

Original Research Article

Anti-anxiety effect of methanol extract of *Pericarpium zanthoxyli* using a strychnine-sensitive glycine receptor model

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Abstract

Purpose: To determine if the methanol extract of *Pericarpium zanthoxyli* exerts anti-anxiety effects and also to explore any probable anti-anxiety mechanism in vivo.

Methods: The staircase test, elevated plus maze test, rota-rod treadmill test and convulsions induced by strychnine and picrotoxin on mice were tested to identify potential mechanism of anti-anxiety activity of the plant extract.

Results: The plant extract (10 mg/kg, p.o.) significantly reduced rearing numbers in the staircase test while it increased the time spent in the open arms as well as the number of entries to the open arms in the elevated plus maze test, suggesting that it has significant anti-anxiety activity. Furthermore, the extract inhibited strychnine-induced convulsion. However, it had little effect on picrotoxin-induced convulsion, suggesting that its anti-anxiety activity may be linked to strychnine-sensitive glycine receptor and not GABA receptor.

Conclusion: These results suggest that the *Pericarpium zanthoxyli* extract may be beneficial for the control of anxiety.

Keywords: Anti-anxiety, *Pericarpium zanthoxyli*, Glycine Receptor, GABA Receptor

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INTRODUCTION

The genus *Zanthoxylum* has more than 200 species belonging to the Rutaceae family. These species have been used as drugs in traditional medicine systems in Central, South, Southeast and East Asia [1]. *Pericarpium zanthoxyli* refers to the rind of dried fruits of *Zanthoxylum bungeanum* Max., *Zanthoxylum schinifolium* Sieb. et Zucc. and *Zanthoxylum piperitum* D.C. was mostly distributed in Korea and China [2]. These species are rich in essential oil that promote absorption of the active components in a prescription for their unique properties [2,3].

In traditional medicines, *Pericarpium zanthoxyli* is widely used for warming the spleen and stomach to relieve pain, killing parasites to relieve itching, cold and painful stomach, diarrhea, indigestion, acute and chronic gastritis, pathogenic wind, epigastric pain, eczema, pruritus, fungal infection, diarrhea, dysentery and toothache [4-9]. It has been reported that *Pericarpium zanthoxyli* has insecticidal activity [10], anti-oxidative activity [2] and inhibits the early stages of adipogenic differentiation [11]. It also has effects on antagonizing ulcer, inflammatory and functional diarrheas, protecting liver and correcting gastrointestinal disorder and analgesic effect to relieve various abdominal pains [9].

Anxiety disorder is the most common psychological behavioral disorder and benzodiazepines (BZD) have been widely used to treat for anxiety. But, continued use of BZD have been reported for side effects such as memory impairment, addiction problem and muscle relaxation [12]. Thus, novel treatment strategies for anti-anxiety with few side effects are essential for the safe management of patients. The aim of the present study was to determine the anti-anxiety effect of *Pericarpium zanthoxyli* extract and its potential mechanisms using animal behavioral experiments.

EXPERIMENTAL

Animals

Male ICR mice (23-28 g) were purchased from Hanlim Experimental Animals Co., Korea. They were housed under standard environmental conditions of temperature at 22 ± 2 °C under a 12-h light/dark cycle and allowed free access to standard certified rodent diet and tap water. All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals' published by the National Research Council of the National Academies, USA and the Animal Care and Use Guidelines of Kyung Hee University, Seoul, KOREA.

Reagents and materials

Strychnine and picrotoxin were purchased from Tocris (USA). Thiopental sodium was kindly provided by JW Pharmaceutical Co. (Korea). All other chemicals were purchased from Sigma Chemical Co. (USA).

Preparation of plant extracts

Dried aerial parts of *Zanthoxylum bungeanum* Maxim collected in autumn were purchased from Kyung-Dong Oriental Market, Seoul, Republic of Korea. They were authenticated by Professor Emeritus Chang-SooYook, Department of Oriental Pharmacy, Kyung Hee University, Seoul, Republic of Korea. A quantity (100 g) was cut into small pieces and extracted three times with 70 % methanol (300 ml) for 3 h via sonication. The resulting methanol extract was concentrated in a rotary evaporator and freeze-dried.

