Evaluation of the Anxiolytic Properties of Tetrahydropalmatine, a *Corydalis Yanhusuo* Compound, in the Male Sprague-Dawley Rat

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The purpose of this study was to investigate the anxiolytic effects of tetrahydropalmatine (THP) and its potential interaction with the benzodiazepine binding site on the γ-aminobutyric acid ($GABA_A$) receptor in the male Sprague-Dawley rat. Tetrahydropalmatine (THP), an active component isolated from the Chinese herbal plant Corydalis yanhusuo, is used in Asia for its analgesic, sedative, and hypnotic properties during herbal therapy. Fifty-five rats were assigned to 1 of 5 groups with 11 rats per group: 1) control (vehicle), 2) THP, 3) midazolam, 4) midazolam with THP, and 5) flumazenil with THP.

In this study, the elevated plus-maze measured the behavioral components of anxiety and motor movements. The data were analyzed using a 2-tailed multivariate analysis of variance to determine if a significant difference existed followed by the least significant difference post hoc test. The findings suggest that THP, 25 mg/kg, given via intraperitoneal injection, results in significant anxiolysis and decreased motor movements. Furthermore, flumazenil, 3 mg/kg, does not fully antagonize the effects of THP.

Keywords: Anxiolysis, *Corydalis yanhusuo*, elevated plus-maze, Sprague-Dawley rat, tetrahydropalmatine.

Anxiety is a feeling of apprehension and fear characterized by physical symptoms such as palpitations, sweating, and feelings of stress. Anxiety disorders are the most common mental disturbances and are prevalent across all ethnic populations; 40 million adults in the United States experience some form of anxiety disorder.[1] They can have a profound, negative effect on a person's general health and well-being and can acutely affect surgical outcomes if untreated.

Anxiety causes particularly complex alterations within the sympathetic nervous system. The sympathetic nervous system responds to acute stress by the release of hormones by the endocrine system, neurotransmitter release via the hypothalamus, regulation of various body functions by the pituitary gland, and stimulation of the adrenal medulla. Activation of the sympathetic nervous system involves release of potent catecholamines such as epinephrine, norepinephrine, and dopamine into the bloodstream. The resulting activation uses a tremendous amount of energy as the cardiovascular, digestive, pulmonary, and other systems mobilize energy stores in a fight-or-flight response.[2] Because of the profound metabolic changes caused by anxiety-induced stress-related catecholamine release, healthcare providers routinely give benzodiazepines, specifically midazolam, as a premedicant for anxiety before initiating many invasive hospital procedures.[3]

Many patients do not notify physicians, anesthesia providers, and nurses about their homeopathic and alternative herbal medication use before hospitalization because of the mistaken belief that herbal preparations are benign. The National Institutes of Health estimates that approximately 40% of American adults and approximately 12% of children used alternative medical treatments within the past 12 months.[4] According to the Dietary Supplement Health and Education Act of 1994, there is no requirement for evidence of efficacy, safety, or quality control standards for supplements, thereby increasing the risk of adverse effects related to

herbal medications. In the United States between 1993 and 1998, the Food and Drug Administration documented approximately 2,600 adverse events, including 100 deaths, related to herbal medications.[5] Currently, there is no central repository for documenting adverse effects associated with herbal remedy interactions; hence, the true number of adverse effects may be much higher than reported. In fact, because of this undisclosed use of herbal medications, the Joint Commission has mandated a screening for herbal medications at each healthcare visit.[6] However, because of a lack of scientific research or rigorous drug controls, patients and clinicians are unaware of possible adverse effects that accompany herbal remedies, especially when used in conjunction with prescribed medications. This lack of data demonstrates the need for scientific research concerning herbal medications and the possible adverse effects and interactions with perioperative medicines. Many herbal products can interact with frequently used medications, including anesthesia, and may cause serious unforeseen consequences or complications.[5,7,8]

