**Pandanus amaryllifolius** Root Extract Prolongs Sleeping Time and Reduces Locomotor Activity in Mice

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**Abstract**

The investigation on locomotor activity and hypnotic effect of the decoction of *Pandanus amaryllifolius* root was performed in mice. The extract at doses of 1-2 g/kg significantly decreased the spontaneous locomotor activity in a dose-dependent manner during 30 minutes after feeding. The extract at the dose of 4 g/kg significantly suppressed the locomotor activity in amphetamine-treated mice but the lower dose cannot. In addition, the extract at doses of 0.5-2 g/kg feeding prolonged the pentobarbital-induced sleeping time in both sexes of mice. This effect was not attenuated by flumazenil (a selective benzodiazepine receptor antagonist). These results suggest that the water extract of *Pandanus amaryllifolius* root suppressed the spontaneous and amphetamine-activated locomotor activity. The extract also potentiated the effect of pentobarbital sodium on sleep which is not implicated with benzodiazepine receptor system.

**Keyword:** *Pandanus amaryllifolius*, Toei-hom, Sleeping time, Flumazenil, Locomotor activity, Methamphetamine

**INTRODUCTION**

*Pandanus amaryllifolius*. (*Pandanus odoratus*, Fragrant screw pine, Pandan; Thai name: Toei-hom, Pandanaceae) is an upright shrub, approximately 0.5-1.0 meter high, consisting of stem and nominal support roots. *Pandanus amaryllifolius* is said to be a restorative, promoting a feeling of well being. Various parts of Toei-hom are used in food and traditional medicine. The leaves are also used as a flavoring for desserts such as pandan cake and sweet beverages. The most current use of hot water extract of *Pandanus* root by traditional practitioner is to treat diabetic patients and is found in the Ayurvedic system of medicine for its hypoglycemic action and it is believed very effective to lower blood glucose level in patients but the traditional practitioners always remind the patients that the extract could not 100 percent guarantee to effectively work on all patients. It was reported that oral administration of aqueous extract of root significantly decreased plasma glucose levels in normal female rats and the alloxan-diabetic female rats. Additionally, the root extract showed hypoglycemic and hypolipidemic effects in the streptozotocin-diabetic rats which the active compound for hypoglycemic effect was 4-Hydroxybenzoic acid. Besides the anti-diabetic property, the other famous traditional usage of this root in Malay culture and also Chinese culture is treating common cold with fever, stop bleeding after parturition. Ethanol extract of the leaf also showed anti-hyperglycemic effect in diabetic mice. It has been further reported that the ethanol extract of the leaves exhibited excellent heat-stable antioxidant property and the leaf and root extract also showed...
the antioxidant activity in topical application\textsuperscript{16}. The leaves have a repellent effect on cockroaches\textsuperscript{17}.

Our researchers have worked on its anti-diabetic effects for a long time. During the hypoglycemic experiment, we observed the sedative effect in the Pandanus-treated animals and little information on the central nervous system (CNS) of Pandan was reported. Thus, we investigated the effect of the decoction of \textit{P.amaryllifolius} root on locomotor activity either with or without methamphetamine activation and sleeping time.

**MATERIALS AND METHODS**

**Animals**

Mice weighed 25-35 g, were obtained from Salaya animal center, Mahidol University. Animals were housed in groups of 5 per cage for at least one week in the laboratory animal room before the experiments with free access to food and water. Housing conditions were maintained temperature at 23 ± 1 °C with 60% humidity and a 12 h light-dark cycle. All experiments were carried out during the months of January-March and during 9-12 am period. The animals are acclimatized to the laboratory conditions for at least 5 days prior to experimentation. All experiments were carried out according to the guidelines for care and use of experimental animals, and were approved by the Mahidol University Animal Care and Use Committee, Faculty of Pharmacy, Mahidol University (14/2548), Thailand.

**Plant material**

The authentic samples of the root of \textit{P. Amaryllifolius} Roxb. used in this study were collected in their natural habitats in Chonburi province, Thailand. They were identified by the Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. A voucher specimen (no. BK 62480) was deposited at the herbarium of the Thai Ministry of Forestry, Thailand.

**Extraction**

Three hundred g of dry powder of the root of \textit{P. amaryllifolius} was extracted with 3 liters of water at 70 °C for 1 day and then filtered. The supernatant was lyophilized after evaporating at 70 °C until the volume was about 400 ml. The lyophilized extract was brown bulky powder weighing about 26 g (yield = 8.7 %) and was kept in cool place. The lyophilized powder was dissolved in water prior to the experiment.

**Chemicals**

Pentobarbital sodium (Sigma chemical Ltd, St. Louis, USA); diazepam, flumazenil (Roche Co., Ltd., Basel) and methamphetamine dihydrochloride (Dainippon Pharmaceutical Co.,Ltd., Osaka) were used.