Staircase test

The stair case test was carried out as described below [13]. A mouse was placed singly on the floor of the box with its back to the staircase. The number of steps (stairs) climbed and the number of rears (rearing) were counted visually over a 3

min period. Climbing was defined as each stair on which the mouse placed all four paws; rearing was defined as each instance the mouse rose on hind legs, either on a stair or leaning against the wall. The number of stairs descended was not taken into account. After each animal had been tested, the box was rapidly cleaned to eliminate any olfactory cue which might modify the behavior of the next animal. The *Pericarpium zanthoxyli* extract (10 or 90 mg/kg) was administered orally using 18 gauge animal feeding needles 60 min before the test. In each experiment, a control group received only saline. Thiopental (25 mg/kg, i.p.) was used as a standard drug.

Elevated plus maze test

The plus maze, consisting of two open arms (50 x 10 cm) and two enclosed arms (50 x 10 x 40 cm) and elevated to a height of 70 cm, was used as described earlier by Pellow *et al* [14]. Mice were treated with saline or *Pericarpium zanthoxyli* extract (10 or 90 mg/kg, p.o.) and 60 min later were placed individually in the center of the plus maze, facing the closed arm. The time spent in the closed and open arms and the number of entries in the closed and open arms during the 5-min test period were recorded visually. Trials were recorded and analyzed using the Noldus Ethovision XT software (Noldus Information Technology, Leesburg, VA).

Strychnine-induced convulsions

Groups of 10 mice within the weight range of 23 and 28 g were used. They were treated orally with saline or *Pericarpium zanthoxyli* extract (10 or 90 mg/kg, p.o.). One hour later the mice were injected with 2 mg/kg strychnine i.p. The time lapse before the occurrence of tonic extensor convulsion and death was noted during a 1-hour period [15].

Picrotoxin-induced convulsions

Groups of 10 mice within the weight range of 23 and 28 g were used. They were treated orally with saline or *Pericarpium zanthoxyli* extract (10 or 90 mg/kg, p.o.). One hour later the mice were injected with 3.5 mg/kg picrotoxin s.c. The time until occurrence of clonic seizure was noted during a 30-min period [16].

Rotarod test

Each mouse was trained to run in a Rotarod (3 cm in diameter, 15 rpm, Rota-Rod Treadmill, Neo Click Co, Seoul, Korea) until it could remain there for 5 min without falling. The mice were then

evaluated in a Rotarod performance test for 5 min, 60 min after saline or *Pericarpium zanthoxyli* (10 mg/kg, p.o.) treatment [17].

Statistical analysis

Data analysis was carried out using the Graphpad Prism 4.0 software (GraphPad Software, Inc., La Jolla, CA), and expressed as mean \pm SEM. Statistical analysis of treatment effects was determined using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test between groups. $P < 0.05$ were considered statistically significant.

RESULTS

Administration of the plant extract (10 mg/kg, p.o.) did not cause any significant changes in the number of steps climbed (Figure 1A) whereas it caused a decrease in the number of rears (Figure 2A) by 45 % compared to control. When the extract was administered at a dose of 90 mg/kg, p.o., the number of steps climbed (Fig. 1B) was not changed, but the number of rears

(Fig. 2B) was decreased by 50 % as compared to control.

The *Pericarpium zanthoxyli* extract had little effect on the number of entries to closed arms (Figure 2B) whereas it caused an increase in the number of open arm entries (Figure 1A) by 2.4-fold. The time spent in open arms (Figure 3A) was also significantly increased by the extract to 2.1-fold compared to control while the time spent in closed arms was not affected (Figure 3B).

