Effect of Thai traditional antinausea remedy on hypnotic and sedative activity in animal experimental models: Interaction with drugs acting at GABA_A receptor

Abstract
Thai traditional antinausea remedy is drug registered in the National List of Essential Medicines for the treatment of blood circulation disorders, dizziness, fatigue, and insomnia. Antinausea remedies have long been used, but their effects and action mechanisms remain poorly understood. Here, hypnotic, sedative, and anxiolytic activities of antinausea remedies were evaluated. This preclinical trial assessed the hypnotic, sedative, and anxiolytic activities of antinausea remedies. Thai traditional antinausea remedy was extracted by decoction in deionized water until exhaustion and concentrated to dryness. Anxiolytic activities were evaluated using elevated plus-maze and open-field tests for vehicle control group compared to treatment groups (50, 100, and 200 mg/kg). Hypnotic and mechanistic studies were performed using thiopental sodium-induced sleeping time with benzodiazepine receptor antagonist test. In the thiopental sodium-induced sleeping time test, vehicle control groups were compared to treatment groups (10, 50, and 100 mg/kg). In addition, GABAergic agonistic effect vehicle control groups were pretreated with 3.5-mg/kg flumazenil before sterile water and compared to the treatment group which also received flumazenil before 100 mg/kg extract. Data were statistically analyzed using analysis of variance followed by multiple comparison testing. The aqueous extract was found to be a hypnotic and sedative agent with a dose response either as latent period or prolonged sleeping time (P < 0.05) and a successive GABAergic agonistic effect. Locomotor determination revealed the tendency to relieve anxiety. Thai traditional antinausea remedy can induce sleep and be safely used to reduce anxiety.

Key words: Antinausea remedy, anxiolytic, hypnotic, sedative

INTRODUCTION
The Kingdom of Thailand has its own historical healing techniques called “Thai traditional medicine” (TTM) which involve the art of compounding medicinal material ingredients derived from plants, animals, or minerals into various dosages as TTM recipes. The Food and Drug Administration (FDA) of Thailand classifies herbal medicinal products into four categories as traditional drugs, modified traditional drugs, modern herbal medicines or phytopharmaceuticals, and new drugs. The National List

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of Essential Medicines was launched in BE 2552 by the National Drug Board. This recommends reviewing primary drug registries as a tool to promote drug use in Thailand in accordance with the sufficiency economy philosophy.\textsuperscript{[2]} The antinausea remedy was named and registered as an essential medicine according to traditional healers for the treatment of blood circulation disorders with indications for the treatment of dizziness, fatigue, and insomnia; however, this does not relate to the name of the remedy. In addition, research regarding the effects and mechanisms of its action remains limited. Thus, here, the Thai antinausea remedy was assessed for hypnotic, sedative, and anxiolytic capabilities. Results may indicate alternative ways of administering medication to patients suffering from insomnia and offer important precautionary information for appropriate medicinal usage.

**MATERIALS AND METHODS**

### Plant material and extraction

Ingredients for the Thai traditional antinausea remedy used here were donated by the Academic and Production Services Centre for Aesthetics and Health, Rajamangala University of Technology Thanyaburi. Antinausea remedy powder (225 g) consisted of Glycyrrhiza glabra L. (32 g), Myristica fragrans Houtt. (24 g), Syzygium aromaticum (L.) Merrill and Perry (12 g), Angelica sinensis (Oliv.) Diels (12 g), Ligusticum chuanxiong Franch (12 g), Chrysophyllum zizanioides (L.) Roberty (12 g), Nelumbo nucifera Gaertn. (12 g), Cinnamomum bejolghota (Buch.-Ham.) Sweet (10 g), Cinnamomum loureiri Nees. (8 g), Cinnamomum verum J. Presl (8 g), Aquilaria crassna Pierre (8 g), Euphorbia antiquorum L. (8 g), Artemisia annua L. (8 g), Terminalia chebula Retz. (8 g), Alyxia reinwardtii Blume (8 g), Dryobalanops aromatica Gaertn. (6 g), Lagerstroemia floribunda Jack (6 g), Mesua ferrea L. (6 g), Minusops elengi L. (6 g), Mammeea siamensis T. Anders. (6 g), Aganosma wallichii G. Don (5 g), Draecena loureiri Gagnep. (4 g), and sodium tetraborate decahydrate (4 g). The dried powder was simmered in deionized water (60°C) until exhaustion. Aqueous extracts were filtered through Whatman number 1 filter paper and then concentrated to dryness using a freeze dryer (Labconco Corporation, USA) to obtain the extract yields (yield = 11.8% w/w). The crude extract was then used for further studies.

