indicum* was also mentioned with sedative effects. Sa-ik Hong et al. investigated the anxiolytic effects of *Chrysanthemum indicum* water extract using the elevated plus maze test in mice. *Chrysanthemum indicum* at dose of 500 mg/kg significantly increased the time spent in the open arms of the elevated plus maze compared to the control group. Moreover, the

effect of *Chrysanthemum indicum* was blocked by bicuculline (a selective GABAA receptor antagonist) and WAY 100635 (a selective 5-HT1A receptor antagonist). Taken together, these findings suggest that the anxiolytic-like effects of *Chrysanthemum indicum* extract might be mediated by the GABAA receptor and the 5-HT1A receptor.¹⁸

In conclusion, our results showed the anxiolytic effects of “Ky cuc dia hoang thang gia vi” remedy. The rationale for the ethnomedicinal use of the herbal remedy “Ky cuc dia hoang thang gia vi” in the management of anxiety was confirmed scientifically in this study.

V. CONCLUSION

The present study demonstrated that the herbal remedy “Ky cuc dia hoang thang gia vi” at doses of 31.2 g/kg and 62.4 g/kg per day exerted anxiolytic effects.

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