Thiopental treatment (26 mg/kg, i.p.) caused a 2-fold increase in the number of entries in open arms (Figure 2C) as compared to control whereas it had little effect on the number of entries in closed arms (Figure 2D). Thiopental also caused a 4-fold increase in the time spent in open arms as compared to control (Figure 3C), but, the time spent in closed arms was decreased by 27 % as compared to control by thiopental (Figure 3D).

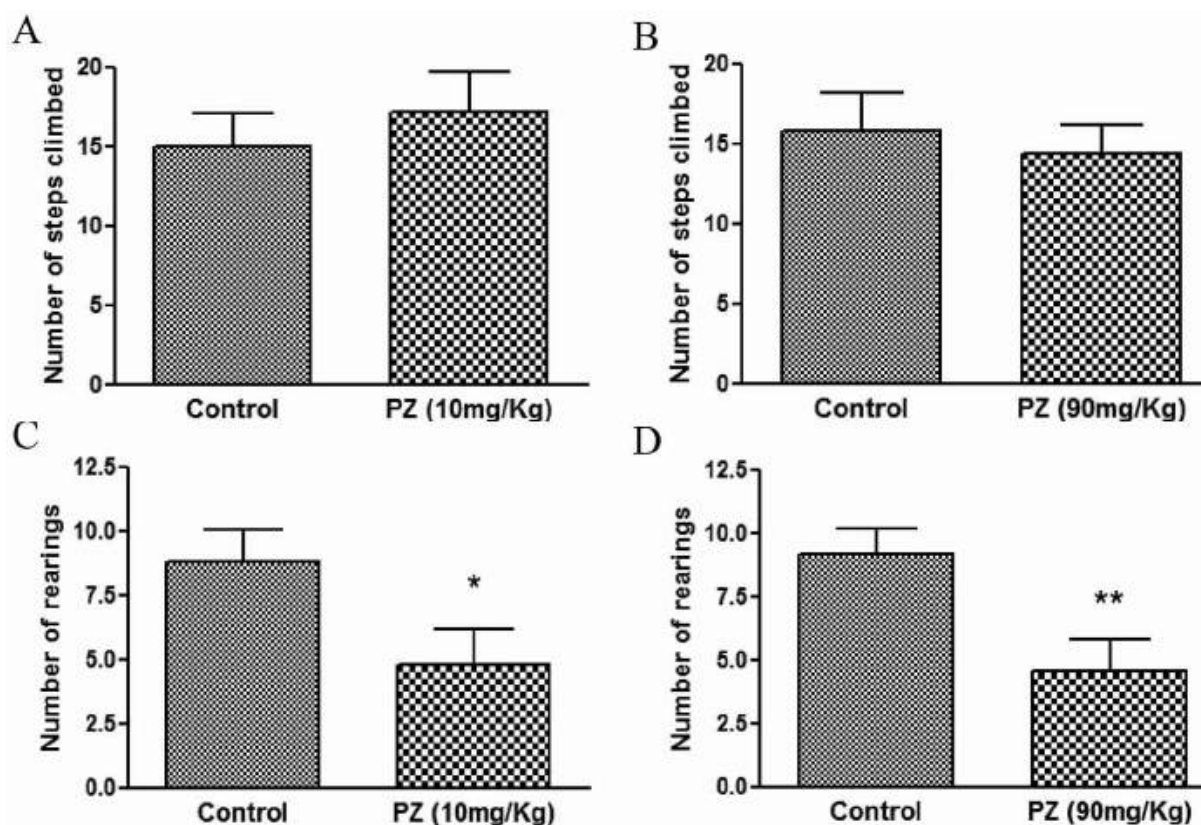


Figure 1: Effect of the *Pericarpium zanthoxyli* extract on anxiety in the staircase test in mice. Following administration of the *Pericarpium zanthoxyli* extract (10 or 90 mg/kg, p.o.), the staircase test was performed as described in the Materials and Methods. Data are expressed as mean \pm SEM. * $p < 0.05$ compared to the control group; ** $p < 0.01$ compared to the control group; (one-way ANOVA followed by Tukey's multiple comparison test, $n = 5$)