Although many herbal preparations such as *Corydalis yanhusuo* show promise in treating various maladies and attenuating symptoms, there are few data regarding how this herb interacts with commonly prescribed medicines. *Corydalis yanhusuo* is a traditional Chinese herbal medicine that is speculated to have analgesic properties,[9] antiseizure activity,[10] and antihypertensive effects.[11,12] It appears that tetrahydropalmatine (THP) is the biologically active extract[13] and has been demonstrated to have antiseizure properties by reducing the seizure activity of kindled rats.[14] THP may also be responsible for the antihypertensive properties of *Corydalis yanhusuo*; it has been shown to antagonize dopamine-2 receptors in the hypothalamus, resulting in hypotension and bradycardia.[15] Two studies have demonstrated that THP inhibits voltage-gated calcium channels[16] and works similarly to verapamil.[17] Wu and colleagues[18] studied cardiac infarct size in the rat model using *Corydalis yanhusuo*. They demonstrated that *Corydalis* provided "significant reduction in infarct size" and improvement in cardiac function after myocardial infarction. Results from a study by Lin and colleagues[14] suggest THP to be an effective antiepileptogenic and anticonvulsant agent. Recently, multiple studies examined the potential role of THP in suppressing brain stimulation reward effects of cocaine[19] and drug addiction therapy involving heroine and opiates.[20,21]

Although there is evidence of central nervous system modulation, only 1 study has evaluated the anxiolytic properties of orally administered THP in the mouse model, which demonstrated a significant decrease in anxiety.[22] The data are limited and inconclusive regarding the potential interactive effects of THP with benzodiazepines. The purposes of this study were to compare the anxiolytic effect of THP with midazolam, a benzodiazepine with known anxiolytic effects in the rat model, and to investigate the possible interaction of THP on the benzodiazepine site of the $GABA_A$ receptor.

Materials and Methods

A prospective, between-subjects experimental design in the rodent model was used to investigate the purposes of this study. The use of laboratory rats in this study was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and received Institutional Animal Care and Use Committee approval from the Department of Clinical Investigations at Brooke Army Medical Center, Fort Sam Houston, Texas.

Fifty-five male Sprague-Dawley rats (Harlan Sprague Dawley Laboratories, Indianapolis, Indiana), weighing 200 to 250 g, were used in this study. They were housed in groups of 3 in clear polycarbonate cages lined with bedding and given free access to food and water. The rats underwent a 10-day adaptation period in a temperature-controlled room (21°-23°C, 60% humidity) with a light-dark cycle of 12 hours of light (6 AM to 6 PM) and 12 hours of darkness (6 PM to 6 AM). The rats were handled by only 1 researcher for weighing, drug administration, and cleaning of cages and were naive to the elevated plus-maze (EPM) apparatus and test room. The EPM is a widely used instrument to measure anxiety in the rodent model and has been validated through research by Pellow and colleagues[23] based on the previous work by Montgomery.[24] Increased time in the open arm represents reduced anxiety in the rat model.

The rats were divided into 5 treatment groups with 11 rats per group receiving intraperitoneal injections of the following: (1) control (saline); (2) THP (Latoxan, Valence, France) at 25 mg/kg,[14] (3) midazolam (Roche, Basel, Switzerland), 1.5 mg/kg; (4) midazolam, 1.5 mg/kg, plus THP, 25mg/kg; and (5) flumazenil (Sigma Chemical Co, St. Louis, Missouri), 3 mg/kg, plus THP, 25 mg/kg. The THP interaction at the benzodiazepine receptor site on the $GABA_A$ receptor was tested by the group receiving flumazenil with THP, a known benzodiazepine receptor antagonist. Potential interactive effects between THP and benzodiazepine agonists were tested by the group receiving midazolam with THP. Midazolam is a well-studied, potent anxiolytic that is given frequently in the hospital surgical setting to relieve or prevent patient anxiety.[25] All rats received 2 injections, each 1 mL in volume. Injections were group-dependent and according to group assignment. The vehicle (solvent) for flumazenil was dimethyl sulfoxide, and the vehicle for THP was saline. Midazolam was obtained premixed. Therefore, group 1 (control) received dimethyl sulfoxide and saline; group 2 received dimethyl sulfoxide and THP; group 3 received dimethyl sulfoxide and midazolam; group 4 received midazolam and THP; and group 5 received flumazenil and THP.