### Experimental animals

Adult male Swiss albino mice (4–6 weeks old with a body weight range of 35–45 g) from the National Laboratory Animal Centre, Mahidol University, Thailand, were used to evaluate pharmacological activity. Mice were housed in the Thai Traditional Medicine College, Rajamangala University of Technology Thanyaburi, Thailand, under standard environmental conditions (24°C ± 1°C, 60%–70% humidity, 12-h light: 12-h dark cycle). Food and water were given ad libitum until 2 h before experiments. All experimental procedures were conducted in accordance with guidelines of the Animal Care and Use Committee, Rajamangala University of Technology Thanyaburi, Thailand (Animal License No. RMUTT.TMC.2018.R002).

### Treatment

Male Swiss albino mice were divided into 13 groups of eight each. Mice in Group I received sterile water at a similar volume to the treatment groups. Mice in Groups II, III, and IV received 10-, 50-, and 100-mg/kg extract. Mice in Groups IV and V were pretreated with 3.5-mg/kg flumazenil (Tokyo Chemical Industry Co., Ltd., Japan) in 10% dimethyl sulfoxide and then received 100-mg/kg extract and vehicle control for pentobarbital-induced sleeping time test, respectively. Mice in Groups VI and X received sterile water at the same volume as the treatment groups and served as the negative control. Groups VII, VIII, and IX received 10-, 50-, and 100-mg/kg extract for the elevated plus-maze test. Groups XI, XII, and XIII received 10-, 50-, and 100-mg/kg extract for the open-field test.

### Thiopeental sodium-induced sleeping time test

Thiopental sodium-induced sleeping time was evaluated following previous literature.\textsuperscript{[3]} The extract was suspended in sterile water and orally administered. Diazepam (30 mg/kg of body weight; GPO, Thailand) was injected intraperitoneally to induce animal sleep 30 min after gavage of the aqueous extract. The latent period and sleeping time were recorded and compared to the control group by observing the righting reflex.\textsuperscript{[4]}

### Elevated plus-maze test

The elevated plus-maze test was performed using an apparatus which consisted of two open arms (10 cm × 50 cm) and two closed arms (10 cm × 50 cm × 40 cm) with a wooden maze located 30 cm above a black floor as described in a previous study.\textsuperscript{[5]} The extract in sterile water and vehicle was orally administered. Mice were placed in the center of the maze and then observed for 5 min. Time (in s) that the animals spent in the open and closed arms was recorded and compared with the control group. The maze was cleaned with nonodor wet tissue paper at the end of each test.

### Open-field test

The open-field test was performed as previously described.\textsuperscript{[6]} The apparatus consisted of a floor 100 cm × 100 cm divided by red lines into 25 squares with white walls 50 cm high. The test room was illuminated at the same intensity as the colony room. The extract in sterile water and vehicle was orally administered. Mice were placed in the center of the open field, and their behavior was observed for 5 min. A number of line crossings, center square entries, stretch postures, defecation/urination, rearing, and grooming (face cleaning, paw licking, fur licking, head scraping, and rubbing) were recorded. The field was cleaned with nonodor wet tissue paper at the end of each test.
Statistical analysis
Results are presented as mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance, followed by Dunnett’s post hoc test using SPSS software, SPSS INC., USA

RESULTS

Hypnotic effects
Hypnotic effects were evaluated on the basis of the thiopental sodium-induced sleeping time test. Aqueous extracts at doses of 10, 50, and 100 mg/kg significantly augmented the latent time between waking and onset of sleep. Sleeping times of mice were also prolonged at the same dose with latent phase. In addition, extract at dosage of 100 mg/kg pretreated with flumazenil reduced latent time compared to negative control and also shortened sleeping times (*P < 0.05) [Figures 1 and 2].