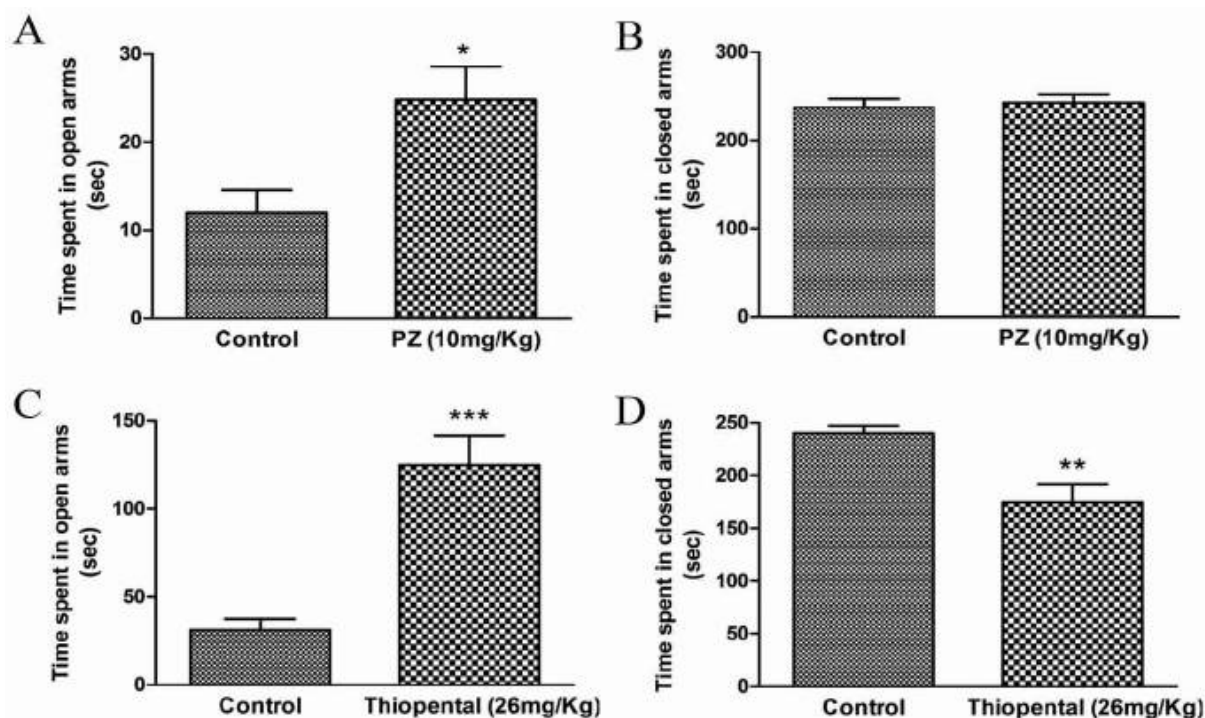


Figure 2: Effects of the *Pericarpium zanthoxyli* extract and thiopental on anxiety in the elevated plus maze test in mice. Following the administration of the *Pericarpium zanthoxyli* extract (10 mg/kg, p.o.) or thiopental (26 mg/kg, i.p.), the elevated plus maze test was performed as described in the Materials and Methods. Data are expressed as mean \pm SEM. * $p < 0.05$, *** $p < 0.001$ compared to the control group (one-way ANOVA followed by Tukey's multiple comparison test, $n = 5$)