Group	Control	THP	Midazolam (Mid)	Flumazenil + THP (Flu+THP)	Midazolam + THP (Mid+THP)
Ratio of open arm time: total time	18.1 (3.3)	82.9 (11.4)*	40.3 (10.0)*	66.2 (14.1)*	74.1 (13.1)*
Basic movements	990.3 (64.5)	30.4 (15)*	394.5 (112.4)*	69.0 (30.3)*	85.0 (62.8)*
Fine movements	703.7 (43.0)	25.0 (11.5)*	271.7 (78.4)*	56.3 (23.1)*	60.2 (45.4)*

Group	Post hoc analysis	
Ratio of open-arm time: total time	THP vs control: $P = .000$	Flum + THP vs control: $P = 0.003$
	THP vs Mid: $P = .009$	Mid + THP vs control: $P = .001$
	Mid vs Mid + THP: $P = .036$	
Basic movements	THP vs control: $P = .000$	Flum + THP vs Mid: $P = .001$
	THP vs Mid: $P = .000$	Mid + THP vs control: $P = .000$
	Mid vs control: $P = .000$	Mid + THP vs Mid: $P = .002$
	Flum + THP vs control: $P = .000$	
Fine movements	THP vs control: $P = .000$	Flum + THP vs Mid: $P = .002$
	THP vs Mid: $P = .000$	Mid + THP vs control: $P = .000$
	Mid vs control: $P = .000$	Mid + THP vs Mid: $P = .002$
	Flum + THP vs control: $P = .000$	

Table. Ratio of Open-Arm Time to Total Maze Time (in seconds) and Number of Motor Movements on Elevated Plus-Maze per Group
Only statistically significant difference data between groups are presented.
Data are presented as mean (SEM)
*Statistically significant difference of $P < .05$
Abbreviation: THP indicates tetrahydropalmatine.

All experiments were conducted in a quiet testing room with overhead fluorescent lights between 8 AM and 3 PM at an ambient temperature of 20° to 22°C to avoid the potential confounding variable of circadian rhythm. Thirty minutes before testing, each rat was randomly assigned to 1 of the 5 groups and received the corresponding injections. After injections, the rats were returned to their cages to reduce any confounding influences or exposure to any additional unfamiliar environments. Each animal was placed in the center of the EPM, facing an open arm, and behavioral responses to anxiety were evaluated by the EPM for 5 minutes. The EPM was networked with MotorMonitor software (Hamilton-Kinder, Poway, California) with laser sensors integrally attached to the EPM, which tracked the number of entries into each arm, time spent in each arm, and total basic and fine motor movements. Basic motor movements are the simple count of beam breaks in the EPM. Each time a photo beam is interrupted the basic movement count is increased. These movements reveal a gross measure of locomotion but do not distinguish what type of activity is being performed. Fine motor movements are a compilation of small animal movements such as grooming, head weaves, or bobs. The EPM was cleaned with soap and water and dried between each animal trial to limit variability related to previous rat scent on the maze. Immediately following the 5-minute test on the EPM, the animals were removed and placed back in their cages.

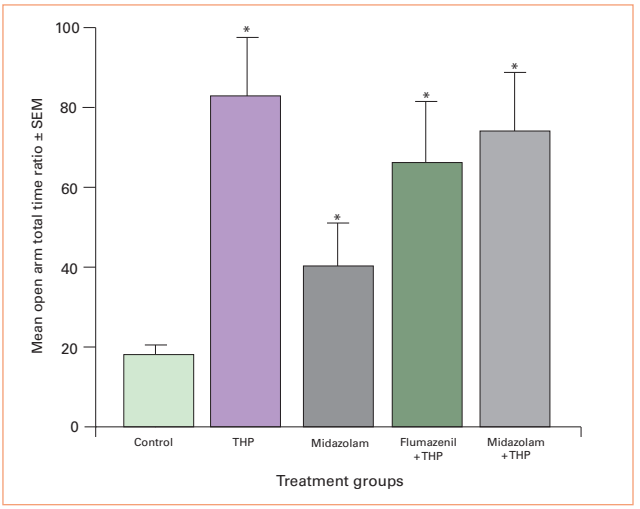

Figure 1. Ratio of Open-Arm Time to Total Time (in seconds) on Elevated Plus-Maze
Each group was composed of 11 rodents. Drugs were injected 30 minutes before testing on the elevated plus-maze.
*Indicates statistically significant difference of $P < .05$; SEM, standard error of the mean.
Abbreviation: THP indicates tetrahydropalmatine.