Anxiolytic and sedative activities
Anxiolytic and sedative activities of the extract were determined using elevated plus-maze and open-field tests. In the elevated plus-maze test, aqueous extract at 10 mg/kg marginally decreased the time rodents spent in the closed arms, whereas 50 and 100 mg/kg greatly increased the time rodents spent in the open arms of the plus maze (*P < 0.05) [Figure 3]. In addition, aqueous extract at 10, 50, and 100 mg/kg resulted in significantly fewer line crossings and center square entries in the open-field test (*P < 0.05) but nonsignificance on rearing, grooming, stretch postures, defecation, and urination [Table 1].

DISCUSSION

Hypnotic, sedative, and anxiolytic effects of an aqueous extract of Thai traditional antinausea remedy were investigated (yield = 11.8% w/w). From the initial observation, no mortality was observed after treatment in 24 h. Results indicated that the aqueous extract had a sedative and hypnotic effect on the central nervous system through GABAergic mechanism at 10, 50, and 100 mg/kg. Moreover, the aqueous extract also showed potential as an antianxiety agent.

In the thiopental sodium-induced sleeping time test, aqueous extract at concentrations of 10, 50, and 100 mg/kg significantly shortened time between the onset and unconscious phase of rodents and also slightly decreased in the pretreatment group [Figure 1]. Moreover, optimized dosage also exploited the negative control by prolongation of the time after rodents approached unconscious phenomenon by righting reflex observation [Figure 2]. Righting reflex or labyrinthine righting reflex in the vestibular system occurs in animals. This response automatically orients the body when it is taken out of normal position.

In the mechanistic study, flumazenil which is a GABA<sub>A</sub> receptor antagonist was orally pretreated for 30 min before treatment with 100-mg/kg extract. Sleeping times were not different compared to the vehicle control group but statistically different to the negative control without GABA<sub>A</sub> receptor antagonist agent which confirmed the aqueous extract function on GABA<sub>A</sub> receptor. This result demonstrated that aqueous extracts generated hypnotic activity with rapid onset and adequate activity.
duration of action through GABA$_A$ receptor. The elevated plus maze measures anxiety in the rodent model and screens the putative anxiolytic compounds based on the aversion to open spaces.[9] In our study, aqueous extracts (50 and 100 mg/kg) affected average time spent in the open arms of the elevated plus maze, indicating an anxiolytic effect, even though the lowest dose showed only a marginal effect. The open-field test is one of the most commonly used platforms to measure habitual behaviors in animal models and investigate pharmacological compounds for anxiolytic effects.[10] The main aspects of open-field behavior are readily characterized by different parameters. Frequency at which rodents cross grid lines or enter the center square with all four paws is one parameter used to monitor levels of anxiety.[10] The aqueous extract showed less potential to influence exploratory behavior. Results indicated that extracts at all concentrations significantly reduced the number of line crossings compared to vehicle control. Similar to the decrease in exploratory behavior, rearing behavior which is categorized as an exploratory behavior has been used as a measurement of anxiety; increased rearing is a sign of decreased anxiety levels.[11] Here, aqueous extracts (10, 50, and 100 mg/kg) showed nonsignificance in rearing numbers. In addition, high frequency in forward elongation of the head and shoulders followed by retraction to the original position and frequency of defecation and urination indicated high levels of anxiety and emotionality, respectively.[12] The aqueous extract gave no significant differences compared to negative control. Conversely, when considering other experimental results, the extract caused drowsiness rather than relaxation. Components of the various types of herbs in this recipe were previously investigated for hypnotic and anxiolytic activities. *G. glabra* or liquorice as the main component in the traditional recipe is documented as a hypnotic and anxiolytic agent. Ethanolic extract of liquorice showed potential for pentobarbital-induced sleeping time through GABA$_A$ receptor and also increased the amount of nonrapid eye movement sleep in mice without decreasing delta activity. This reaction is caused by the active compound called glabrol.[13] In addition, *M. Fragrans*, the second highest quantity in the recipe, was also previously examined for hypnotic activity. *M. fragrans* showed potential for both phenobarbitone- and pentobarbitone-induced sleeping times. Significant sedative properties might result from myristicin which is a natural organic compound and a major component of nutmeg seed.[14,15]

CONCLUSION

The aqueous extract has potential as a hypnotic and sedative agent with anxiolytic effects. Our findings can be used to ensure that proper precautions are taken and the drug is used correctly. Further studies are required to determine the pharmacodynamics of Thai traditional antinausea remedy and analyze its chemical metabolism to discover drug absorption and elimination mechanisms.

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Conflicts of interest

There are no conflicts of interest.

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