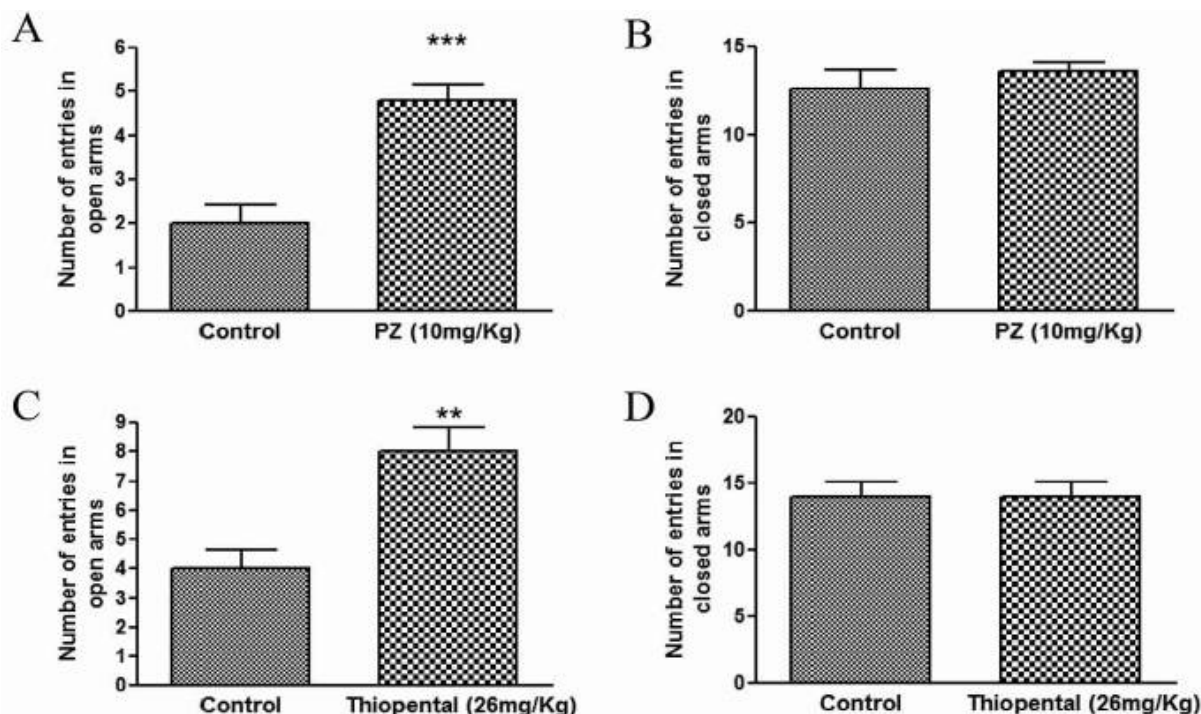


Figure 3: Effects of the *Pericarpium zanthoxyli* extract and thiopental on anxiety in the elevated plus maze test in mice. Following the administration of the *Pericarpium zanthoxyli* extract (10 mg/kg, p.o.) or thiopental (26 mg/kg, i.p.), the elevated plus maze test was performed as described in the Materials and Methods. Data are expressed as mean \pm SEM. ** $p < 0.01$, *** $p < 0.001$ compared to the control group (one-way ANOVA followed by Tukey's multiple comparison test, $n = 5$)