Results

A 2-tailed multivariate analysis of variance (MANOVA) was performed followed by a least significant difference post hoc test. Analysis of the ratio of open-arm time to total time spent in the EPM displayed a statistically sig-

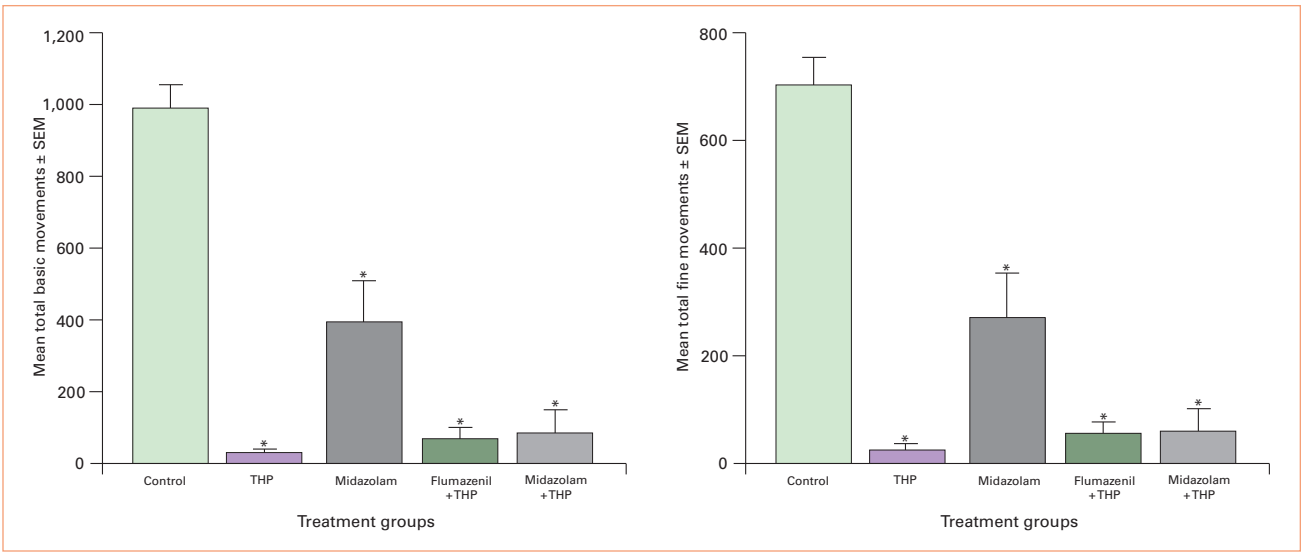

Figure 2. Basic and Fine Motor Movements on Elevated Plus-Maze

Each group was composed of 11 rodents. Drugs were injected 30 minutes before testing on the elevated plus-maze.
*Indicates statistically significant difference of $P < .05$; SEM, standard error of the mean.
Abbreviation: THP indicates tetrahydropalmatine.

nificant difference between groups. The findings showed a significant increase in time spent in open arms with the THP group compared with the control group (P = .000) and the midazolam group (P = .009). Moreover, the THP group spent 48.6% more time in the open arm than the midazolam group. The midazolam with THP group also spent significantly increased time in the open arm compared with the control group (P = .001) and the midazolam alone group (P = .036). The flumazenil with THP group also demonstrated significant increases in time spent in open arms compared with the control group (P = .003) (Table, Figure 1). There was no statistically significant difference found between the THP and midazolam with THP groups.