**Locomotor Activity measurements**

Locomotor activities were measured in \textit{P. amaryllifolius}-fed groups of normal mice and methamphetamine-treated mice. Two hours prior to the experiments, mice were housed in the experiment room in order to get used to new habitat. Water (control) or \textit{P. amaryllifolius} extract was orally administered to the mouse. Then each mouse was immediately placed in round cage with small-animal activity monitor (Animate, MATYS, Toyo Sangyo L.t.d., Japan). Infared photocell beams along both horizontal planes formed a 4 x 7 grid within cage. The beam was 3.5 cm apart and 2 cm above the floor of the cage. Spontaneous locomotor activity was automatically counted and recorded the number of photobeam interruptions. Cumulative photobeam interruptions for 30-min period for each animal were subject to statistical analysis in the experiments. In methamphetamine-treated experiment, 30 min after water or \textit{P. amaryllifolius} was orally administered to each mouse, methamphetamine 0.5 mg/kg was intraperitoneally injected and then the mouse was immediately placed in a round cage and locomotor activity was recorded in the same procedure.

**Measurement of pentobarbital-induced sleep**

Pentobarbital-induced sleeping time was measured in \textit{P. amaryllifolius} -fed mice comparing to water-fed mice (control). Thirty minutes after feeding with water or various doses of \textit{P. amaryllifolius} extract, pentobarbital-sodium (50 mg/kg) was intraperitoneally injected to each mouse. Sleeping
time was taken as the period between the loss of the righting reflex and its return.

Statistical Analysis

All values expressed as the mean ± S.E. were obtained from a number of experiments (n). Unpaired, Student-t test was performed to evaluate the statistical differences between the control and the experimental samples, and p values of 0.05 or less were considered significant.

RESULTS

Effect on locomotor activity

In the present study, some neuropharmacological effects of water extract of the root and rhizome of *P. amaryllifolius* in mice were reported. Firstly, the root extract at doses of 1 and 2 g/kg also significantly decreased the spontaneous locomotor activity during 30 minutes after feeding from 696.6 ± 53.3 counts/min (control value) to 457.6 ± 46.7 (34 % decrease, P<0.05) and 248.6 ± 41.3 counts/min (64 % decrease, P<0.01), respectively (Figure 1). Moreover, the root extract at higher dose (4 g/kg) significantly decrease the locomotor activity after methamphetamine injection from 1369.3 ± 61.1 counts/min (control value) to 1196.2 ± 51.0 counts/min (13 % decrease, P<0.05), but the lower dose (2 g/kg) cannot (Figure 2).

Effect on sleeping time

The *P. amaryllifolius* root extract prolonged the pentobarbital-induced sleeping time in both sexes of mice (Figure 3). The root extract at doses of 0.5 and 1 g/kg alone did not induce sleep in both sexes of mice. In male mice, the root extract at doses of 0.5, 1 and 2 g/kg significantly prolonged the sleeping time from 45.1 ± 2.6 minutes (control group) to 62.6 ± 1.5, 50.0 ± 1.9 and 59.6 ± 5.0 minutes (38, 11 and 32% increase, P<0.05), respectively. In female mice, the extract at doses of 0.5, 1 and 2 g/kg significantly prolonged the sleeping time from 51.1 ± 3.9 minutes (control group) to 58.4 ± 4.9, 59.5 ± 2.6 and 58.4 ± 3.4 minutes (14, 16 and 12 % increase, P<0.05), respectively. The effect of flumazenil on the pentobarbital-induced sleeping time was shown in Figure 4. Flumazenil (1mg/kg), without affecting the sleep by itself, also did not attenuate the effect of *P. amaryllifolius* root extract on the pentobarbital sleep but flumazenil abolished the prolonging effect of 0.1 mg/kg diazepam.

![Figure 1](image-url)  
* P<0.05 **P<0.01 vs Control

Figure 1. Effect of *P. amaryllifolius* root extract on the spontaneous locomotor activity in male mice

Water (control) or *P. amaryllifolius* extract was orally administered to each mouse. Then each mouse was immediately placed in a round cage with small-animal activity monitor. Spontaneous locomotor activity was automatically counted and recorded the number of photobeam interruptions in term of cumulative photobeam interruptions for 30-min period. Each datum represents the mean ± SE. Number in bars indicates the number of mice in each experiment.
Water (control) or *P. amaryllifolius* extract was orally administered to each mouse. After 30 minutes, amphetamine (0.5 mg/kg i.p.) was injected. Then each mouse was immediately placed in a round cage with small-animal activity monitor. The locomotor activity was automatically counted and recorded the number of photobeam interruptions in terms of cumulative photobeam interruptions for 30-min period. Each datum represents the mean ± SE. Number in bars indicates the number of mice in each experiment.

**Figure 2.** Effect of *P. amaryllifolius* root extract on the locomotor activity activated by amphetamine (MAP) in male mice

Water (control) or *P. amaryllifolius* extract was orally administered to each mouse. After 30 minutes, pentobarbital (50 mg/kg i.p.) was injected and the duration of pentobarbital-induced sleep was measured as the period between the loss of the righting reflex and its return. Each datum represents the mean ± SE. Number in bars indicates the number of mice in each experiment.