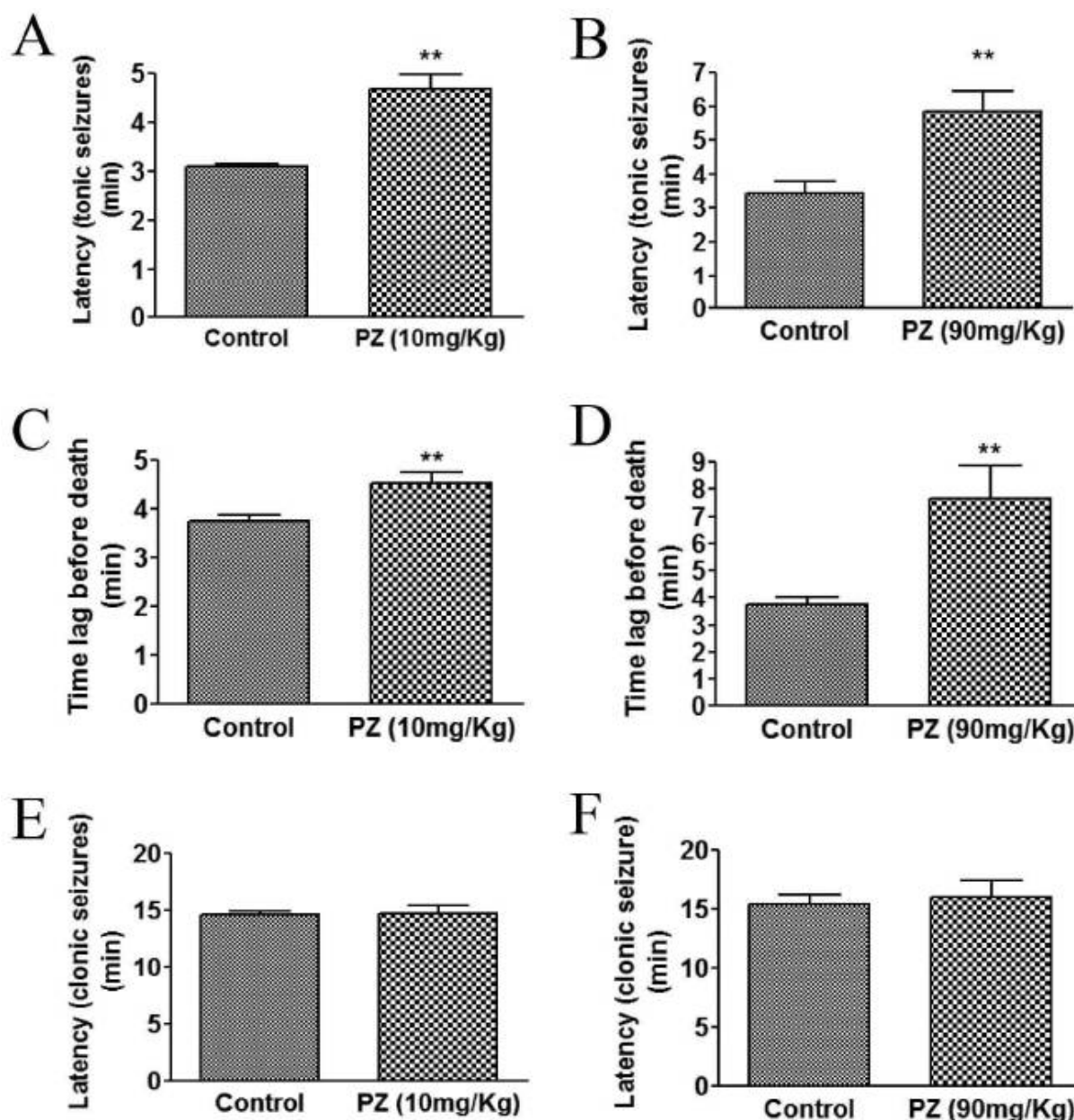


Figure 4: Effect of the *Pericarpium zanthoxyli* extract on the strychnine- and picrotoxin-induced convulsions in mice. Following the *Pericarpium zanthoxyli* extract administration (10 or 90 mg/kg, p.o.), convulsion was induced by strychnine or picrotoxin as described in the Materials and Methods. Latency to convulsion and the time lag before death was measured. Data are expressed as mean \pm SEM. ** $p < 0.01$ compared to the control group (one-way ANOVA followed by Tukey's multiple comparison test, $n = 5$)

The *Pericarpium zanthoxyli* extract (10 and 90 mg/kg, p.o.) increased the latency to the onset of the first clonic seizure caused by strychnine by 51 and 71 % as compared to control, respectively (Figure 4, A-B). It prolonged the induction time of death by 1.21-fold and 2.06-fold respectively as compared to control, (Figure 4, C-4). However, the *Pericarpium zanthoxyli* extract (10 or 90 mg/kg) had not showed any significant effect on the latency to the onset of convulsion caused by picrotoxin (Figure 4, E-F).

The extract (10 mg/kg) exerted no effect in the Rotarod test as the time spent on rotarod for both control and extracted-treated rats was about 250 s.