Analysis of basic and fine motor movements also displayed statistically significant differences between groups (See Table; Figure 2). The THP group demonstrated a decrease in basic and fine motor movements compared with the control group and midazolam group (P = .000). Similarly, the midazolam group showed a significant decrease in movements when compared with the control group (P = .000). The flumazenil with THP group also demonstrated a significant decrease in basic and fine movements when compared with the control group (P = .000), and when compared with the midazolam-only group, also demonstrated decreased basic movements (P = .001) and fine movements (P = .002). When midazolam was combined with THP, this group demonstrated significantly decreased basic and fine motor movements when compared with the control group (P = .000) and the midazolam-only group (P = .002).

Discussion

There is a lack of research concerning THP in the Western pharmacological community; most research has been primarily conducted in China. Research presented by Lin and colleagues[14] found that THP, given at 20 to 30 mg/kg, inhibited amygdaloidal dopamine release in the rat model and so prevented picrotoxin-induced epileptic attacks. THP appeared to inhibit voltage-dependent calcium channels as found in the research conducted by Zhao's group.[16] Research by Chueh et al[15] using male Sprague-Dawley rats gave evidence that THP at 1 to 10 mg/kg reduced serotonin release from the hypothalamus with proportional decreases in heart rate and mean arterial pressure. Leung et al[22] conducted a study that showed the effect of THP was abolished when flumazenil, 1.25 mg/kg, was given with THP, 1 mg/kg, in the mouse model, demonstrating that THP appears to bind to the benzodiazepine site of the $GABA_A$ receptor.

As stated earlier, the THP group spent a mean average of 83% of the time in open arms, whereas the midazolam with THP group time in open arms was 74%. These groups did not show a significant difference, which may be a result of the THP having an overwhelming effect, thus masking the effects of midazolam. The current study tested 3 mg/kg flumazenil with THP at 25 mg/kg, and this group spent 66% of the time in open arms. These findings showed a 20% decrease in mean open-arm time compared with the group given THP alone. Again, a possible explanation is that THP at 25 mg/kg, given after flumazenil, 3 mg/kg, may suggest a possible overwhelming effect of THP on the benzodiazepine site of the $GABA_A$ receptor. Flumazenil is a competitive antagonist at the benzodiazepine receptor site on the $GABA_A$ receptor and reverses benzodiazepine agonists. The difference between the THP alone and THP-flumazenil groups was not statistically significant, and a possible explanation

for the lack of significance between the THP-alone and THP-flumazenil groups is that the THP dose of 25 mg/kg may have overcome the flumazenil antagonism at the benzodiazepine binding site because of a large THP concentration gradient. Another interesting speculation is a possible dose-dependent, anxiolytic-like activity on areas other than the benzodiazepine site of the $GABA_A$ receptor. The rat motor movements were significantly depressed after a single dose of THP (mean of 30 compared with a mean of 394 with midazolam and a mean of 990 with the control). A possible explanation is that THP at 25 mg/kg may modulate central motor control areas or the neuromuscular junction. Interestingly, after the rats receiving THP were removed from the EPM, their motor reflexes, such as grasping and climbing, appeared intact during handling. Therefore, it is speculated that the myorelaxant effects may be a central nervous system effect rather than peripheral modulation. Additional neurobehavioral studies to evaluate the decreased motor movement findings and myorelaxant properties of THP to include rotarod horizontal wire test and inverted screen test should be conducted.

There are limited data regarding the potential interactions of THP with other medications used in hospital settings and its exact mechanism of action on muscle movement. Future studies warrant using additional motor movement evaluative instruments (horizontal-wire test and rotarod apparatus) to determine dose-dependent effects of THP on the neuromuscular system, motor reflex components, and the extent of central nervous system motor suppression. Additional investigations should include a dose-response study of THP effect on anxiety and a dose-dependent THP effect with flumazenil in order to determine the extent of the interaction of THP on the $GABA_A$ benzodiazepine site. Lastly, the use of molecular binding assays may provide important data to help determine precisely how THP modulates various other receptors such as $GABA_A$, serotonin, dopamine, and voltage-dependent calcium channels.

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