**Figure 3.** Effect of *P. amaryllifolius* root extract on the sleeping time induced by pentobarbital sodium 50 mg/kg i.p. in both sex of mice

Water (control) or *P. amaryllifolius* extract was orally administered to each mouse. After 30 minutes, pentobarbital (50 mg/kg i.p.) was injected and the duration of pentobarbital-induced sleep was measured as the period between the loss of the righting reflex and its return. Each datum represents the mean ± SE. Number in bars indicates the number of mice in each experiment.
Water (control) or *P. amaryllifolius* extract or the reference drug diazepam was orally administered to each mouse. Immediately before the administration of test agents, flumazenil (1 mg/kg i.p.) was injected. Thirty minutes later, pentobarbital (50 mg/kg i.p.) was injected to each mouse and the duration of pentobarbital-induced sleep was measured as the period between the loss of the righting reflex and its return. Each datum represents the mean ± SE. Number in bars indicates the number of mice in each experiment.

**Figure 4.** Effect of flumazenil on *P. amaryllifolius* root extract and diazepam modulation of pentobarbital-induced sleep

DISCUSSION

It is generally accepted that sedative/CNS depressant effect of drugs can be evaluated by measurement of spontaneous motor activity and sodium pentobarbital-induced sleeping time in laboratory animals and Fujimori (1995) proposed that the enhancement of barbital hypnosis is a good index of CNS depressant activity. This study examined the effect on locomotor activity and hypnotic effect of *P. amaryllifolius* root in mice to indicate the sedative effect of the plant. The *P. amaryllifolius* root extract showed a suppressive effect on spontaneous locomotor activity (Figure 1) which was one of central nervous system function. The locomotor activity was automatically counted and recorded in terms of the number of photobeam interruptions by the animal movements. It could relatively show the animal movement and activity. The spontaneous locomotor activity, especially the control group, of the experiment was quite high because of the excitement of animal after feeding and immediately placed in the instrument. *P. amaryllifolius* root extract alone decreased this activity during 30 minutes after feeding about 34 - 64 % from control level. These data suggested that *P. amaryllifolius* root might suppress the activity of central nervous system. The hyperactive model or treated with methamphetamine, methamphetamine activates CNS activity on serotonin (5-hydroxytryptamine/5-HT), norepinephrine and dopamine transporters, though the primary mechanism of action is on the dopamine transporter (DAT), resulted in an increased concentration of extracellular dopamine primarily in the limbic system (particularly in the striatal nucleus accumbens) causing locomotor activation. In the hyperactivative model, the extract also significantly suppressed the locomotor activity by 13 % at a high dose,
4 g/kg p.o., as evaluated by measurement of locomotor activity.

Sleeping time was a common model for a hypnotic study; in this case pentobarbital sodium (intermediate-acting sedative drug) was used as a sleep inducer. Oral administration of the water extract of *P. amaryllifolius* root alone could not induce a sleep but it had a prolonging effect on the pentobarbital-induced sleeping time in both sexes of mice, however, in this dose range, the activity was not a dose-response manner (Figure 3). The activity was not different among the extract-treated group and the lowest dose used (0.5 g/kg) showed the highest activity. This dose range might be in the supramaximal range or the different among doses might be too little. In this study, 1 g/kg flumazenil, a selective benzodiazepine receptor antagonist, was used to find whether benzodiazepine receptor system is implicated in the mechanism of action of this plant extract on sleep. It was found that flumazenil at this dose abolished the prolonging effect of diazepam, a reference sedative agent, but flumazenil failed to attenuate the effect of *P. amaryllifolius* extract on pentobarbital-induced sleep (Figure 4). Thus, *P. amaryllifolius* extract action on sleep is not implicated with GABA-benzodiazepine receptor system. The hypnotic effect and decreasing locomotor activity of the extract might be its pharmacological activities or this might represent its neurotoxicity. Fortunately, the effective dose range used in this experiment was 0.5 - 4 g/kg which was lower than the reported oral LD$_{50}$ of the extract >8 g/kg$^{17}$. Thus, both hypnotic effect and decreasing locomotor activity represent its CNS depressive activity of the extract. The phytochemical screening tests of this root extract showed the presence of flavonoids and coumarin in the root extract of *P. amaryllifolius*.$^{18}$ However, the flavonoid and/or phenolic compounds in the root extract might be the active constituents because of its structure might easily pass through the blood brain barrier into the central nervous system to show this action. Based on the previous study of *P. odoratissimus*, two phenolics, four lignin type compounds and a new benzofuran-derivatives were found in the root.$^{18}$ The compounds include pinoresinol and 3,4-bis(4-hydroxy-3-methoxybenzyl) tetrahydrofuran. There are four new pyrrolidine alkaloids that have been found, which are pandamarilactone-E, -F, -F-N-oxide and G, together with pandamarilactones-A to –D.$^{19}$

In conclusion, the present results suggest that *P. amaryllifolius* root suppressed the spontaneous locomotor activity and methamphetamine-activated locomotor activity. The extract also potentiated the effect of pentobarbital sodium on sleep which is not implicated with benzodiazepine receptor system. Clarification of the exact mechanisms of action of the extract will require further investigations.

**REFERENCES**

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