DISCUSSION

Pericarpium zanthoxyli has been widely used as a spice in Asian countries since it has unique aroma and taste [1]. In addition, it has been

found that *Pericarpium zanthoxyli* has various pharmacological effects such as analgesic, anti-microbial, anti-oxidative and anti-inflammatory actions [2,7-10,18-24]. It also has essential oils up to 11 % and their aroma affects a patient's mood [25]. The above findings prompted us to investigate whether the methanolic extract of *Pericarpium zanthoxyli* has any anti-anxiety activity in experimental animals.

In the present study, *Pericarpium zanthoxyli* extract has a significant anti-anxiety activity in mice as evidenced by behavioral experiments such as the elevated plus maze test and staircase test. The elevated plus maze test and staircase test are used by many investigators as common tools for the study of anxiety of rodents such as mice and rats. In the staircase test, the rearing number is considered as an index of anxiety whereas the number of steps climbed is representative of the explorative activity of animals [13]. The *Pericarpium zanthoxyli* extract at a concentration of 10 mg/kg significantly decreased the number of rears while the number of steps climbed was not affected by the extract, suggesting that the *Pericarpium zanthoxyli* extract has the ability to exert anti-anxiety action with little effect on the explorative activity. Based upon these results, it is reasonable to propose that the *Pericarpium zanthoxyli* extract's anti-anxiety activity was not due to a decrease of explorative action but due to bona fide CNS sedative action. To further support the *Pericarpium zanthoxyli* extract's anti-anxiety action, elevated plus maze tests were also performed. In the elevated plus maze test, the time spent in the open arms and the number of entries to open arms are considered to be an anti-anxiety activity [14] whereas the number of entries to closed arms and the time spent in closed arms are regarded as an explorative action. Like the results observed in the staircase tests, the *Pericarpium zanthoxyli* extract caused clear inhibition of anxiety as evidenced by the fact that the *Pericarpium zanthoxyli* extract increased the time spent in the open arms and the number of entries to the open arms. In contrast, the time spent in the closed arms and the number of entries to closed arms was not affected by the administration of the *Pericarpium zanthoxyli* extract.

In terms of the potential involvement of GABA and glycine receptor *in vivo*, convulsion experiments employing picrotoxin and strychnine were carried out to explore the mechanism of anti-anxiety action of the extract. It is well known that picrotoxin binds to the GABA receptor complex and inhibits the associated chloride channel activity, thereby inducing convulsion

[26]. In the present study, the *Pericarpium zanthoxyli* extract had no effect on the picrotoxin-induced convulsion, indicating its anti-anxiety action may not be mediated by GABA receptor. On the other hand, strychnine acts as a specific antagonist of glycine receptor and binding of strychnine to glycine receptor causes the inhibition of chloride channel associated with the glycine receptor, which eventually leads to convulsion [27]. In contrast to the GABA receptor-related experiments where *Pericarpium zanthoxyli* had no effect on the onset time of convulsion as well as the induction time of death, it significantly increased the latency of convulsion and the time lag before death in the strychnine-induced convulsion experiments. These results strongly suggest that some active principles in the extract may readily bind glycine receptor in the brain, activate the glycine receptor which induces opening of chloride channel, and inhibit the strychnine-induced convulsion.

In the Rotarod test, the *Pericarpium zanthoxyli* extract did not affect the animal's ability to remain on the Rotarod, suggesting it has little muscle relaxant activity. The present results suggest that the extract may activate strychnine-sensitive glycine receptor in mouse brain, and thereby induce anti-anxiety effect *in vivo*. However, we cannot rule out the possibility that the extract may affect other proteins that are important in the regulation of anxiety.

CONCLUSION

Pericarpium zanthoxyli extract exerts a significant anti-anxiety action. Such an action may be due to its binding to and activation of strychnine-sensitive glycine receptor *in vivo*; however, it has little effect on the GABA receptor system under the experimental conditions used in the present study. Taken together, these results suggest that the *Pericarpium zanthoxyli* extract may be beneficial for the control of anxiety in clinical situations but further studies are required to ascertain this.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

CONTRIBUTION OF AUTHORS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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